

DIFFERENTIAL CONTRIBUTIONS OF *BRCA1* AND *BRCA2* TO EARLY-ONSET BREAST CANCER

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ABSTRACT

Background Germ-line mutations in the *BRCA1* and *BRCA2* genes predispose women to breast cancer. *BRCA1* mutations are found in approximately 12 percent of women with breast cancer of early onset, and the specific mutation causing a deletion of adenine and guanine (185delAG), which is present in 1 percent of the Ashkenazi Jewish population, contributes to 21 percent of breast cancers among young Jewish women. The contribution of *BRCA2* mutations to breast cancer of early onset is unknown.

Methods Lymphocyte specimens from 73 women with breast cancer diagnosed by the age of 32 were studied for heterozygous mutations of *BRCA2* by a complementary-DNA-based protein-truncation assay, followed by automated nucleotide sequencing. In addition, specimens from 39 Jewish women with breast cancer diagnosed by the age of 40 were tested for specific mutations by an allele-specific polymerase chain reaction.

Results Definite *BRCA2* mutations were found in 2 of the 73 women with early-onset breast cancer (2.7 percent; 95 percent confidence interval, 0.4 to 9.6 percent), suggesting that *BRCA2* is associated with fewer cases than *BRCA1* ($P=0.03$). The specific *BRCA2* mutation causing a deletion of thymine (6174delT), which is found in 1.3 percent of the Ashkenazi Jewish population, was observed in 1 of the 39 young Jewish women with breast cancer (2.6 percent; 95 percent confidence interval, 0.09 to 13.5 percent), indicating that it has a small role as a risk factor for early-onset breast cancer. Among young women with breast cancer, there are *BRCA2* mutations that cause truncation of the extreme C terminus of the protein and that may be functionally silent, along with definite truncating mutations.

Conclusions Germ-line mutations in *BRCA2* contribute to fewer cases of breast cancer among young women than do mutations in *BRCA1*. Carriers of *BRCA2* mutations may have a smaller increase in the risk of early-onset breast cancer. (N Engl J Med 1997; 336:1416-21.)

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ilies in which breast cancer is linked to a *BRCA1* mutation also have increased susceptibility to ovarian cancer,¹ and families in which the link is to a *BRCA2* mutation include cases of male breast cancer.⁷ As in other syndromes involving a predisposition to cancer, familial cases of breast cancer occur at a considerably younger age than in the general population.^{8,9} In families with breast cancer, age-specific penetrance refers to the likelihood that breast cancer will develop in a carrier of a predisposing mutation by a certain age. For example, in these families this likelihood is approximately 20 percent by the age of 40 and 80 percent by the age of 70.^{5,6} However, it is difficult to infer the age-specific penetrance of *BRCA1* and *BRCA2* mutations in the general population from an analysis of kindreds with breast cancer, since these families were selected specifically because they have multiple affected members. In the general population, however, women with breast cancer at an early age are more likely than others to have a genetic predisposition, and we can define the relative contributions of mutant *BRCA1* and *BRCA2* genes in these women.

Mutations in the *BRCA1* gene have been linked to half of all cases of familial breast cancer.^{2,10-14} Among women with cancer of early onset, who were not selected because of a family history, the frequency of germ-line *BRCA1* mutations is approximately 12 percent.^{15,16} The frequency of such mutations in the general population is unknown, making it difficult to calculate the increase in the risk of breast cancer in a carrier who does not have a strong family history of breast cancer. However, the frequency of a specific *BRCA1* mutation has been measured in the Ashkenazi Jewish population, both in otherwise unselected subjects and in a cohort of Jewish women with early-onset breast cancer. This mutation, which causes a deletion of adenine and guanine (185delAG), has been found in 1 percent of the Ashkenazi Jewish population¹⁷ and 21 percent of young Jewish women

STUDIES of large families many of whose members have cancer have led to the identification of two genes, *BRCA1* and *BRCA2*, that, when mutated, predispose family members to breast cancer.¹⁻⁴ Members of such kindreds who are heterozygous carriers of a germ-line mutation have a high probability of breast cancer.^{5,6} Fam-

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with breast cancer.^{16,18-20} It is calculated that the risk of early-onset breast cancer among carriers of the 185delAG mutation is 27 times higher than among noncarriers, suggesting that the age-specific penetrance of this *BRCA1* mutation in the Jewish population generally is similar to that predicted from analyses of kindreds with breast cancer.^{16,17}

