Familial Risks, Early-Onset Breast Cancer, and BRCA1 and BRCA2 Germline Mutations

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Background: **Having a family history of breast cancer, particularly if it involves early-onset disease, is a risk factor for breast cancer, but little is known about specific causes of this association. Consequently, we studied mothers, sisters, and aunts of an age-stratified sample of 1567 unselected case patients diagnosed with breast cancer before age 60 years, recruited to a population-based, case–control-family study, in which case patients, control subjects, and their relatives were administered the same questionnaire.** ***Methods:*** **Extensive BRCA1 and BRCA2 mutation testing was carried out for 788 case patients diagnosed before age 40 years, including manual sequencing of DNA from 72 patients with two or more affected relatives. Standardized morbidity ratios, age-specific cumulative risks, and hazard ratios were calculated for groupings of relatives.** ***Results:*** **Cumulative risks of breast cancer to age 50 years in the sisters, mothers, and aunts of the case patients, respectively, were 6, 3, and 2 times the population risk if the case patient was younger than age 40 years at diagnosis but were considerably lower if the case patient was older at diagnosis. When relatives of the case patients with a BRCA1 or BRCA2 mutation were excluded, these risks fell by, at most, 20%. Sisters and aunts, but not mothers, who had an additional first-degree relative with breast cancer were at increased risk, and the risk was greater when that relative was younger at diagnosis. Hazard ratios were 10.7 (95% confidence interval [CI] = 4.2 to 26.8) for sisters and 4.2 (95% CI = 2.2 to 8.1) for aunts, if the relative was aged 40 years at diagnosis. Fewer than one-third of the excess of breast cancers in relatives of case patients diagnosed before age 40 years that are attributed to familial factors are BRCA1- or BRCA2-related.** ***Conclusion:*** **Mutations in genes other than BRCA1 and BRCA2 may be associated with a high risk of breast cancer, especially in young women. [J Natl Cancer Inst 2003; 95:448–57]**

For an unaffected woman, having a family history of breast cancer is associated with an increased risk for the disease. This familial risk is greater if the unaffected woman has a first-degree relative who was diagnosed with breast cancer at a young age or if she has more than one first-degree relative with breast cancer *(1,2).* Understanding the clustering of breast cancer in families (familial aggregation), in the sense above, is important because it could not occur without there being strong underlying risk factors that, either individually or collectively, are shared among relatives and that the average risk associated with such factors for women in the highest quartile of underlying familial risk

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must be at least 20 times that for women in the lowest quartile *(3,4)*.

Little is known about the specific causes of this familial aggregation of breast cancer. Established lifestyle and environmental risk factors have a modest effect on risk. Some of these factors are ubiquitous exposures that have a narrow range, are at best moderately correlated in relatives (correlation coefficients ≤ 0.4), and appear to explain only a small amount of familial aggregation *(4)*. These factors may be poorly measured surrogates of, for example, strong underlying hormonal risk factors, which may themselves be correlated in relatives because of hormonal variations influenced by genetic and/or environmental factors that are shared by family members.

Genetic risk factors may, therefore, explain the major part of familial aggregation. Specific BRCA1 and BRCA2 gene mutations are associated with increased breast cancer risks of 10-fold or more. Even though these mutations may explain a substantial proportion of opportunistically sampled families with the most extreme cancer histories [e.g., *see* Ford et al. *(5)*], they are rare and, consequently, mathematical models predict that they will explain only a small portion of familial aggregation in the sense above *(3)*. This raises two questions: 1) What is the actual portion of this familial aggregation not explained by BRCA1 and BRCA2 mutations? and 2) What are the typical characteristics of this unexplained portion of familial aggregation?

In this article, we use the Australian Breast Cancer Family Study to identify a population-based sample of 1567 women, unselected for a family history of breast cancer, who were diagnosed with incident primary breast cancer before the age of 60 years, half of whom were diagnosed before the age of 40 years. We assembled cohorts of sisters, mothers, and aunts of the case patients and determined their risk of breast cancer. Specifically, we 1) collected data on second- and first-degree relatives by interviewing relatives as well as the case patients; 2) studied families of women with breast cancer diagnosed in three age groups, with emphasis on early-onset disease; 3) performed extensive mutation testing, including manual sequencing of the complete coding regions of the BRCA1 and BRCA2 genes for the case patients diagnosed when younger than age 40 years who had two or more affected relatives; and 4) reassessed the characteristics of familial aggregation, after excluding the relatives of the detected mutation carriers.

