

## GERM-LINE *BRCA1* MUTATIONS IN JEWISH AND NON-JEWISH WOMEN WITH EARLY-ONSET BREAST CANCER

MICHAEL G. FITZGERALD, B.A., DEBORAH J. MACDONALD, R.N., M.S., MICHAEL KRAINER, M.D.,  
 INGRID HOOVER, B.A., ERIN O'NEIL, B.A., HILAL UNSAL, M.D., SANDRA SILVA-ARRIETO, B.A.,  
 DIANNE M. FINKELSTEIN, PH.D., PEGGY BEER-ROMERO, M.S., CHRISTOPH ENGLERT, PH.D.,  
 DENNIS C. SGROI, M.D., BARBARA L. SMITH, M.D., PH.D., JERRY W. YOUNGER, M.D., JUDY E. GARBER, M.D.,  
 ROSEMARY B. DUDA, M.D., KATHLEEN A. MAYZEL, M.D., KURT J. ISSELBACHER, M.D.,  
 STEPHEN H. FRIEND, M.D., PH.D., AND DANIEL A. HABER, M.D., PH.D.

**Abstract Background.** Mutations in a germ-line allele of the *BRCA1* gene contribute to the familial breast cancer syndrome. However, the prevalence of these mutations is unknown in women with breast cancer who do not have the features of this familial syndrome. We sought *BRCA1* mutations in women who were given a diagnosis of breast cancer at an early age, because early onset is characteristic of a genetic predisposition to cancer.

**Methods.** Clinical information and peripheral-blood mononuclear cells were obtained from 418 women from the Boston metropolitan area in whom breast cancer was diagnosed at or before the age of 40. A comprehensive *BRCA1* mutational analysis, involving automated nucleotide sequencing and a protein-truncation assay, was undertaken in 30 of these women, who had breast cancer before the age of 30. In addition, the *BRCA1* mutation 185delAG, which is prevalent in the Ashkenazi Jewish population, was sought with an allele-specific polymer-

ase-chain-reaction assay in 39 Jewish women among the 418 women who had breast cancer at or before the age of 40.

**Results.** Among 30 women with breast cancer before the age of 30, 4 (13 percent) had definite, chain-terminating mutations and 1 had a missense mutation. Two of the four Jewish women in this cohort had the 185delAG mutation. Among the 39 Jewish women with breast cancer at or before the age of 40, 8 (21 percent) carried the 185delAG mutation (95 percent confidence interval, 9 to 36 percent).

**Conclusions.** Germ-line *BRCA1* mutations can be present in young women with breast cancer who do not belong to families with multiple affected members. The specific *BRCA1* mutation known as 185delAG is strongly associated with the onset of breast cancer in Jewish women before the age of 40. (N Engl J Med 1996;334:143-9.)

©1996, Massachusetts Medical Society.

THE *BRCA1* gene has been identified at the chromosome 17q locus, which is linked to familial breast and ovarian cancer.<sup>1,2</sup> *BRCA1*, a potential tumor-suppressor gene, encodes a predicted 200-kd protein whose function is unknown. Genetic susceptibility to breast cancer results from the inactivation of one *BRCA1* allele in the germ line, followed by the loss of the remaining allele in somatic breast tissue.<sup>1,3</sup> Unlike mutations in other known tumor-suppressor genes, *BRCA1* mutations have only been detected in specimens of breast-cancer tissue when there is a mutated germ-line allele. The role of *BRCA1* mutations in breast cancer is therefore limited to women with a genetic susceptibility to this tumor.

Molecular analysis of familial breast cancer has identified *BRCA1* mutations in 31 of 100 families, and genetic-linkage studies suggest that in half of kindreds with breast cancer and in 80 percent of families with a history of both breast and ovarian cancer, there is in-

heritance of a mutated *BRCA1* allele.<sup>1,3-7</sup> It has been estimated that in such kindreds the inheritance of a mutated *BRCA1* allele confers a 59 percent chance that breast or ovarian cancer will appear by the age of 50 and an 82 percent chance that one of these cancers will appear by the age of 70.<sup>8,9</sup>

We do not know the incidence of germ-line *BRCA1* mutations in women in the general population who may be at increased risk for breast cancer but who do not belong to large families with multiple affected members. About 6 to 19 percent of women with breast cancer have an affected relative,<sup>10,11</sup> but this in itself does not reliably identify women with a high risk for a *BRCA1* mutation. Coincidental cases in large families may reflect only the high incidence of sporadic breast cancer, and in small families the absence of multiple affected family members cannot rule out the presence of a *BRCA1* mutation. Clinical features that suggest a genetic predisposition include bilateral breast cancer; bilateral premalignant lesions, such as lobular carcinoma in situ; and unusually young age at presentation.<sup>12</sup>

Early age at diagnosis is a useful marker of genetic susceptibility to cancer. In children predisposed to retinoblastoma or Wilms' tumor by a germ-line mutation in the *RB* or *WT1* genes, cancer develops earlier than in patients with sporadic disease<sup>13,14</sup>; in adults with familial polyposis who carry a germ-line mutation in the *APC* gene, polyps and colonic carcinoma appear early

From the Center for Cancer Risk Analysis and the Massachusetts General Hospital Cancer Center, Charlestown, Mass. (M.G.F., D.J.M., M.K., I.H., E.O., H.U., S.S.-A., P.B.-R., C.E., K.J.I., S.H.F., D.A.H.); and the Departments of Pathology (D.C.S.) and Medical and Surgical Oncology (D.M.F., B.L.S., J.W.Y., D.A.H.), Massachusetts General Hospital; the Department of Biostatistics, Harvard School of Public Health (D.M.F.); Dana-Farber Cancer Institute (J.E.G.); Beth Israel Hospital (R.B.D.); and Faulkner Hospital (K.A.M.) — all in Boston. Address reprint requests to Dr. Haber at MGH Cancer Center, CNY7, Bldg. 149, Charlestown, MA 02129.