Genetic-linkage analysis initially suggested that mutations in *BRCA2* account for most cases of familial breast cancer in which there is no mutation of *BRCA1*.^{3,4,7} However, *BRCA2* mutations have been found in only a fraction of kindreds in which *BRCA1* mutations are absent,^{21,22} suggesting that the number of familial cases attributed to *BRCA2* mutations has been overestimated. In the kindreds studied to date, *BRCA2* mutations appear to confer a high probability of early-onset breast cancer.^{3,4,21,22} However, a specific *BRCA2* mutation that causes a deletion of thymine (6174delT) has been reported in 1.3 percent of the Ashkenazi population^{23,24} but only 8 percent of Ashkenazi Jewish women with early-onset breast cancer, suggesting that its age-specific penetrance is lower than predicted.²⁵ The relative contributions of *BRCA1* and *BRCA2* mutations to breast cancer in the general population are thus uncertain. To address this question, we screened the entire coding region of *BRCA2* for truncating mutations in a cohort of women with breast cancer diagnosed at or before the age of 32; these women are presumed to be highly likely to have a genetic predisposition.

METHODS

Study Patients

A cohort of 418 women with early-onset breast cancer (age at diagnosis, ≤ 40 years) has been described.¹⁶ This cohort was drawn from women with breast cancer diagnosed between 1981 and 1992 at Boston-area hospitals who provided blood samples for genetic analysis and to whom the results of the analyses were not disclosed. Pathological confirmation of the diagnosis and a clinical and family history were available in every case, along with lymphoblasts immortalized by infection with the Epstein-Barr virus (EBV). The entire *BRCA2* coding region was screened for truncating mutations in 73 women with breast cancer diagnosed at or before the age of 32, who represented the subgroup at highest risk for a genetic predisposition. The specific *BRCA1* and *BRCA2* mutations that are prevalent in the Ashkenazi population were sought in 39 Jewish women with breast cancer diagnosed at or before the age of 40. To determine the population prevalence of the C-terminus-truncating mutations in *BRCA2* that appear to be silent polymorphisms, we screened specimens from 130 anonymous blood donors. All the specimens used in the study were coded, and the patients' confidentiality was preserved, in accordance with the guidelines for studies of human subjects.

Analysis of Mutations

Since virtually all the *BRCA2* mutations reported to date in kindreds with breast cancer result in premature termination of the polypeptide chain, we developed a complementary DNA (cDNA)-based protein-truncation assay to screen EBV-immortalized lymphoblasts from women with breast cancer. Total cellular messenger RNA (mRNA) was isolated by standard procedures and reverse-transcribed into cDNA with random hexanucleotide primers.

The entire coding region of *BRCA2* spans 10 kilobases (kb);

the region was divided into three overlapping fragments (Fig. 1) and amplified by the polymerase chain reaction (PCR) in a single multiplex reaction. The primers used were as follows: for fragment I, TGACCGGCGCGGTTTTTGTGTCAGC (sense) and TGATTATCTAATGCCAAGGTATT (antisense); for fragment II, CTGTCAATCCAGACTCTGAAG (sense) and CAGAAATTC-TTGACCAGGTGCGG (antisense); and for fragment III, AACTGACAGATTCTAAACTGC (sense) and AACTGGAAAGGTTA-AGCGTCA (antisense). The PCR conditions consisted of 5 cycles (94°C for 20 seconds, 64°C for 1 minute, and 68°C for 8 minutes) followed by 30 cycles (94°C for 20 seconds, 60°C for 1 minute, and 68°C for 7.5 minutes).

