Prevalence of BRCA1 and BRCA2 Gene Mutations in Patients With Early-Onset Breast Cancer

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Background: **Mutations in the BRCA1 and BRCA2 genes are found in most families with cases of both breast and ovarian cancer or with many cases of early-onset breast cancer. However, in an outbred population, the prevalence of BRCA1 and BRCA2 mutations in patients with breast cancer who were unselected for a family history of this disease has not been determined.** ***Methods:*** **Mutations in the BRCA1 and BRCA2 genes were detected in blood samples from two population-based series of young patients with breast cancer from Britain.** ***Results:*** **Mutations were detected in 15 (5.9%) of 254 women diagnosed with breast cancer before age 36 years (nine [3.5%] in BRCA1 and six [2.4%] in BRCA2) and in 15 (4.1%) of 363 women diagnosed from ages 36 through 45 years (seven [1.9%] in BRCA1 and eight [2.2%] in BRCA2). Eleven percent (six of 55) of patients with a first-degree relative who developed ovarian cancer or breast cancer by age 60 years were mutation carriers, compared with 45% (five of 11) of patients with two or more affected first- or second-degree relatives. The standardized incidence ratio for breast cancer in mothers and sisters was 365 (five observed and 1.37 expected) for 30 mutation carriers and 199 (64 observed and 32.13 expected) for 587 noncarriers. If we assume recent penetrance estimates, the respective proportions of BRCA1 and BRCA2 mutation carriers are 3.1% and 3.0%, respectively, of patients with breast cancer who are younger than age 50 years, 0.49% and 0.84% of patients with breast cancer who are age 50 years or older, and 0.11% and 0.12% of women in the general population.** ***Conclusions:*** **Mutations in the BRCA1 and BRCA2 genes make approximately equal contributions to early-onset breast cancer in Britain and account for a small proportion of the familial risk of breast cancer. [J Natl Cancer Inst 1999;91:943–9]**

After the localization *(1,2)* and isolation *(3,4)* of the breast cancer susceptibility genes BRCA1 and BRCA2, the spectrum of disease-associated mutations in various populations has been investigated in detail (Breast Information Core at http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/). The risks of breast and ovarian cancers associated with mutations in the genes have been estimated *(5)*, and other cancer types to which mutation carriers are predisposed have been identified *(6)*. Variations in cancer risks between different mutations in the genes have been described *(7,8)*, and the pathologic features of breast and ovarian cancers arising in mutation carriers have been characterized *(9)*.

The contribution of mutations in BRCA1 and BRCA2 genes to the population incidence of breast cancer has not been accurately estimated. This has been predominantly attributable to the complexity of analysis (due to the large sizes of the two genes and diversity of mutations) and the substantial numbers of samples required to obtain reliable estimates. The information is important, however, because it determines the cost–benefit implications of genetic testing on a population scale for individuals, health services, and insurance companies.

In certain populations or ethnic groups who have a restricted number of BRCA1 and BRCA2 founding mutations, estimates of the prevalence of mutations in breast cancer patients have been obtained. For example, in Ashkenazi Jews, most mutations in these genes are BRCA1 185delAG, BRCA1 5382insC, or BRCA2 6174delT. BRCA1 185delAG is found in 20% of Ashkenazi Jewish women with breast cancer diagnosed before age 42 years and BRCA2 6174delT accounts for 8% of the cases *(10,11)*. Therefore, among Ashkenazi Jews, BRCA1 mutations appear to make a greater contribution to early-onset breast cancer than do BRCA2 mutations. Conversely, in Iceland, a single BRCA2 mutation, 999del5, is found in 24% of the women with breast cancer diagnosed before the age of 40 years, whereas BRCA1 makes a very small contribution *(12,13)*. The discrepancy between these observations is a consequence of the histories of the Ashkenazi and Icelandic populations, which have resulted in founder effects, possibly coupled to mutation-specific variations in the penetrance of the genes. These population distortions mean that the results of these studies are not directly applicable to large outbred populations.

To clarify the contribution of mutations in BRCA1 and BRCA2 to breast cancer incidence in outbred populations, we have examined a large population-based series of patients with early-onset breast cancer from the U.K. for mutations in both BRCA1 and BRCA2 genes.