Supported in part by grants (to the Center for Cancer Risk Analysis) from the Massachusetts General Hospital and Oncor.

in life.<sup>15,16</sup> In women with familial breast cancer, tumors usually appear before the age of 45, a considerably younger age than is typical in the general population.<sup>17</sup> An epidemiologic model predicts that 36 percent of all women in whom breast cancer appears between the ages of 20 and 29 have a genetic predisposition, as compared with 1 percent of women with breast cancer diagnosed at the age of 80 or older.<sup>18</sup> The annual incidence of breast cancer in women 20 to 29 years old is 1.7 per 100,000 women; these young patients account for 1.5 percent of all cases of breast cancer in the United States.<sup>17</sup> To test the hypothesis that women who have breast cancer at a young age have a high probability of carrying a *BRCA1* mutation, we analyzed the prevalence of germ-line *BRCA1* mutations in women with early-onset breast cancer. In addition, we studied Jewish women who had breast cancer at an early age to determine the presence of a specific *BRCA1* allele with a mutation at position 185 involving a deletion of adenine and guanine (185delAG) that has recently been found in 1 percent of the Ashkenazi Jewish population.<sup>19</sup>

## METHODS

### Study Patients

Women in whom breast cancer developed between 1981 and 1992 at or before the age of 40 were identified by retrospectively reviewing the medical records at four breast-cancer referral centers in Boston — Massachusetts General Hospital, Dana-Farber Cancer Institute, Beth Israel Hospital, and Faulkner Hospital. Among 850 eligible women, 418 agreed to donate blood for an analysis of genes for susceptibility to breast cancer, the results of which would not be disclosed. The blood samples were coded, and the confidentiality of the patients was preserved, in accordance with the guidelines for studies of human subjects of the collaborating hospitals. Medical and family histories and demographic variables (including religion and ethnic origin) were obtained by direct interviews, and pathological diagnoses were confirmed by a review of the medical records. In a rigorous determination of the presence of *BRCA1* mutations in women with early-onset breast cancer, the entire coding region of the *BRCA1* gene was analyzed in a subgroup of 30 women in whom breast cancer developed before the age of 30. In addition, 39 Jewish

women among the 418 women who had breast cancer at or before the age of 40 were screened for the 185delAG mutation.

### Blood Samples and Molecular Strategies

Blood samples were obtained for the extraction of genomic DNA and polyA messenger RNA (mRNA), which were isolated from peripheral-blood mononuclear cells by standard procedures. First-strand complementary DNA (cDNA) was synthesized from the polyA mRNA with random hexamers (Pharmacia). B lymphoblasts immortalized by the Epstein-Barr virus were also prepared from all specimens, and when appropriate, paraffin-embedded blocks of the primary tumor were obtained for a mutational analysis based on the polymerase chain reaction (PCR).

### Protein Transcription-Translation Assay

Protein transcription-translation (PTT) analysis detects premature stop codons in a single reaction by amplifying DNA fragments of the coding region, transcribing the fragments into mRNA, and translating the mRNA into radiolabeled peptides.<sup>16,20</sup> The results of genomic DNA-based PTT analysis of *BRCA1* exon 11 were reported while this study was in preparation,<sup>21</sup> and we undertook to screen the entire *BRCA1* coding region using both mRNA and genomic DNA-based PTT analysis. *BRCA1* contains 24 exons, the first of which is noncoding; exon 4 consists of a repetitive sequence of the Alu family that is omitted from most transcripts; and exon 11 contains 3.5 kb of the entire 5.6-kb coding sequence.<sup>1</sup> The *BRCA1* gene transcript was therefore divided into six overlapping fragments (Fig. 1, top panel): the 5' end, containing exons 2 through 10 and the 5' end of exon 11 (amino acids 1 through 265), and the 3' end, containing exons 12 through 24 and the 3' end of exon 11 (amino acids 1354 through 1864), were amplified as single fragments from randomly primed cDNA. Exon 11 was first amplified as a single 3.5-kb fragment from genomic DNA by long-range PCR, with ExTaq (Takara), followed by nested PCR amplification of four overlapping fragments (AP, containing amino acids 224 through 499; BP, containing amino acids 461 through 749; CP, containing amino acids 712 through 1056; and DP, containing amino acids 1020 through 1366). The 5' oligonucleotide for each of the six PCR fragments contained a T7 polymerase recognition site, a Kozak consensus sequence, and a start codon, to allow the transcription and translation of uncloned PCR products. The analysis of uncloned PCR products ensured that both *BRCA1* alleles would be represented and reduced the possibility of any polymerase-induced error that might be present in an individual PCR product. One microgram of the PCR product was incubated with T7 polymerase and rabbit reticulocyte lysate in a transcription-translation mix (Promega) in the presence of <sup>35</sup>S-labeled methionine (Amersham), followed by electrophoresis with 12 percent and 15 percent

Figure 1. Mutational Analysis of *BRCA1*.