The three products of the multiplex PCR were then used in a nested, internal PCR to generate eight overlapping fragments of approximately 1.5 kb each. Each sense primer used in this reaction contained the 5' extension GGATCCTAATACGACTCACTATAG-GGAGACCACCATG, which encoded a T7 RNA polymerase recognition site, a start codon, and a Kozak translation-initiation consensus sequence, to allow efficient in vitro transcription and translation. The primers used were as follows: fragment 1, ATGCCTATTG-GATCCAAAGAGAG (sense) and GATACCCTGAAATGAAGAA-GCCAC (antisense); fragment 2, TCTATATTCAGATAAAGAGA-ATC (sense) and TGATTATCTAATGCCAAGGTATT (antisense); fragment 3, TTAGGAAATACTAAGGAACCTCA (sense) and AT-TACCATGCATGCTTCTTGA (antisense); fragment 4, ACTGA-TCAGCACAACATATGTC (sense) and GGATTTTACCACCTGG-CTATCCCTA (antisense); fragment 5, CCACAAACTGIAAATG-AAGATAT (sense) and ATGATGCATAAACAATCTTCGA (anti-sense); fragment 6, AAGGCTTCAAAAAGCACTCCA (sense) and AACTGGGCCTTAACAGCATAACC (antisense); fragment 7, ACA-CTGTCTCTGTGTTTCT (sense) and TCTGCTTCATTGCA-AAGTATGT (antisense); and fragment 8, GAAAGAGCTAACAT-ACAGTTAGCA (sense) and AACTGGAAAGGTTAAGCGTCA (antisense). The PCR conditions consisted of 45 cycles (94°C for 30 seconds, 55 to 61°C for 30 seconds, and 72°C for 3 minutes).

One microgram of the PCR product was incubated with T7 polymerase, rabbit reticulocyte lysate, and [³⁵S]methionine (Amersham) in a coupled transcription-translation reaction (Promega), followed by electrophoresis with 12 percent polyacrylamide-sodium dodecyl sulfate gel. The PCR products that gave rise to truncated peptides were analyzed by automated nucleotide sequencing of both strands, with dye-labeled dideoxy terminators (Applied Biosystems). Uncolored PCR products were analyzed to ensure that both alleles were represented and to avoid *Taq* polymerase-induced errors that might be present in individual PCR products. When necessary, potential mutations were confirmed by sequencing multiple cloned PCR products.

To detect the 6174delT mutation, an allele-specific PCR reaction was designed that used four primers in a single reaction. The primers were as follows: primer 1, TGGCAGGTTGTACGAG-GCATTGG (sense); primer 2, TAAATGTTCTGGAGTACGTAT-AGC (antisense); primer 3, GTGGGATTTTTAGCACAGCAA-GTG (sense); and primer 4, CTGATACCTGGACAGATTTCCCT (antisense). The PCR conditions consisted of 40 cycles (94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds). This reaction produces a PCR fragment containing 194 base pairs (bp) that is specific for the wild-type allele, a fragment of 299 bp that is specific for the mutant allele, and a control fragment of 448 or 449 bp that is common to both alleles.

To detect the mutation that causes a substitution of guanine for cytosine (C10152G), an allele-specific reverse-transcription PCR was designed that used the following primers: primer 1, TCTGTTTCCACACCTGTCTCAGC (sense); primer 2, TTTAT-TGTCGCCCTTTGCAAATGC (antisense); primer 3, ACCAAGG-AGTTGTGGCACCACAAATAC (sense); and primer 4, CAGTTC-TTTTTCTTTATGGGTGTTTCC (antisense). The conditions were identical to those of the 6174delT PCR, except that the annealing temperature was 61°C. This reaction produces a 381-bp fragment specific for the wild-type allele, a 235-bp fragment specific for the mutant allele, and a control fragment of 566 bp common to both alleles.

The mutation that causes a substitution of thymine for adenine at position 10,204 (A10204T) was also detected by an allele-specific reverse-transcription PCR that used primers 1 and 2 as described in the preceding paragraph along with TTCTCCTCAGATGACTCCATTTA (sense) and TTCCAAAAGAGAAATTCATTGAATTTTTTA (antisense). The PCR conditions were identical to those of the 6174delT reaction. This reaction produces a 321-bp fragment specific for the wild-type allele, a 290-bp fragment specific for the mutant allele, and a control fragment of 566 bp common to both alleles.

The *BRCA1* mutation at position 5382 that causes an insertion of cytosine (5382insC) was detected by an allele-specific genomic PCR that used the following primers: primer 1, TAAAATG-GACGTGTCTGCT (sense); primer 2, CTGCAAAGGGGAGTGGAAATACAG (antisense); primer 3, CAAAGCGAGCAAGAAATCCCA (sense); and primer 4, GAGCTTTACCTTTCTGTCC-TGGGGA (antisense). The PCR conditions were identical to those of the 6174delT reaction. This reaction produces a 100-bp fragment specific for the wild-type allele, a 175-bp fragment specific for the mutant allele, and a control fragment of 233 or 234 bp common to both alleles.