Patients and Methods

Patients With Breast Cancer

The main study protocol of the U.K. National Case Control Study Group has been detailed elsewhere *(14,15)*. Briefly, two population-based, case–control studies of Caucasian women were performed sequentially from 1982 through 1989. The first study included 755 women diagnosed with breast cancer before the age of years and registered from 1982 through 1985. The second study included 644 women diagnosed from 36 years through 45 years of age and registered from 1988 through 1989. Patients were ascertained through regional

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See "Notes" following "References."

cancer registries throughout Britain in the first study and in South Thames, Oxford, and Yorkshire in the second. All patients were interviewed in their homes by trained interviewers. The interview included questions on basic demographic details, reproductive and contraceptive histories, medical history (including history of benign breast disease), and details of personal habits, such as alcohol and tobacco consumption. A family history of breast and ovarian cancers was also obtained. Information on all mothers and sisters, including full name, date of birth, date and cause of death, and details of any cancers, was obtained from the patient up to the date of interview or to the date when the patient lost contact with her relative. Details of all cancers in second-degree female relatives were obtained only in the second study. Death certificates were sought for all reported deaths, and all mothers and sisters who could be traced in the National Health Service Central Register were flagged for notification of emigration, cancer diagnosis, and death.

Women who had taken part in these studies were contacted again in 1992 to obtain a blood sample and again in 1993 to obtain specific permission to use the sample for genetic studies. All patients included in the study gave informed consent for these analyses, and the protocol was approved by the Ethical Committee of the Royal Marsden Hospital National Health Service Trust.

Mutation Detection

DNA was extracted from blood by isolation of leukocyte nuclei through sucrose lysis of cell membranes, nuclear lysis by detergent, removal of proteins by sodium chloride, and ethanol precipitation. The full coding sequences and splice junctions of BRCA1 and BRCA2 genes were amplified by polymerase chain reaction (PCR). Both PCR primers were end labeled with adenosine 5′-[γ-^{32}P]triphosphate by T4 polynucleotide kinase, and the amplified fragments were analyzed by conformation-sensitive gel electrophoresis *(16).* PCR products from samples that showed migration shifts were directly sequenced in forward and reverse directions by use of a model 377 DNA sequencer (Applied Biosystems, Foster City, CA). Mutations were numbered according to accepted conventions.

Statistical Analysis

All significance levels were two-sided. Fisher's exact test (doubled to give a two-sided test) was used to compare proportions, and survival curves were compared with the use of the logrank test. Expected numbers of cancers and deaths that occurred before age 80 years among mothers and sisters were calculated from incidence and death rates for the general population of England and Wales by using the person-years program *(17).* Cancer incidence data were obtained from the Office for National Statistics (London, U.K.), because the published rates are substantially too low, particularly from 1980 through 1987 *(18).* Mothers were followed from the date of birth of the index patient, and sisters were followed from age 20 years. Flagged patients were censored at the end of 1996 for mortality, at the end of 1991 for cancer incidence, or at emigration. The remaining patients were censored at the date of interview or last contact with the patient. Observed numbers were compared with these expected values assuming a Poisson distribution.

The Breast Cancer Linkage Consortium (BCLC) has published penetrance estimates for BRCA1 and BRCA2 carriers at ages 30 years, 40 years, 50 years, 60 years, and 70 years *(5).* Two penetrance estimates for BRCA2 were reported. We used the higher estimate at young ages, which minimizes the difference between BRCA1 and BRCA2 and results in a lower estimate of the proportion of BRCA2 carriers in older breast cancer patients. The corresponding breast cancer incidence rates for carriers in each 5-year age group from 25–29 years through 65–69 years were calculated from these penetrance estimates on the assumption that the incidence ratio for carriers compared with the general population of England and Wales (1991 rates) changes linearly with age from 30–34 years through 45–49 years and from 50–54 years through 65–69 years. If we assume that carriers have normal mortality from causes other than breast cancer, the fraction (f_i) of all breast cancers in the ith 5-year age group that arise in carriers is given by

$$f_i = p(c_i - c_{i-1})/(g_i - g_{i-1}),$$

where p is the proportion of carriers at birth (i.e., approximately double the allele frequency) and c_i and g_i denote penetrance (cumulative breast cancer risk in the absence of other causes of death) at the end of the ith 5-year age interval for carriers and the general population, respectively. These fractions were calculated from the carrier and general population incidence rates. Multiplying f_i by n_i (the number of index cases diagnosed in the ith age group in our study) and summing over all ages thus give an expression for the expected number of carriers in our sample of

$$p \sum_i n_i(c_i - c_{i-1})/(g_i - g_{i-1}).$$

Equating this expression to the observed number of carriers provides an estimate of the carrier frequency p for each gene and hence of the fraction f_i of all breast cancers that occur in carriers in each age group.