The top panel shows the genomic structure of *BRCA1* and the strategies for the protein transcription-translation (PTT) assay and nucleotide sequencing. Exons 1 and 4 are not shown because they are noncoding exons. When possible, fragments of the *BRCA1* coding region were amplified by PCR from reverse-transcribed mRNA (cDNA). However, the presence of extensive alternative splicing at the 5' end of the *BRCA1* transcript required that individual exons be amplified from genomic DNA by PCR; the large exon 11 was amplified most readily from genomic DNA.

The middle panel (boxed) shows the results of an electrophoretic analysis of peptides produced by PCR-amplified fragments of *BRCA1* from eight patients. The PTT assay revealed a heterozygous mutation in Patient 99. Half the DNA fragments amplified from Patient 99 contained a premature stop codon resulting from a mutation in one of the *BRCA1* alleles. The transcription of the DNA fragments into mRNA and subsequent translation into radioactively labeled protein yielded a truncated peptide (arrow), along with the full-length normal product.

The bottom panel shows an analysis of the heterozygous mutation in Patient 99 by automated nucleotide sequencing. The wild-type *BRCA1* sequence was 5'GAAACATGTAA3' (underlined). The deletion of AT in one of the two alleles resulted in a shift in the reading frame and a premature stop codon. Both alleles are analyzed together here. In the plus strand, the nucleotide sequence reads from left to right. The arrowhead indicates the site of the deletion of the two nucleotides in one allele and the resulting unsynchronized sequence: to the right of the arrowhead, two nucleotides are recognized at each position, with a corresponding reduction in the signal intensity of each. The nomenclature used to indicate the presence of two nucleotides in the same position is as follows: R denotes A+G; W, A+T; S, G+C; K, G+T; and Y, C+T. For the minus strand, the sequence (converted from antisense to sense nucleotides in this analysis) reads from right to left; the arrowhead again marks the site of the heterozygous deletion, and the sequence to the left of the arrowhead is unsynchronized.

polyacrylamide–sodium dodecyl sulfate gels. The sequence of all primers used and the optimal PCR conditions have been submitted to the Breast Cancer Information Core electronic data base (Internet address: [http://www.nchgr.nih.gov/dir/lab\\_transfer/bic/](http://www.nchgr.nih.gov/dir/lab_transfer/bic/)).

**Automated Nucleotide Sequencing**

The sequence of the entire 5.6-kb coding region of *BRCA1* was determined for each germ-line specimen with automated sequencing of both strands of uncloned PCR products using dye-labeled dideoxy terminators (Applied Biosystems). The use of uncloned PCR products allowed both alleles to be analyzed in a single reaction and min-

imized artifactual errors in any one product. The most direct approach, analysis of reverse-transcriptase PCR products spanning the entire *BRCA1* transcript, was complicated by the low level of *BRCA1* mRNA expression in peripheral-blood mononuclear cells and by alternative mRNA splicing at the 5' end of the transcript. Conditions were therefore optimal for sequencing analysis with templates derived by PCR amplification of both genomic DNA and cDNA. The 5' end of *BRCA1* (exons 2 through 5, 7 through 10, and 13 and 14) was amplified from genomic DNA with primers complementary to the intron sequences flanking each exon. These exons could not be sequenced successfully from cDNA as a single fragment because of