To identify definite mutations in the *BRCA1* gene in 43 women who had breast cancer diagnosed between the ages of 30 and 32, we used a novel yeast-based truncation assay.²⁶ In brief, the *BRCA1* transcript was divided into three overlapping fragments (comprising codons 1 to 263, 224 to 1365, and 1324 to 1863) that were amplified by reverse-transcription PCR and inserted in the correct reading frame of the yeast gene *URA3* by homologous recombination. The *URA3* gene confers the ability to grow in the absence of uracil; yeast cells that contain a *BRCA1-URA3* fusion protein derived from wild-type *BRCA1* grow in the absence of uracil, whereas those containing a fusion protein derived from a *BRCA1* fragment with a truncating mutation depend for their growth on the presence of uracil. This assay identifies carriers of heterozygous *BRCA1* mutations, since in such carriers an

average (\pm SD) of 49 ± 6 percent of the yeast cells containing the *BRCA1-URA3* fusion protein require uracil for growth.

Statistical Analysis

The prevalence of *BRCA1* mutations in the study patients was compared with that of *BRCA2* mutations by Fisher's exact test. Exact 95 percent confidence intervals for these prevalence rates were calculated with StatXact 3 (Cytel Software). The risks associated with the two mutations were compared by testing the homogeneity of the odds ratios from tables comparing the rates of each mutation among cases and controls (cohorts of Ashkenazi Jewish women). This test assumes that the mutations are independent (an assumption that is believed to be biologically sound). A test that did not make this assumption but instead assumed that the cohorts are large enough to provide population rates gave similar results.

RESULTS

We have previously described the clinical characteristics of a cohort of 418 women with breast cancer diagnosed at or before the age of 40.¹⁶ Among these women, bilateral breast cancer (in 5 percent) and a strong family history of breast cancer (in 7 percent) were clinical features indicating an increased risk for a genetic predisposition. Since virtually all the known *BRCA2* mutations (32 of 33) in kindreds with breast cancer result in premature chain terminations,^{3,4,21,22} we used a protein-truncation assay that detects premature stop codons in the coding sequence of the gene, as described in the Methods section. Mutations identified by this approach were confirmed by nucleotide sequencing.

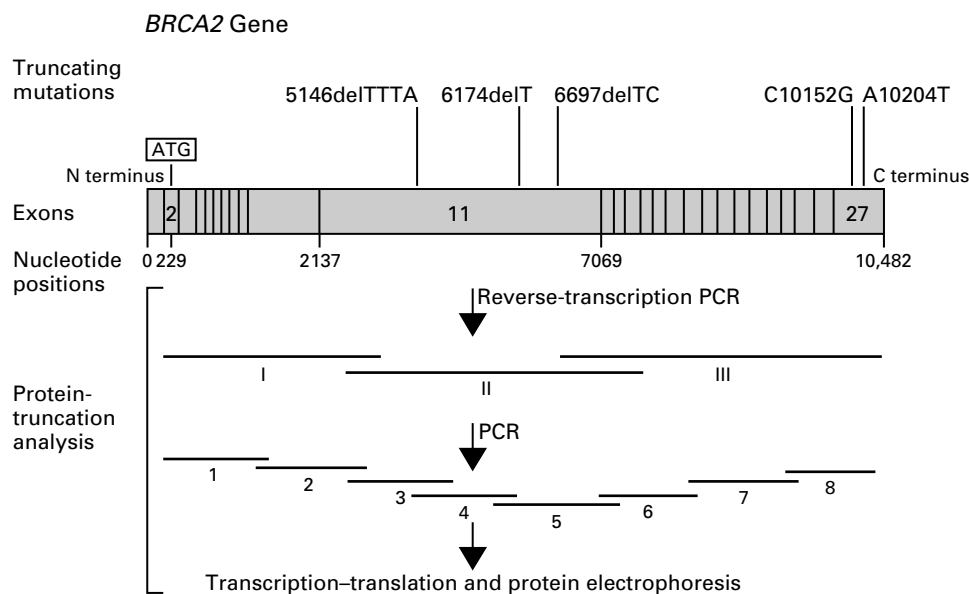


Figure 1. Identification and Location of *BRCA2* Mutations That Truncate the Polypeptide Chain.