The penetrance function for carriers of the relevant gene c_i and the penetrance function for the general population g_i (linearly interpolated within each 5-year age range) were averaged to give the age-specific penetrance for mothers and sisters of carriers. Each woman's probability of developing breast cancer was then calculated as the change in this average penetrance function from her age at entry (20 years old for sisters and age at index patient's birth for mothers) through her age at exit, divided by unity minus penetrance at entry. The exit date was defined for this purpose as the earliest of the following dates: the date of death from causes other than breast cancer, the 70th birthday, and the censoring date for cancer incidence, as described above. These probabilities were used to calculate the exact probability of obtaining the observed number of cases of breast cancer or fewer in relatives of carriers, which was doubled to give a two-sided significance level. The calculation was repeated with the combined penetrance estimate for the three common Ashkenazi mutations *(19).*

RESULTS

Patients in the Study

In the original U.K. National Case Control Studies of breast cancer, 755 women diagnosed before the age of 36 years and 644 women diagnosed from ages 36 years through 45 years were interviewed. When contacted again in 1992, 336 patients diagnosed before age 36 years and 450 patients diagnosed from ages 36 years through 45 years (total = 786 patients) returned a sample. Subsequently, these patients were contacted again to ask specific permission for genetic analyses, and 254 patients diagnosed before age 36 years and 363 patients diagnosed from ages 36 years through 45 years gave consent. These 617 samples were analyzed for mutations. The most common reason that a patient did not supply a blood sample was because she was dead or seriously ill (272 women diagnosed before age 36 years and 69 women diagnosed from ages 36 years through 45 years). Replies were not received from 69 patients diagnosed before age 36 years and 47 patients diagnosed from ages 36 years through 45 years. Forty-nine patients diagnosed before age 36 years and 53 women diagnosed from ages 36 years through 45 years refused to donate a blood sample. The remaining losses (29 women diagnosed before age 36 years and 25 women diagnosed from ages 36 years through 45 years) were patients who could not be traced, patients for whom the general practitioner refused permission to approach, and patients for whom blood sampling was not successful.

To evaluate whether the subset of patients tested for mutations in BRCA1 and BRCA2 was representative of the sample originally interviewed, we compared the family histories of the tested set and the untested set. In the study of patients diagnosed before age 36 years, 6.7% (17 of 254) of tested patients and 10.8% (54 of 501) of untested patients had a family history of breast cancer (defined as a mother or sister affected with breast cancer before age 60 years). The corresponding proportions of patients diagnosed from ages 36 years through 45 years were 9.1% (33 of 363) and 9.3% (26 of 281), respectively. This deficit of patients diagnosed before age 36 years who had a family history of breast cancer, although not statistically significant (P = .09), could be due at least partly to reduced survival, because

there was a delay of 5–8 years between interview and blood collection in the first study. The study design precludes analysis of survival time from diagnosis, but the 5-year survival from date of original interview was statistically significantly lower in patients with a family history (64%) than in those without (74%; $P = .03$, age stratified).

Mutations in BRCA1 and BRCA2 Genes

Sixteen BRCA1 mutations and 14 BRCA2 mutations that are predicted to encode truncated proteins were detected (Table 1). These mutations are associated with predisposition to the disease and, therefore, have been classified as disease associated. Eleven of 16 BRCA1 mutations and eight of 14 BRCA2 mutations have been previously registered in the Breast Information Core database (http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/), almost all of these having been detected in families with multiple cases of breast cancer. Several base substitutions that encode missense amino acid changes were encountered. Previously, none have been unambiguously classified as disease associated, and others have been reported as neutral polymorphisms. Fifteen rare missense changes (four in BRCA1 and 11 in BRCA2) have not been previously registered as polymorphisms to our knowledge. Three hundred twenty control DNA samples from healthy women were screened for the presence of these sequence variants. None of four missense changes in BRCA1 and two of 11 missense changes in BRCA2 were found in the control series and, therefore, were classified as polymorphisms. An additional one of four amino acids that were variant in BRCA1 and three of 11 amino acids that were variant in BRCA2 were not identical to corresponding residues in the mouse sequences. These variants, therefore, were classified as likely polymorphisms. Of the amino acid residues in which the remaining three missense changes in BRCA1 and six missense changes in BRCA2 were observed, none in BRCA1 and only one in BRCA2 were located in regions of the protein that exhibit more than 80% sequence identity between the human and mouse proteins. Their functional importance is questionable and, therefore, none have been included as disease-associated mutations. A 6-base-pair BRCA1 in-frame deletion also has not been classified as disease associated, because it does not generate a substantially truncated protein. This deletion was not found in previous mutational screens of several hundred samples, was not detected in our screen of 320 control samples from healthy women, and was not reported or registered previously in the Breast Information Core. This deletion results in the removal of Glu-1000 and Glu-1001; Glu-1000 is identical in mouse BRCA1, but Glu-1001 is not. This variant may in reality be disease associated, but the evidence in favor of this classification is currently insufficient. Thus, this variant has been classified as unknown.