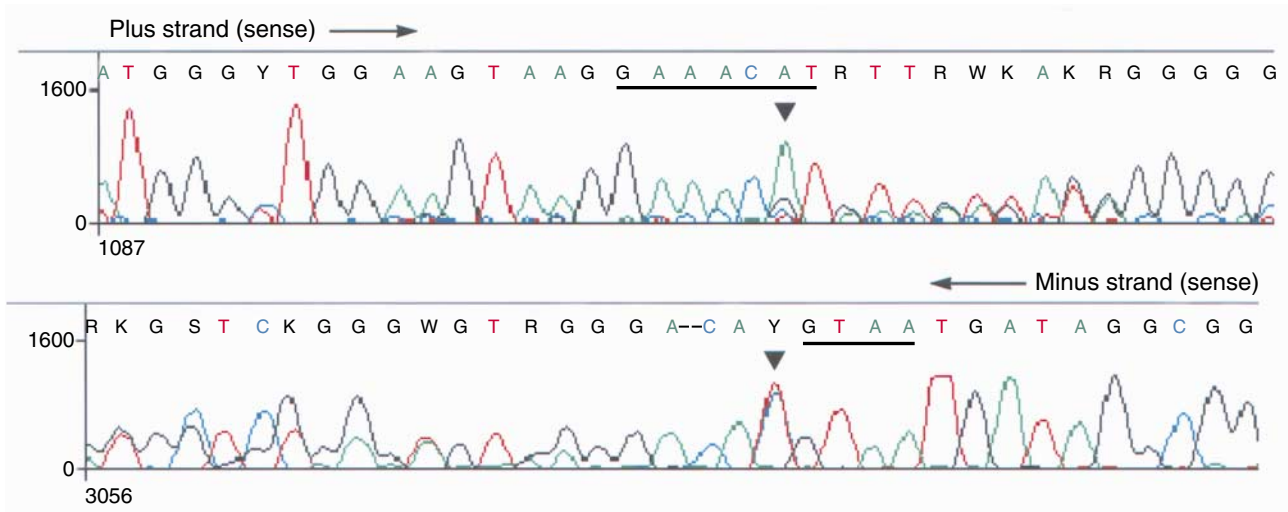
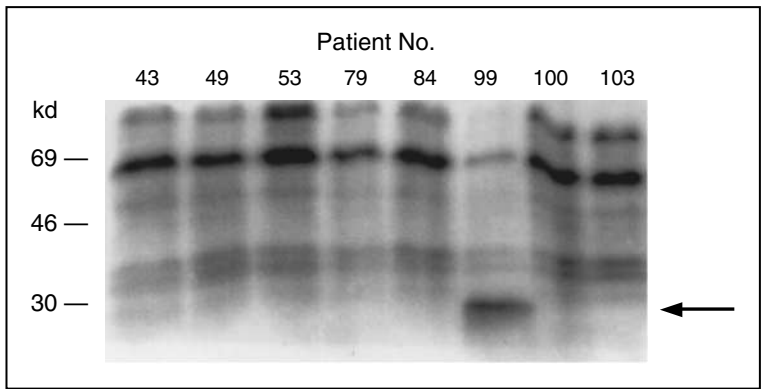
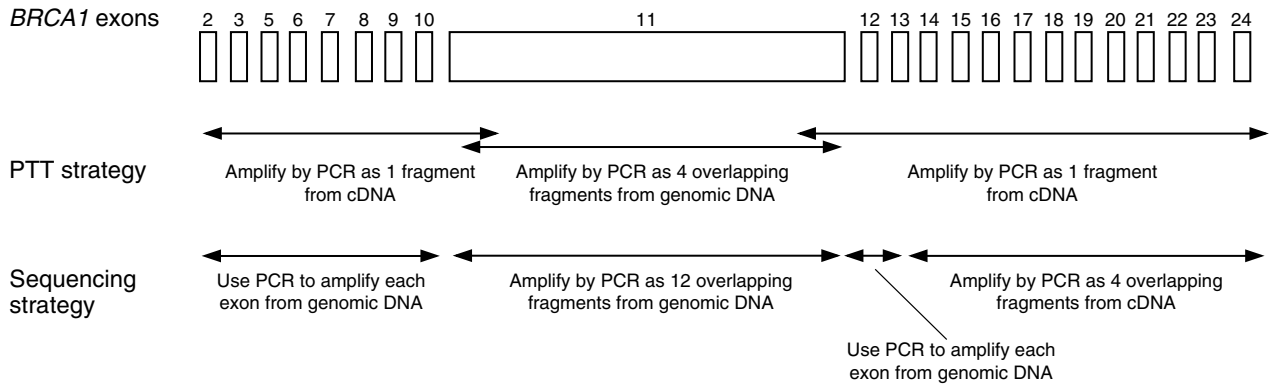


Table 1. Clinical Characteristics of 418 Women with Early-Onset Breast Cancer.

AGE AT DIAGNOSIS (YR)	NO. OF WOMEN	ASSOCIATED LCIS*	BILATERAL BREAST CANCER	FAMILY HISTORY OF BREAST CANCER			FAMILY HISTORY OF OVARIAN CANCER
				DEFINITE†	1ST-DEGREE RELATIVE	1ST- OR 2ND-DEGREE RELATIVE	
<30	30	0	3 (10)	3 (10)	6 (20)	10 (33)	1 (3)
30 to 40	388	8 (2)	19 (5)	26 (7)	73 (19)	158 (41)	27 (7)
Total	418	8 (2)	22 (5)	29 (7)	79 (19)	168 (40)	28 (7)

\*LCIS denotes lobular carcinoma in situ.

†A definite family history was defined as the presence of breast cancer in three or more paternal or maternal relatives in two or more generations.

the extensive alternative splicing of the *BRCA1* transcript in peripheral-blood mononuclear cells (alternative splicing of exons 5, 9 and 10 and alternative use of splice junctions in exons 8 and 14). Exon 6 was amplified from cDNA, because the flanking intron sequences were not optimal for analysis from genomic DNA; an initial reverse-transcriptase PCR spanning exons 2 through 11 was followed by a nested PCR containing exon 6. Exon 11 was amplified in 12 overlapping fragments from genomic DNA, as follows: 11A, from the intronic sequence through nucleotide 939; 11B, nucleotides 871 through 1251; 11C, nucleotides 1193 through 1537; 11D, nucleotides 1480 through 1850; 11E, nucleotides 1769 through 2134; 11F, nucleotides 2077 through 2438; 11G, nucleotides 2358 through 2759; 11H, nucleotides 2667 through 3012; 11I, nucleotides 2944 through 3299; 11J, nucleotides 3233 through 3590; 11K, nucleotides 3533 through 3907; and 11L, nucleotide 3820 through the intronic sequence. Exons 12 and 13 were amplified individually from genomic DNA with primers complementary to the flanking introns. The 3' end of *BRCA1* (exons 13 through 24) was analyzed by reverse-transcriptase PCR amplification. An initial reverse-transcriptase PCR spanning exons 12 through 24 was used, followed by a nested PCR that produced four overlapping fragments spanning exons 13 through 16, 15 through 18, 17 through 22, and 21 through 24.