The *BRCA2* gene is encoded by 27 exons, the second of which contains the initiator codon (ATG) at nucleotide position 229. The coding sequence spans 10,254 nucleotides, encoding 3418 amino acids. Each truncating mutation results in the insertion of a premature stop codon in the sequence. Detecting these mutations by a protein-truncation assay involves making a complementary DNA copy of the *BRCA2* transcript, performing two rounds of nested PCR amplification, and synthesizing messenger RNA and protein *in vitro* from the PCR-generated templates. Abnormally shortened protein products are created when there is a truncating mutation in one of the two *BRCA2* alleles; these products can be detected by electrophoresis.

We initially studied the *BRCA2* gene in the youngest subgroup of our cohort, 30 women who had breast cancer diagnosed before the age of 30 and had previously been screened for *BRCA1* mutations.¹⁶ No *BRCA2* mutations were detected. We therefore studied an additional 43 women whose breast cancer was diagnosed between the ages of 30 and 32. We detected two definite *BRCA2* mutations in this group. Patient 110 had a deletion of four nucleotides, and Patient 420 had a deletion of two nucleotides. Both deletions were in exon 11 (Table 1), and both caused shifts in the codon reading frame that resulted in premature stop codons. Both women had a history of bilateral breast cancer, but neither had a family history of breast cancer.

In addition to these two definite *BRCA2* mutations, we detected two mutations in the last exon of the gene that resulted in truncation of the extreme C terminus of the protein (Table 1 and Fig. 1). One of these two nonsense mutations in exon 27, A10204T, causes a deletion of 93 amino acids from the BRCA2 protein, which contains 3418 amino acids. The second nonsense mutation, C10152G, causes a deletion of 111 amino acids. A10204T (also called 3326ter, since it causes a deletion from amino acid 3326 to the C terminus) has recently been described as a silent polymorphism because a European study has found it to have a prevalence of 2.2 percent in both the general population and the population with breast cancer.²⁷ This mutation was also reported in 1 of 115 controls from the United States,²⁷ but we did not detect it in 130 healthy women in the Boston area. C10152G has not been reported previously, nor did we detect it in the 130 controls we studied. However, since this mutation causes truncation of the extreme C terminus of the BRCA2 protein, as does A10204T, C10152G is also likely to be functionally silent.

We compared the frequency of *BRCA1* mutations with that of *BRCA2* mutations in the cohort of 73 women with breast cancer diagnosed at or before the age of 32. We have previously reported¹⁶ four definite *BRCA1* mutations in 30 women with breast cancer diagnosed before the age of 30. Using a yeast-based assay for truncating mutations,²⁶ we identified *BRCA1* mutations in 5 of 43 women with breast cancer diagnosed between the ages of 30 and 32 (Table 2). Unlike mutations in the extreme C terminus of *BRCA2*, those in the extreme C terminus of *BRCA1* are associated with a high likelihood of breast cancer (Breast Cancer Information Core electronic data base; Internet address, <http://www.nchgr.nih.gov/dir/lab_transfer/bic/>), indicating that all *BRCA1* mutations that cause protein truncation can be considered definite mutations.

The presence of common *BRCA1* and *BRCA2* mutations in the Ashkenazi Jewish population allows the relative risk of breast cancer associated with specific mutations to be calculated. We used an allele-specific PCR technique to test for specific mutations in 39 Jewish women who had breast cancer diagnosed at or before the age of 40. The *BRCA2* mutation 6174delT, found in 1.3 percent of the Ashkenazi population overall,^{23,24} was detected in 1 of the 39 Jewish women with early-onset breast cancer (2.6 percent; 95 percent confidence interval, 0.09 to 13.5 percent). Two recurrent *BRCA1* mutations in the Ashkenazi population are more strongly associated with breast cancer. The 5382insC mutation, which is present in 0.1 percent of the Ashkenazi population,²³ was found in 1 of the 39 Jewish women with early-onset breast cancer (2.6 percent), and we have previously reported finding the 185delAG mutation, present in 1 percent of the Ashkenazi Jewish population,¹⁷ in 8 of these 39 women (21 percent)¹⁶ (Table 3).

TABLE 1. *BRCA2* MUTATIONS IN 73 WOMEN WITH EARLY-ONSET BREAST CANCER.