The age at diagnosis for patients with disease-associated mutations is reported in Table 1. Mutations were detected in 15 (5.9%) of 254 women diagnosed with breast cancer before age 36 years (nine [3.5%] in BRCA1 and six [2.4%] in BRCA2) and in 15 (4.1%) of 363 women diagnosed between ages 36 years and 45 years (seven [1.9%] in BRCA1 and eight [2.2%] in BRCA2). A direct comparison of age distribution between BRCA1 and BRCA2 mutation carriers was not statistically significant ($P = .22$; Wilcoxon test). However, BRCA1 mutation carriers tended to be diagnosed at younger ages than noncarriers ($P = .04$), whereas BRCA2 mutation carriers did not ($P = .95$). BRCA1 mutations may thus be more penetrant at young ages than BRCA2 mutations.

Family Histories of Cancer in BRCA1 and BRCA2 Mutation Carriers

Family histories of cancer in carriers of mutations are shown in Table 1. Six of the 30 mutation carriers (five BRCA1 and one BRCA2) had a history of breast and/or ovarian cancers in first-degree female relatives. Six first-degree female relatives of BRCA1 mutation carriers were affected. Two carriers had mothers with breast cancer and one carrier had a mother with ovarian cancer. Two sisters of BRCA1 mutation carriers had ovarian cancer and one sister had breast cancer. One BRCA1 mutation carrier had a mother with breast cancer and a sister with ovarian cancer. One BRCA2 mutation carrier had both a mother and a sister with breast cancer. Family histories of cancer involving second-degree and more distant relatives were asked about only in the study of patients diagnosed from ages 36 years through 45 years. Eleven of 15 mutation carriers diagnosed from ages 36 years through 45 years (five of seven BRCA1 and six of eight BRCA2) had first- or second-degree female relatives with breast and/or ovarian cancers, and another BRCA2 mutation carrier had a cousin diagnosed with breast cancer at age 34 years.

Proportion of Familial Relative Risk of Breast Cancer Attributable to BRCA1 and BRCA2 Genes

The observed and expected numbers of breast cancers in first-degree female relatives of patients are shown in Table 2. Sixty-nine women diagnosed with breast cancer at any age up to 80 years were identified among mothers and sisters of screened patients compared with an expected number of 33.50 women, based on national incidence rates. Five mothers or sisters of BRCA1 or BRCA2 mutation carriers developed breast cancer compared with an expected 1.37 mothers or sisters (for a standardized incidence ratio of 365); in contrast, among mothers and sisters of noncarriers, there were 64 cases of breast cancer diagnosed compared with 32.13 cases expected (for a standardized incidence ratio of 199). From the BCLC analyses *(5)*, the sensitivity of our mutation detection method is about 63%. If the number of mutation carriers is adjusted accordingly, these data suggest that about 16% of the excess risk to relatives (observed minus expected number) is attributable to BRCA1 and BRCA2 mutations. This is the case even for relatives diagnosed at an early age. There were 33 mothers or sisters diagnosed with cancer before age 50 years compared with 12.62 mothers or sisters expected, three (0.58 expected) of whom were related to mutation carriers.

Familial clustering of breast and ovarian cancers according to carrier status and numbers of affected relatives is shown in Table 3. For breast cancer before the age of 60 years and ovarian cancer at any age, the proportion of carriers was 45% (five of 11; two BRCA1 and three BRCA2) for patients with two or more affected first- or second-degree relatives and 11% (six of 55; five BRCA1 and one BRCA2) for patients with at least one affected first-degree relative. Therefore, families with multiple cases of breast cancer diagnosed before the age of 60 years or ovarian cancer favor the presence of mutations in the known genes.