All PCR products were purified by electrophoresis on 2 percent low-melting-point agarose (FMC), and appropriate fragments were extracted by incubation at 65°C and treatment with 1 U of Agarase (Sigma). Cycle sequencing with *Taq* polymerase and dye-labeled dideoxy terminators was performed according to the manufacturer's instructions. The reaction products were analyzed with a DNA sequencer, and the sequence files were edited with the Factura program (Applied Biosystems). Base positions with a secondary peak at least half the height of the primary peak were marked with use of the degeneracy codes of the International Union of Biochemistry. For each PCR fragment, Factura-processed sense and antisense sequence files were aligned with the wild-type *BRCA1* sequence with the Sequence Navigator program (Applied Biosystems). The primer sequences and PCR conditions were adjusted to provide optimal resolution for automated sequencing analysis (this information is available through the Breast Cancer Information Core data base). For PCR fragments that yielded unreliable results of automated sequencing analysis, a 5' tail containing M13-derived sequencing primers was attached to the amplification primers, and these standard primers were then used in the sequencing.

#### Detection of the 185delAG Mutation

To screen for the 185delAG mutation in *BRCA1* exon 2, genomic DNA was amplified with four primers in a single PCR (annealing temperature, 58°C), as follows: primer 1 (sense), complementary to the 5' intron sequence 5'GAAGTTGTCATTTATAAACCTTT3'; primer 2 (antisense), complementary to the 185delAG mutation 5'TGACTTACCAGATGGGACACTA3'; primer 3 (sense), complementary to the wild-type exon 2 sequence 5'ATTAATGCTATGCAGAAAA-TCTTAGAG3'; and primer 4 (antisense), complementary to the 3' intron sequence 5'GTATGTAAGGTCAATTCTGTTC3'. For each specimen, this combination of primers produced bands of 170 nucleotides (185delAG specific), 118 nucleotides (wild-type specific),

and 282 or 284 nucleotides (control for the mutated and wild-type alleles, respectively).

#### Statistical Analysis

Ninety-five percent confidence intervals for the estimates of risk and the odds ratios were calculated with StatXact (Cytel). The association between familial risk and the rate of mutation was tested by Fisher's exact test. The attributable risk was estimated by methods used in retrospective studies under the assumption that the disease was rare (and therefore that the odds ratio was an approximation of the relative risk).<sup>22</sup> The prevalence of the 185delAG mutation in the Jewish population was estimated to be 8 per 858 people, according to data reported elsewhere.<sup>19</sup> Also, we assumed that the cumulative risk of breast cancer by the age of 40 was 1 in 217 women.<sup>17</sup>

#### RESULTS

Clinical data were recorded and peripheral-blood specimens obtained for 418 women from the Boston metropolitan area in whom breast cancer was diagnosed at the age of 40 or earlier. To undertake comprehensive mutational screening of the *BRCA1* gene, we first selected the 30 women who we presumed had the highest risk of a genetic predisposition, because they had breast cancer before the age of 30. Most of these women had been affected between the ages of 20 and 29 with infiltrating ductal carcinoma, with or without ductal carcinoma in situ, but tumors with less common histologic features were also noted, including infiltrating lobular, medullary, and anaplastic carcinomas; one five-year-old child had a juvenile secretory adenocarcinoma. In addition to diagnosis at an early age, 5 of the 30 women had bilateral breast cancer (2 patients), a definite family history of breast cancer (one in which three or more paternal or maternal relatives are affected and are from two or more generations; 2 patients), or both (1 patient) (Table 1). These clinical features, which strongly suggest a genetic predisposition, were similar in the women with onset of disease before the age of 30 and those with onset between 30 and 40. Among all 418 women who had breast cancer at the age of 40 or earlier, 168 (40 percent) had at least one first- or second-degree affected relative (95 percent confidence interval, 36 to 46 percent), a figure that contrasts with estimates of 6 to 19 percent for the general population.<sup>10,11</sup> Thus, most women in our study did not belong to characteristic breast-and-ovarian-cancer pedigrees, but they represented a subgroup of the general population that has an increased

risk of a genetic predisposition to breast cancer.

### Screening for *BRCA1* Mutations by the PTT Assay

Since the large size of the *BRCA1* gene complicates mutational studies, we used two approaches: the PTT assay, a rapid screening test that can detect chain-terminating mutations,<sup>16,20,21</sup> and automated nucleotide sequencing combined with the use of software to facilitate the identification of heterozygous mutations, a method that can detect all nucleotide substitutions in the *BRCA1* coding region.