VARIABLE	MUTATION*	EXON	CODON	NUCLEOTIDE CHANGE †	AMINO ACID CHANGE	EFFECT ON PROTEIN
Definite mutations						
Patient 110	5146delTTTA	11	1639	4-base deletion of nucleotides 4917–4920	Frame shift	Truncation at codon 1639
Patient 420	6697delTC	11	2156	2-base deletion of nucleotides 6468 and 6469	Frame shift	Truncation at codon 2174
Probable polymorphisms						
Patient 236	C10152G	27	3308	C→G at nucleotide 9923	Tyr→stop	Truncation at codon 3308
Patient 333	A10204T (also known as 3326ter)	27	3326	A→T at nucleotide 9975	Lys→stop	Truncation at codon 3326

*The names of the mutations include the nucleotide number, starting with the first position in the sequence, as described by Wooster et al.³ and Tavtigian et al.⁴

†The numbering of the nucleotides shown is based on the location of the initiator codon, which is at position 229 in the published sequences.

TABLE 2. DEFINITE *BRCA1* MUTATIONS IN 73 WOMEN WITH EARLY-ONSET BREAST CANCER.

VARIABLE	TRUNCATING MUTATION*
Breast cancer before age 30 (n=30)†	
Patient 99	2-Base deletion of nucleotides 981 and 982
Patient 231	2-Base deletion of nucleotides 68 and 69
Patient 253	2-Base deletion of nucleotides 68 and 69
Patient 364	C→T at nucleotide 1687
Breast cancer at age 30–32 (n=43)‡	
Patient 60	Deletion of exon 22 (nucleotides 5333 to 5406)
Patient 268	2-Base deletion of nucleotides 2515 and 2516
Patient 316	2-Base deletion of nucleotides 68 and 69
Patient 342	22-Base deletion of nucleotides 192 to 213
Patient 376	2-Base deletion of nucleotides 68 and 69

*The numbering of the nucleotides shown is based on the location of the initiator codon (exon 2). The mutation that causes a two-base deletion of nucleotides 68 and 69 is commonly known as 185delAG.

†The mutations in these patients have been previously reported¹⁶ and were detected by the protein-truncation assay and by nucleotide sequencing of the entire *BRCA1* coding region.

‡The mutations in these patients were detected by a yeast-based assay²⁶ that identifies truncating mutations in fragments of the *BRCA1* transcript, followed by nucleotide sequencing.

TABLE 3. *BRCA1* AND *BRCA2* MUTATIONS IN WOMEN WITH EARLY-ONSET BREAST CANCER.*

POPULATION AND VARIABLE	<i>BRCA1</i> MUTATIONS		<i>BRCA2</i> MUTATIONS	
	ALL	SPECIFIC TO ASHKENAZI POPULATION	ALL	SPECIFIC TO ASHKENAZI POPULATION
		185delAG 5382insC		6174delT
Early-onset breast cancer				
No. affected	9	8	1	2
No. studied	73	39	39	73
Percent	12	21	2.6	2.7
General population (% affected)	—	1	0.1	—

*The frequency of all *BRCA1* and *BRCA2* mutations in the general population is unknown. The prevalence rates of specific mutations in the Ashkenazi Jewish population were derived from Struewing et al.,¹⁷ Roa et al.,²³ and Oddoux et al.²⁴ The frequency of *BRCA2* mutations was compared with that of *BRCA1* mutations in 73 women with breast cancer diagnosed at or before the age of 32. The analysis of *BRCA1* mutations included 30 women with breast cancer diagnosed at or below the age of 30¹⁶ and 43 women with breast cancer diagnosed between the ages of 30 and 32 who were screened with a yeast-based truncation assay.²⁶ The frequency of specific *BRCA1* and *BRCA2* mutations was compared in a cohort of 39 Jewish women with breast cancer diagnosed at or before the age of 40. The clinical characteristics of the entire cohort of 418 women with breast cancer diagnosed at or before the age of 40 have been described previously.¹⁶