There was no overall excess of ovarian cancer among mothers and sisters (six deaths observed and 6.14 expected [Table 2]), but there were three deaths observed (0.24 expected) in relatives of carriers (all BRCA1) and a chance deficit among relatives of

Table 1. Detection of BRCA1 and BRCA2 mutations*

Age at diagnosis, y	Mutation	Mutation type	Mother (age at diagnosis)	Sister (age at diagnosis)	Other family history of breast or ovarian cancer (age at diagnosis)	Other family history of cancer (age at diagnosis)
		A. BRCA1 mutations detected in 617 women with breast cancer diagnosed before age 46 y				
38	G200-1C	Splice site			Maternal aunt, breast (<55 y)	
26	377insT	Frameshift				
39	1475delA	Frameshift	Ovary (34 y)			
33	2080delA	Frameshift				
40	2080delA	Frameshift	Breast (40 y)	Ovary (41 y)	Paternal grandmother, breast (60 y)	Maternal grandmother, lung (62 y)
34	2187delA	Frameshift				
35	2800delAA	Frameshift				
39	3347delAG	Frameshift				Father, liver (62 y)
43	3450delCAAG	Frameshift		Ovary (38 y)		Mother, colorectal (82 y)
34	3695insT	Frameshift				
40	4184delTCAA	Frameshift				
38	4184delTCAA	Frameshift		Breast (44 y)	Paternal grandmother, breast (52 y)	
35	4184delTCAA	Frameshift				
30	4401delA	Frameshift				
28	G5272A Trp 1718 Stop	Nonsense				
28	5382insC	Frameshift	Breast (56 y)			
		B. BRCA2 mutations detected in 617 women with breast cancer diagnosed before age 46 y				
32	763insAT	Frameshift				Mother, "womb or cervix" (50 y) Sister, ovarian tumor of uncertain behavior (35 y)
43	763insAT	Frameshift			Paternal aunt, breast (?)	
43	983delACAG	Frameshift			Paternal aunt, breast (35 y) Paternal aunt, breast (43 y) Paternal cousin, breast (52 y)	
41	A3058T Lys944Stop	Nonsense				Maternal aunt, bowel (67 y)
44	3758delACAG	Frameshift			Paternal grandmother, breast (?)	Father, lung (74 y) Paternal uncle, prostate (70 y) Paternal cousin, uterus (50 y)
45	3908delTG	Frameshift			Maternal cousin, breast (34 y)	
31	4706delAAAG	Frameshift				
41	5804delTTAA	Frameshift	Breast (62 y)	Breast (42 y)	Maternal aunt, breast (45 y) Maternal grandmother, breast (?)	
40	C6137A Ser1970Stop	Nonsense			Paternal grandmother, breast (75 y) Paternal aunt, breast (?)	Father, prostate (66 y)
44	6174delT	Frameshift			Maternal aunt, breast (40 y) Maternal aunt, breast (40 y) Maternal aunt, breast (54 y) Maternal cousin, breast (30 y)	Mother, pancreas (?)
34	6305insA	Frameshift				
33	8803delC	Frameshift				Sister, CIS, eye (38 y)
25	9132delC	Frameshift				
25	9132delC	Frameshift				

*ins = insertion; del = deletion; Trp = tryptophan; Lys = lysine; Ser = serine; CIS = carcinoma *in situ.*

noncarriers (three deaths observed and 5.90 expected). Of six families with at least one patient with ovarian cancer, three families (50%) had mutations in BRCA1 and none had mutations in BRCA2. It has been suggested that there is an elevated risk of ovarian cancer associated with BRCA1 mutations located on the 5′ side of nucleotide 1440 compared with mutations on the 3′ side of this position *(8).* The three BRCA1 mutations identified in patients with ovarian cancer fall to the 5′ side of

Table 2. Breast cancer incidence and cause-specific mortality, up to age 80 years, in mothers and sisters of patients with early-onset breast cancer (expected figures calculated from national rates from England and Wales)

Age at event, y	Relatives of all 617 tested patients (n = 1188)*		Relatives of 587 noncarriers (n = 1136)*		Relatives of 30 mutation carriers (n = 52)*	
	Obs	Exp	Obs	Exp	Obs	Exp
Breast cancer incidence in mothers and sisters						
<40	10	3.57	10	3.41	0	0.16
40–49	23	9.05	20	8.63	3	0.42
50–59	17	8.80	16	8.44	1	0.36
60–69	14	8.45	13	8.11	1	0.34
70–79	5	3.64	5	3.55	0	0.09
Total	69	33.50	64	32.13	5	1.37
Breast cancer mortality in mothers and sisters						
<40	3	1.08	3	1.04	0	0.04
40–49	10	3.53	8	3.36	2	0.17
50–59	11	5.17	10	4.96	1	0.21
60–69	7	5.29	6	5.09	1	0.20
70–79	3	3.43	3	3.32	0	0.11
Total	34	18.50	30	17.77	4	0.73
Ovarian cancer mortality in mothers and sisters						
<80	6	6.14	3	5.90	3	0.24
Mortality from all other causes in mothers and sisters						
<80	196	203.90	186	196.31	10	7.59

*Obs = observed; Exp = expected.

nucleotide 1440. Mortality from other causes was close to that expected irrespective of carrier status.