Patients and Methods

The Australian Breast Cancer Family Study is a population-based, case–control-family study of breast cancer (in which case patients, control subjects, and their relatives were administered the same questionnaire) with an emphasis on early-onset disease, that was carried out in Melbourne and Sydney, Australia *(6–8)*. Approval for the study was obtained from the ethics committees of The University of Melbourne and The Cancer Councils of Victoria and New South Wales. All subjects provided written informed consent for participation in the study.

Case Patients

Potential case patients were adult women living in the metropolitan areas of Melbourne and Sydney who were diagnosed with a histologically confirmed first primary cancer of the breast. From January 1, 1992, through September 30, 1999, in Melbourne and from January 1, 1993, through December 31, 1998, in Sydney, women aged younger than 40 years at diagnosis were selected; after January 1, 1996, random samples of women aged 40–49 years and 50–59 years at diagnosis were selected.

Case patients were identified by use of the Victorian and New South Wales cancer registries, to which notification of cancer diagnoses is a legislative requirement. Recruitment into the study began with a letter to the attending doctor requesting permission to approach the case patient. If permission was granted, her participation was sought by a letter. The letters to the doctors and potential case patients emphasized that participation of all eligible women was being sought, irrespective of family history of breast cancer. From January 1, 1992, through July 31, 1995, case patients were restricted to women who could speak English; from January 1, 1996, onward, non-English speaking women were included. Overall, we approached 2303 eligible case patients (ages at diagnosis: 1208 = <40 years, 551 = 40–49 years, 544 = 50–59 years). Attrition by death (2%), refusal by the attending doctor (8%), refusal by the case patient (16%), nonresponse by the attending doctor (1%), nonresponse by the case patient (1%), or the case patient having moved and being unable to locate (2%) resulted in 1578 case patients (856 = <40 years, 367 = 40–49 years, 355 = 50–59 years) being interviewed (participation: 69% overall, 71% = <40 years, 67% = 40–49 years, 65% = 50–59 years).

Control Subjects

Potential control subjects were adult women living in the metropolitan areas of Melbourne and Sydney; these women were approached with a letter similar to that used for case patients *(7)*. During the study period from January 1, 1992, through September 30, 1999, in Melbourne and from January 1, 1993, through December 31, 1998, in Sydney, women were selected from the electoral roll (to which registration of adults is compulsory in Australia) by use of proportional random sampling based on the expected age distribution of the case patients. We approached 1531 eligible control subjects (913 = <40 years, 298 = 40–49 years, 320 = 50–59 years), 1021 (67%) agreed to participate (600 = <40 years, 205 = 40–49 years, 216 = 50–59 years), 432 (28%) refused, and 78 (5%) did not respond. As with case patients, before July 31, 1995, participation was restricted to control subjects who could speak English, and from January 1, 1996, onward, non-English speakers were included.

Data Collection

Participating case patients and control subjects completed an interviewer-administered risk factor questionnaire and family cancer history questionnaire involving construction of a pedigree covering all known first- and second-degree adult relatives, and we requested a blood sample from each participant. The in-person interview was conducted in the subject's home. The median time between diagnosis and interview was 8 months for case patients, and the median time between recruitment and interview was 2 months for control subjects. In addition, each subject was asked to obtain permission from specific male and female adult relatives for their participation. Participation involved completing the same risk factor questionnaire and providing additional information for the pedigree and family history questionnaire, typically during a telephone interview. Thus, reports of cancer in relatives of the subjects often came from multiple sources within the family, so that the completed pedigree information for each individual was based on a self-report

or report(s) from their first-degree relatives and rarely was based only on a report from a second-degree relative. For relatives who could not be interviewed (because we were not granted permission to contact them or because they were deceased or unable or unwilling to participate), proxy information was obtained with an abridged version of the risk factor questionnaire that was completed by their closest available relative. We sought to verify all cancers reported in relatives through cancer registries and death certificates.