DISCUSSION

We detected definite germ-line mutations in *BRCA2* in 2 of 73 women with breast cancer diagnosed at or before the age of 32 (2.7 percent; 95 percent confidence interval, 0.4 to 9.6 percent). By contrast, in our analysis of the same cohort, we identified definite *BRCA1* mutations in 9 of 73 women (12 percent; 95 percent confidence interval, 5.8 to 22 percent), including 4 of 30 women with breast cancer diagnosed before the age of 30¹⁶ and 5 of 43 women with breast cancer diagnosed between the ages of 30 and 32. Germ-line mutations in *BRCA2* appear to contribute to a smaller fraction of cases of early-onset breast cancer in the general population than do germ-line mutations in *BRCA1* (P=0.03). Although this observation is unexpected, it is consistent with recent studies of kindreds with breast cancer. *BRCA1* mutations are present in approximately half of such kindreds,^{2,10-14} but two recent studies found *BRCA2* mutations in only 10 of 75 kindreds in which *BRCA1* mutations were absent.^{21,22} Among patients with early-onset breast cancer in the general population, as well as in selected kindreds with breast cancer, *BRCA2* mutations therefore occur approximately one fifth as often as *BRCA1* mutations.

The infrequency of *BRCA2* mutations among women with early-onset breast cancer may result simply from a low prevalence of these mutations in the population. From epidemiologic models, a frequency ranging from 1 in 400 to 1 in 800 has been calculated for all mutations predisposing women to breast cancer,⁵ but this estimate has yet to be confirmed by molecular studies, and the relative distribution of *BRCA1* and *BRCA2* mutations is unknown. However, studies of recurrent mutations in the Ashkenazi Jewish population suggest that mutations in these two genes differ not in their prevalence in the population, but rather in their associated risk for the development of breast cancer. Our analysis of three different mutations in 39 Jewish women with breast cancer diagnosed at or before the age of 40 showed considerable differences in age-specific penetrance. The common *BRCA1* mutation 185delAG (which appears in 1 percent of the population¹⁷) is associated with an increase in the risk of early-onset breast cancer by a factor of 27 (95 percent confidence interval, 8 to 89). With the less common *BRCA1* mutation 5382insC (which appears in 0.1 percent of the population²³), the risk may increase by a factor of 20 (95 percent confidence interval, 0.4 to 212). In contrast, the risk of early-onset breast cancer associated with the common *BRCA2* mutation 6174delT (which appears in 1.3 percent of the population^{23,24}) is probably only doubled (95 percent confidence interval, 0.05 to 12) (P=0.0166 for the comparison between the relative risks associated with the two common mutations, 185delAG in *BRCA1* and 6174delT in *BRCA2*). The proportion of *BRCA2* mutations as compared with

BRCA1 mutations in our cohort of Jewish women with early-onset breast cancer is consistent with that in a large study of familial cases²⁸ and somewhat lower than that in another study of cases with early onset.²⁵ Taken together, these observations suggest that the risk of early-onset breast cancer associated with the *BRCA2* mutation 6174delT is 1/10 to 1/5 of the risk associated with recurrent *BRCA1* mutations in the Ashkenazi population.

The fact that virtually all *BRCA2* mutations cause premature chain termination suggests that their effect on protein function may depend on where they truncate the protein. Like the *BRCA2* mutations in Patients 110 and 420 (Table 1), the 6174delT mutation occurs within the large exon 11 and results in the loss of half the encoded protein (Fig. 1). For this reason, it and many other *BRCA2* mutations that truncate protein segments of comparable size probably have the same age-specific penetrance. In contrast, mutations that truncate the protein at the extreme C terminus, such as C10152G and A10204T in the terminal exon 27, may affect protein function minimally and thus have no effect on the development of breast cancer. These two adjacent mutations were each present in 1 of the 73 women with early-onset breast cancer whom we studied (1.4 percent). Although we did not detect either mutation in 130 healthy persons from the Boston area, A10204T has recently been reported in 2.2 percent of both patients with breast cancer and controls in a study in the United Kingdom, indicating that it is not a risk factor for breast cancer, but rather a silent polymorphism.²⁶

In the absence of information on the functional domains of the protein encoded by *BRCA2*, further studies will be needed to determine whether silent mutations are restricted to exon 27 or whether other mutations in distal *BRCA2* exons are associated with intermediate increases in the risk of breast cancer. The possibilities that the increase in the risk of early-onset breast cancer is smaller with *BRCA2* mutations than with *BRCA1* mutations and that a number of C terminus-truncating mutations in *BRCA2* may be functionally silent call for caution in formulating clinical recommendations for carriers of *BRCA2* mutations.

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