Risks of Breast Cancer Associated With Mutations in BRCA1 and BRCA2 Genes

From the BCLC penetrance estimates published recently by the BCLC based on multiple-case families, 13.5 cases of breast cancer would be expected among mothers and sisters of patients compared with five cases observed ($P = .004$). The expected number of cases based on penetrance estimates obtained from relatives of mutation carriers identified in a series of unaffected Ashkenazi Jewish individuals *(19)* was 10.7, which is not quite statistically significantly different from the observed five cases ($P = .053$).

Prevalence of BRCA1 and BRCA2 Mutations and Their Contribution to Overall Breast Cancer Incidence

Our data and the BCLC penetrance estimates provide estimates of the proportion of individuals who are carriers of BRCA1 or BRCA2 mutations in the general population and, hence, among patients with breast cancer at each age (Table 4). The estimated prevalences of BRCA1 and BRCA2 carriers, assuming 63% sensitivity of mutation detection, are 0.11% and 0.12%, respectively, at birth in the general population. Among breast cancer patients, the respective prevalences are 3.1% and 3.0% for those diagnosed before age 50 years, 0.49% and 0.84% for those diagnosed from ages 50 years through 69 years, and 1.3% and 1.5% for all patients who are less than 70 years old at diagnosis.

Discussion

To our knowledge, this is the first report of the prevalence of both BRCA1 and BRCA2 mutations in a large population-based series of patients with early-onset breast cancer from an outbred population (in which recurrent [founder] mutations constitute a small proportion of the total). Fifteen (5.9%) of 254 women diagnosed with breast cancer before age 36 years and 15 (4.1%) of 363 women diagnosed from ages 36 years through 45 years carried mutations in BRCA1 or BRCA2. These observed prevalences are likely to be underestimates because the sensitivity of the mutation detection technique used is not complete. Large genomic deletions, mutations in introns or in promoter regions, and a small minority of base substitutions are not detected. Moreover, some of the rare missense amino acid changes that have been classified as of unknown importance may be disease causing. By comparing the number of mutations found in families clearly linked to either gene, the sensitivity of conformation-sensitive gel electrophoresis and other similar techniques has been estimated at 63% *(5)*. If we adjust for incomplete sensitivity, our estimate of the proportion of breast cancers that are due to these genes is 9.4% before age 36 years and 6.6% from ages 36 years through 45 years.

There was also a slightly lower prevalence of patients with a family history of breast cancer diagnosed before age 60 years (50 [8.1%] of 617 patients) among those analyzed for BRCA1 and BRCA2 mutations compared with those not tested (80 [10.2%] of 782). This underascertainment may be at least partly due to the statistically significantly poorer survival ($P = .03$) of patients with a family history of disease of breast cancer.

The prevalence of BRCA1 mutations among patients with breast cancer in the U.K. has previously been estimated at 7.5% for those diagnosed before age 30 years, 5.1% for those diagnosed from ages 30 years through 39 years, and 2.2% for those diagnosed from ages 40 years through 49 years *(20)*. (These estimates were obtained by using the penetrance estimates for breast and ovarian cancers in BRCA1 mutation carriers derived from the multiple-case BCLC linkage families and by assuming that the excesses of ovarian cancer in relatives of patients with breast cancer and of breast cancer in relatives of ovarian cancer are due to BRCA1 mutations.) The observed frequencies of 3.5% (nine of 254) patients diagnosed before age 36 years and 1.9% (seven of 363) patients diagnosed from ages 36 years through 45 years for BRCA1 are comparable to these predictions, particularly after adjustment for the sensitivity of mutation detection. The results obtained in this study are not statistically significantly lower than recently published results for BRCA1 in a U.S. population-based study of an outbred population in which 6.2% (12 of 193) patients diagnosed before age 35 years carried BRCA1 mutations *(21)*.