Seventy to 80 percent of *BRCA1* mutations identified in kindreds with breast cancer are nonsense mutations or small insertions and deletions that shift the codon reading frame, causing premature protein termination.<sup>1,3-7</sup> These mutations should be detectable by PTT analysis, in which overlapping fragments spanning the entire *BRCA1* coding region are amplified by PCR, transcribed into mRNA, and translated into protein (Fig. 1). Abnormally shortened peptides, indicative of the premature termination of translation, were sought among the protein fragments. For example, Patient 99 had both a truncated peptide and the expected full-size protein fragment, a finding consistent with a heterozygous mutation (Fig. 1, middle panel). In this case, nucleotide sequencing showed a deletion of two bases at codon 327 in one of the two *BRCA1* alleles. This mutation shifted the codon reading frame and accounted for premature chain termination (Fig. 1, bottom panel). In Patient 364, a truncated protein product was traced to a nonsense mutation at codon 563 (Table 2).

### Automated Sequencing of the *BRCA1* Coding Region

We sequenced the entire coding region of the *BRCA1* in the 30 women who had breast cancer before the age of 30 (Table 2). In addition to Patients 99 and 364, two unrelated patients, Patients 231 and 253, had the same mutation, a heterozygous deletion of two nucleotides at codon 23 that leads to a frame shift and premature chain termination. The mutation was not detected by the PTT assay, because it was in the first coding exon and encoded a peptide only 39 amino acids long. This mutation, called 185delAG, has been found in patients with *BRCA1*-linked familial breast cancer.<sup>7,23</sup> It was also found in 1 percent of 858 samples from people of Ashkenazi Jewish origin living in the United States and Israel — a prevalence consistent with the hypothesis that the mutation was inherited from a common ancestor in this Jewish population.<sup>19</sup>

The presence of 4 women with definite *BRCA1* mutations (i.e., mutations resulting in unambiguous inacti-

Table 2. *BRCA1* Mutations in Women with Breast Cancer before the Age of 30.\*

VARIABLE	EXON	CODON	NUCLEOTIDE CHANGE AND POSITION	EFFECT ON PROTEIN
Frame shifts				
Patient 231	2	23	2-bp deletion at 68	Truncation at codon 39
Patient 253	2	23	2-bp deletion at 68	Truncation at codon 39
Patient 99	11	327	2-bp deletion at 981	Truncation at codon 329
Nonsense mutations				
Patient 364	11	563	C→T at 1687	Truncation at codon 563
Missense mutations				
Patient 285	16	1606	A→G at 4816	Lys→Glu
Polymorphisms				
In 6 patients	11	694	C→T at 2082	None
In 11 patients	11	771	T→C at 2311	None
In 12 patients	11	871	C→T at 2612	Pro→Leu
In 10 patients	11	1038	A→G at 3113	Glu→Gly
In 8 patients	11	1183	A→G at 3548	Lys→Arg
In 5 patients	13	1436	T→C at 4308	None

\*Mutations were identified by automated sequencing of both strands from uncloned PCR products derived from the *BRCA1* coding region. Nucleotide position was defined in relation to the initiation codon (exon 2). The mutation at codon 23 (nucleotide 68) was identical to the 185delAG mutation<sup>19</sup> (position 185 refers to the position in the initially published *BRCA1* nucleotide sequence,<sup>1</sup> which included a 5' noncoding sequence of 117 nucleotides). Polymorphisms were identified from the Breast Cancer Information Core data base.

vation of the gene product) among 30 women who had breast cancer before age 30 yielded a significantly higher proportion (13 percent; 95 percent confidence interval, 4 to 31 percent) than did the 1 in 833 calculated for the general white population<sup>24</sup> ( $P < 0.001$ ). Surprisingly, none of three women with definite family histories of breast cancer and only one of three women with bilateral tumors had *BRCA1* mutations, indicating that *BRCA1* contributes to the genetic predisposition to breast cancer in some cases but that other genetic factors also play a part.

Nucleotide-sequencing analysis also identified a missense mutation in Patient 285: a substitution of guanosine for adenosine at codon 1606, which produces a substitution of glutamic acid for lysine in the predicted protein sequence. This mutation has not been observed in *BRCA1*-linked breast cancer families, nor has it been reported as a common polymorphism in the general population<sup>3-7</sup> (and the Breast Cancer Information Core electronic data base). This patient had no relatives with breast cancer who could be tested for the mutant allele. In her primary tumor, homozygosity for the mutant allele, which is characteristic of disease-associated mutations, was not found. The functional consequences of this amino acid substitution are therefore unknown. We detected six other nucleotide substitutions in *BRCA1* in several patients (Table 2). Three of these substitutions were silent, and three resulted in amino acid changes. The common occurrence of these *BRCA1* substitutions in the general population<sup>3-7</sup> (and the Breast Cancer Information Core data base) suggests that they are polymorphic variants with no clinical relevance.