An important conclusion from the present study is that, in the U.K., mutations in BRCA2 (14 [2.3%] of 617 patients) and BRCA1 (16 [2.6%] of 617 patients) contribute similarly to early-onset breast cancer. Currently, there is no published information on the prevalence of BRCA2 in other outbred population-based series of cancer patients. In a hospital-based series of 73 patients diagnosed before age 32 years, BRCA2 mutations were detected in 2.7% patients compared with 12.3% patients with mutations in BRCA1 *(22,23)*. However, this study was conducted in the Boston area, which has a high Ashkenazi Jewish population, and four of the mutations detected in BRCA1 were 185delAG, which has a high prevalence among Ashkenazi Jews. Therefore, the wider relevance of this study is questionable. Studies of families with multiple cases of early-onset breast cancer have usually detected more mutations in BRCA1 than in BRCA2. However,

Table 3. Family history of breast and ovarian cancers in relatives of the 617 patients with breast cancer analyzed for BRCA1 and BRCA2 mutations*

	Patients diagnosed before age 36 y and from age 36 y through 45 y				Patients diagnosed from age 36 y through 45 y			
	Mother affected	First-degree relatives			First- and second-degree relatives			
		0	1	2	0	1	2	3 or more
Breast cancer at any age, No.	54	547	67	3	248	87	22	6
	(2, 1)	(13, 13)	(3, 0)	(0, 1)	(4, 2)	(1, 2)	(2, 2)	(0, 2)
Breast cancer before age 60 y, No.	32	567	49	1	296	59	8	0
	(2, 0)	(13, 13)	(3, 1)	(0, 0)	(4, 5)	(2, 0)	(1, 3)	(0, 0)
Breast cancer before age 60 y and/or ovarian cancer, No.	36	562	52	3	291	61	10	1
	(3, 0)	(11, 13)	(4, 1)	(1, 0)	(2, 5)	(3, 0)	(2, 3)	(0, 0)
Ovarian cancer, No.	4	611	5	1	357	4	1	1
	(1, 0)	(13, 14)	(3, 0)	(0, 0)	(4, 8)	(3, 0)	(0, 0)	(0, 0)

*Numbers of first-degree relatives are presented together for both series of patients. Information on second-degree relatives was obtained only from the study of patients diagnosed between ages 36 years and 45 years; therefore, these are presented separately. The numbers of BRCA1 and BRCA2 mutations are indicated in parentheses (BRCA1, BRCA2).

Table 4. Annual numbers of breast cancers by age at diagnosis in Britain in 1991 showing estimated numbers and percentages with BRCA1 and BRCA2 mutations

	Age 0–49 y	Age 50–69 y	Age 0–69 y
Overall, No.	6726	15 907	22 633
BRCA1 carriers, No.	209	78	287
BRCA2 carriers, No.	202	134	336
BRCA1 prevalence, %	3.1	0.49	1.3
BRCA2 prevalence, %	3.0	0.84	1.5

many such series are biased toward families with ovarian cancer, which clearly selects for BRCA1 mutations, and toward multiple cases of very early-onset breast cancer, which may also select for BRCA1 mutations. In the most recent analysis by the BCLC of families with breast cancer only, approximately equal numbers of the families had mutations in BRCA1 and BRCA2 genes *(5)*.

With the BCLC BRCA1 and BRCA2 penetrance estimates and the prevalence estimates obtained in this study, we have estimated the prevalence of mutations in the two genes in the U.K. population and in patients with breast cancer diagnosed at later ages. A population frequency for carriers of 0.0011 was obtained for BRCA1 mutations and of 0.0012 for BRCA2 mutations. The estimate for BRCA1 mutations is similar to the value (0.0012) previously derived from the familial association of breast and ovarian cancers *(20)*. We predict that, in the U.K., BRCA2 mutations are likely to account for more cases of breast cancer than BRCA1 mutations in patients diagnosed at age 50 years and older and in patients with breast cancer overall. This result is predominantly due to the lower penetrance of BRCA2 at young ages. Although absolute estimates of penetrance differ among studies of breast cancer families, of unaffected individuals, and of patients with breast cancer, most indicate that the lifetime penetrance of BRCA1 and BRCA2 mutations are approximately equal and that the penetrance at ages younger than 50 years is less for BRCA2 mutations *(5)*. The lower penetrance of BRCA2 mutations at young ages is supported by this study because the age distribution of BRCA1 mutation carriers was statistically significantly skewed toward younger ages compared with noncarriers ($P = .04$; Wilcoxon test), but the age distribution of BRCA2 mutation carriers was not ($P = .95$).

The results indicate that a considerable proportion of the familial risk of breast cancer is not attributable to mutations in BRCA1 and BRCA2 genes. Sixty-four mothers and sisters of patients not carrying mutations were diagnosed with breast cancer compared with 32.13 expected from national rates, whereas only five first-degree female relatives of BRCA1 and BRCA2 mutation carriers were affected compared with 1.37 expected. Therefore, only about 16% of the excess risk to mothers and sisters of patients with breast cancer in this series was attributable to mutations in the two genes (when adjusted for sensitivity of mutation detection). The familial risk that is not due to BRCA1 and BRCA2 mutations is also present at young ages, because 30 mothers and sisters of noncarrier patients were affected by breast cancer before age 50 years compared with 12.04 expected. Even if the likelihood of missed mutations and other biases is taken into account, most of the familial risk of breast cancer appears to be due to genes other than BRCA1 and BRCA2.

The observation of a substantial residual familial risk that is not explained by BRCA1 and BRCA2 mutations is consistent with the results of recent studies carried out by the BCLC on families with multiple cases of breast cancer *(5)*. Although almost all families with multiple cases of breast and ovarian cancers and most very large families with multiple cases of breast cancer carry BRCA1 and BRCA2 mutations, only one third of families with only four or five cases of breast cancer and no cases of ovarian cancer carry mutations in either BRCA1 or BRCA2. Because smaller familial clusters are much more common than families with large numbers of cases, the indication from these studies is also that a substantial proportion of familial clustering is not accounted for by mutations in BRCA1 and BRCA2 genes. The BCLC analyses also suggest that mutations in the remaining genes are likely to have lower penetrance than BRCA1 and BRCA2 mutations. Our results support this hypothesis. Although only about 16% of the overall familial risk to mothers and sisters of patients appears to be due to BRCA1 and BRCA2 mutations, five (45%) of 11 families with two or more first- or second-degree relatives with ovarian cancer or breast cancer diagnosed before age 60 years carried BRCA1 or BRCA2 mutations. Therefore, the more substantial the familial clustering, the greater the enrichment for mutations in the known genes, indicating that they confer a higher risk than the remaining susceptibility genes.

Of six patients who reported at least one case of ovarian cancer among first- or second-degree relatives, three patients carried a mutation in BRCA1. This is consistent with results from the BCLC indicating that almost all families with multiple

cases of breast and ovarian cancers carry mutations in BRCA1 or BRCA2 genes and supports the higher risk of ovarian cancer associated with BRCA1 mutations compared with BRCA2 mutations.

There has recently been considerable discussion of the risks of breast cancer associated with mutations in BRCA1 and BRCA2, particularly the differences between estimates obtained from analyses of families with multiple cases of breast cancer and the generally lower estimates obtained from analyses of patients with breast cancer collected without regard to family history or from series of unaffected individuals *(24)*. In this study, there were only five cases of breast cancer in mothers and sisters of BRCA1 or BRCA2 mutation carriers. This number is statistically significantly less than the expected number of 13.5, calculated from the BCLC penetrance analysis that is based on multiple-case families ($P = .004$). This low penetrance may, however, be due at least partly to the inferior survival of patients with an affected first-degree relative ($P = .03$), which presumably resulted in some reduction in the observed risks to relatives of both carriers and noncarriers. The effect would be most marked for the first study, where there was a delay of 5–8 years between interview and blood collection and where 36% (272 of 755) of patients could not be tested because of serious illness or death. A further bias among mothers relates to the low risk of breast cancer associated with high parity. The probability that a woman will have a daughter with breast cancer (or any disease) is roughly proportional to the number of daughters she has, so mothers of patients with breast cancer will tend to have high parity. The numbers of affected mothers and sisters also may have been reduced by chance. Complete data on cancers in second-degree relatives were available only for women diagnosed with breast cancer from ages 36 years through 45 years. Only three of these 15 patients had a first-degree relative with breast cancer (Table 1; two with BRCA1 mutations and one with a BRCA2 mutation), but six more (two with BRCA1 mutations and four with BRCA2 mutations) had at least one second-degree relative with breast cancer. Thus, although our data suggest a somewhat lower breast cancer penetrance than estimates derived from multiple-case families, the difference may not be very marked.

The results have implications for the cost-effectiveness of wider implementation of BRCA1 and BRCA2 mutation analysis. Only a small proportion of patients with early-onset breast cancer carry a mutation in one or the other gene, and only a small proportion of the familial risk of breast cancer is attributable to these genes. However, a substantial proportion (45% in our data) of young patients with two or more affected relatives are mutation carriers, and perhaps such women should be offered the opportunity of a test for mutations in BRCA1 and BRCA2 genes.

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