### Prevalence of the 185delAG Mutation in Jewish Women with Early-Onset Breast Cancer

The 1 percent prevalence of the *BRCA1* mutation 185delAG (at codon 23) in the Jewish population of

Table 3. Incidence of the *BRCA1* Mutation 185delAG among Jewish Women with the Onset of Breast Cancer at or before the Age of 40.\*

185delAG PRESENT	NO. OF WOMEN (%)	MEAN AGE AT ONSET  yr	BILATERAL BREAST CANCER	FAMILY HISTORY OF BREAST CANCER			RELATIVES WITH BREAST CANCER†
				DEFINITE‡	1ST-DEGREE RELATIVE	1ST- OR 2ND-DEGREE RELATIVE	
Yes	8 (21)	36	2 (25)	0	4 (50)	7 (88)	10/43 (23)
No	31 (79)	36	1 (3)	3 (10)	11 (35)	13 (42)§	20/168 (12)¶
Total	39 (100)	36	3 (8)	3 (8)	15 (38)	20 (51)	30/211 (14)

\*The 185delAG mutation was identified by allele-specific PCR amplification in 39 Jewish women with breast cancer diagnosed at or before the age of 40. The number of cases of breast cancer among the first- and second-degree female relatives of known 185delAG carriers was obtained from the family history.

†Values shown are the numbers of female relatives with breast cancer as a fraction of all female first- and second-degree relatives.

‡A definite family history was defined as the presence of breast cancer in three or more paternal or maternal relatives in two or more generations.

§P=0.02 for the comparison with women with the 185delAG mutation.

¶P=0.06 for the comparison with women with the 185delAG mutation.

Eastern European ancestry<sup>19</sup> has aroused concern about its predictive value for breast cancer. It is possible that most Jewish women with the mutation do not have the same risk of breast cancer as women with a clearly defined history of familial cancer.<sup>24</sup> We determined the prevalence of the 185delAG mutation in Jewish women with early-onset breast cancer, using an allele-specific PCR assay whose results were verified by nucleotide sequencing. Among the 30 women who had breast cancer diagnosed before the age of 30, 2 of 4 Jewish women carried the 185delAG mutation. We extended our analysis to the 388 women who were given a diagnosis of breast cancer between ages of 30 and 40. We found that 6 of 35 Jewish women in this group carried the mutation.

None of the eight Jewish women with the 185delAG mutation had a definite family history of breast cancer. However, 7 of the 8 had at least one affected first- or second-degree relative, as compared with 13 of the 31 Jewish women without this mutation (P=0.02) (Table 3). Furthermore, breast cancer was diagnosed in 10 of 43 women (23 percent) who were first- or second-degree relatives of the carriers of the 185delAG mutation, as compared with 20 of 168 women (12 percent) who were related to the Jewish women without the mutation (P=0.06). Thus, the 185delAG mutation was associated with a family history of breast cancer, although large-scale population studies will be needed to determine the exact penetrance of this mutant *BRCA1* allele.

## DISCUSSION

We found an increased prevalence of germ-line mutations in the *BRCA1* gene among women with early-onset breast cancer. Among 30 women in whom breast cancer was diagnosed before the age of 30, we identified 4 who had definite germ-line *BRCA1* mutations that caused premature termination of protein translation (13 percent) and 1 who had a missense mutation. The mutations in 3 of the 26 non-Jewish women were distributed throughout the *BRCA1* coding sequence, a feature characteristic of the inactivation of tumor-susceptibility genes. The broad distribution of these muta-

tions greatly complicates their detection. In addition, caution is required in interpreting amino acid substitutions, because distinguishing novel missense mutations from clinically unimportant polymorphisms is difficult. Therefore, only *BRCA1* mutations that cause premature chain termination and missense mutations previously documented in familial breast and ovarian cancer should be considered definite mutations.

In contrast to the varied *BRCA1* mutations in the non-Jewish population, the specific *BRCA1* mutation we studied, 185delAG, was found frequently among young Jewish women with breast cancer. Given that this mutation has a prevalence of 1 percent in the Jewish population and a prevalence of 21 percent (95 percent confidence interval, 9 to 36 percent) among Jewish women with breast cancer at or before the age of 40, carriers of the 185delAG mutation appear to have a 27-fold increase in the risk of early-onset breast cancer (lower bound of the 97.5 percent confidence interval, 10). This indicates an attributable risk (the fraction of early-onset breast cancer in Jewish women that is due to this mutation) of approximately 20 percent (95 percent confidence interval, 6 to 31 percent). Caution has been urged in interpreting the risk of cancer associated with the 185delAG mutation in women who do not have a history of familial cancer.<sup>19,24</sup> The penetrance of this allele is unknown in members of the general population who have not been selected on the basis of such history. It has been thought unlikely that 1 percent of Jewish families might share the severe cancer phenotype characteristic of *BRCA1*-associated familial breast cancer. Only large, prospective population studies can define the risk of breast and ovarian cancer associated with the 185delAG mutation. Given that the incidence of breast cancer by the age of 40 in the U.S. population is 1 in 217 (0.5 percent),<sup>17</sup> we calculate that the predictive value of the 185delAG mutation for the development of breast cancer at or before the age of 40 is 9 percent. This estimate could be higher if the rate of early-onset breast cancer in the Jewish population exceeds that in the non-Jewish population. The lifetime risk of breast cancer associated with the 185delAG mutation in the general population is

uncertain. Our calculation that 20 percent (range, 6 to 31 percent) of cases of breast cancer at or before the age of 40 in Jewish women are linked to the 185delAG mutation is similar to the attributable risk of 16 percent (range, 7 to 23 percent) predicted by Struewing and co-workers,<sup>19</sup> who used age-specific penetrance rates derived from kindreds with familial breast cancer. In the general population, therefore, it is possible that the lifetime risk of cancer associated with the 185delAG mutation approaches that observed in such families.

Since the relative contribution of genetic factors to breast cancer declines markedly with age, the prevalence of the 185delAG mutation among older Jewish women with breast cancer (those over 40) is likely to be considerably lower. Genetic testing is therefore likely to be most effective in young Jewish women with breast cancer, among whom it may open the possibility of cancer prevention, early detection of second tumors, and genetic counseling for the women and their first- and second-degree relatives. The increased risk of early-onset breast cancer among carriers of the 185delAG mutation also suggests that intensified screening for breast cancer should start at an early age. However, testing for the 185delAG mutation in the general Ashkenazi Jewish population should await larger population-based studies that confirm the penetrance of 185delAG and the formulation of guidelines for the genetic counseling and clinical care of women who carry the mutation.

We are indebted to the women who participated in this study and to their referring physicians; to Mel Kronick and Morgan Conrad (of Applied Biosystems) for advice and for providing sequencing-analysis programs still in development; to Marga White and Lisa Sadzewicz (of OncorMed) for sharing reagents and information about *BRCA1* sequencing protocols; and to the members of the Breast Cancer Information Core data base, in particular Roger Wiseman and Barbara Weber for sharing sequencing information and data on *BRCA1* polymorphisms.

#### REFERENCES

- 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.
- Hall JM, Lee MK, Newman B, et al. Linkage of early-onset familial breast cancer to chromosome 17q21. *Science* 1990;250:1684-9.
- Futreal PA, Liu Q, Shattuck-Eidens D, et al. *BRCA1* mutations in primary breast and ovarian carcinomas. *Science* 1994;266:120-2.
- 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.
- 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.
- 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.
- 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.
- 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.
- Rowell S, Newman B, Boyd J, King MC. Inherited predisposition to breast and ovarian cancer. *Am J Hum Genet* 1994;55:861-5.
- Colditz GA, Willett WC, Hunter DJ, et al. Family history, age, and risk of breast cancer: prospective data from the Nurses' Health Study. *JAMA* 1993;270:338-43. [Erratum, *JAMA* 1993;270:1548.]
- Slattery ML, Kerber RA. A comprehensive evaluation of family history and breast cancer risk: the Utah Population Database. *JAMA* 1993;270:1563-8.
- Harris JR, Lippman ME, Veronesi U, Willett W. Breast cancer. *N Engl J Med* 1992;327:319-28, 390-8, 473-80.
- Knudson AG Jr. Mutation and cancer: a statistical study of retinoblastoma. *Proc Natl Acad Sci U S A* 1971;68:820-3.
- Haber DA, Housman DE. Rate-limiting steps: the genetics of pediatric cancers. *Cell* 1991;64:5-8.
- Cannon-Albright LA, Skolnick MH, Bishop DT, Lee RG, Burt RW. Common inheritance of susceptibility to colonic adenomatous polyps and associated colorectal cancers. *N Engl J Med* 1988;319:533-7.
- Powell SM, Petersen GM, Krush AJ, et al. Molecular diagnosis of familial adenomatous polyposis. *N Engl J Med* 1993;329:1982-7.
- Ries LAG, Miller BA, Hankey BR, Kosary CL, Hurray A, Edwards BK, eds. SEER cancer statistics review, 1973-1991: tables and graphs. Bethesda, Md.: National Cancer Institute, 1994. (NIH publication no. 94-2789.)
- 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.
- 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.
- van der Luijt R, Khan PM, Vasen H, et al. Rapid detection of translation-terminating mutations at the adenomatous polyposis coli (*APC*) gene by direct protein truncation test. *Genomics* 1994;20:1-4.
- Hogervorst FBL, Cornelis RS, Bout M, et al. Rapid detection of *BRCA1* mutations by the protein truncation test. *Nat Genet* 1995;10:208-12.
- Fleiss JL. *Statistical methods for rates and proportions*. 2nd ed. New York: John Wiley, 1981.
- Struewing JP, Brody LC, Erdos MR, et al. Detection of eight *BRCA1* mutations in 10 breast/ovarian cancer families, including 1 family with male breast cancer. *Am J Hum Genet* 1995;57:1-7.
- Goldgar DE, Reilly PR. A common *BRCA1* mutation in the Ashkenazim. *Nat Genet* 1995;11:113-4.

#### IMAGES IN CLINICAL MEDICINE

Images in Clinical Medicine, a weekly *Journal* feature, presents clinically important visual images, emphasizing those a doctor might encounter in an average day at the office, the emergency department, or the hospital. If you have an original unpublished, high-quality color or black-and-white photograph representing such a typical image that you would like considered for publication, send it with a descriptive legend to Kim Eagle, M.D., University of Michigan Medical Center, Division of Cardiology, 3910 Taubman Center, Box 0366, 1500 East Medical Center Drive, Ann Arbor, MI 48109. For details about the size and labeling of the photographs, the requirements for the legend, and authorship, please contact Dr. Eagle at 313-936-5275 (phone) or 313-936-5256 (fax), or the *New England Journal of Medicine* at [images@edit.nejm.org](mailto:images@edit.nejm.org) (e-mail).