Relatives of Case Patients and Control Subjects

Data for the sisters, mothers, and aunts of both case patients and control subjects were used for the present study. The variables were relationship to the case patient or control subject, year of birth, year and age at completion of the family history questionnaire (or year of death and age at death if deceased), breast cancer status at the time of the case patient's diagnosis or at the time of the control subject's recruitment into the study, year and age at breast cancer diagnosis if affected, the presence of breast cancer in at least one first-degree relative other than the case patient or control subject (for sisters, this meant in any sister[s] or the mother; for mothers, this meant in any sister[s], the maternal grandmother, or any maternal aunt[s]; for maternal aunts, this meant in the mother, any other maternal aunt[s], or the maternal grandmother; and for paternal aunts, this meant in any other paternal aunt[s] or the paternal grandmother), and youngest age at diagnosis of any affected first-degree relative.

Because complete family records for the specific variables were required for these analyses, missing values were estimated by use of a defined protocol. If an exact age was not known but an age range was provided, age was estimated as the midpoint of that range. In the absence of any age information, it was assumed that both parents of a common child were born in the same year, that a parent was aged 20 years at birth of a first child, and that there were 2 years between the births of children. For relatives for whom vital status was unknown, ages were censored at the time they were last known to be alive (e.g., at the birth of their last child). Age at cancer diagnosis was estimated as age of death if the relative was deceased or as the mean age at onset for the cancer type *(9).* If no information was available regarding the birth order and year of birth of maternal or paternal aunts, they were given the same birth year as the case patient's mother or father. Eleven of the 1578 case families and 25 of the 1021 control families were omitted because the case patients or control subjects were adopted or had refused any participation by family members and, thus, there was no information on the biologic relatives, which was needed for this analysis.

Table 1 shows that a total of 8622 mothers, sisters, and aunts of case patients contributed 505 743 person-years. Families in which the case patient was younger than 40 years at diagnosis or recruitment contributed 55% of the total number of relatives and 51% of the total person-years. For the relatives of control subjects, 5464 mothers, sisters, and aunts contributed 311 681 person-years.

Of the 50 (25 = <40 years, 12 = 40–49 years, 13 = 50–59 years) reports of breast cancer in sisters of case patients, we verified 74% (72% = <40 years, 67% = 40–49 years, 85% = 50–59 years) by cancer registry reports and 2% (4% = <40 years, 0% = 40–49 years, 0% = 50–59 years) by medical records or death certificates. There were 150 (81 = <40 years, 35 = 40–49 years, 34 = 50–59 years) reports of breast cancer in mothers of case patients; we verified 55% (65% = <40 years, 48% = 40–49 years, 38% = 50–59 years) by cancer registry reports and 13% (10% = <40 years, 20% = 40–49 years, 12% = 50–59 years) by medical records or death certificates. There were 270 (139 = <40 years, 67 = 40–49 years, 64 = 50–59 years) reports of breast cancer in aunts of case patients; we verified 34% (31% = <40 years, 37% = 40–49 years, 39% = 50–59 years) by cancer registry reports and 12% (12% = <40 years, 15% = 40–49 years, 9% = 50–59 years) by medical records or death certificates.

Similar verification rates were obtained in the relatives of control subjects: 60.7% in mothers and sisters and 41.5% in aunts. Reasons for being unable to perform the verification check included insufficient identifying information to search registries for the relative, diagnosis occurring before cancer registry records, or diagnosis occurring in areas in which we had no contact with a cancer registry.

Age at interview or death was unknown for 0% (0% = <40 years, 0% = 40–49 years, 2% = 50–59 years) of sisters of case patients, 1% (0% = <40 years, 0% = 40–49 years, 1% = 50–59 years) of mothers of case patients, and 17% (12% = <40 years, 16% = 40–49 years, 28% = 50–59 years) of aunts of case patients. Age at breast cancer diagnosis was known for all affected sisters and mothers, except for two mothers in the 50- to 59-year age group and was unknown for 13% (7% = <40 years, 10% = 40–49 years, 28% = 50–59 years) of affected aunts. For control subjects, age at interview or death was unknown for 1%

Table 1. Number of relatives and person-years of observation by relationship to case patients with breast cancer and by the age group of the case patient*

Relationship to case patient	No. of relatives	No. of relatives per case patient	No. of person-years	No. of person-years per relative	No. of person-years per case patient
Case patient younger than 40 y at diagnosis					
Sisters	1114	1.3 ± 1.3	40 939	36.8 ± 7.3	48.2 ± 51.2
Mothers	850	1.0 ± 0.0	52 825	62.1 ± 8.1	62.1 ± 8.1
Aunts	2596	3.0 ± 2.3	158 060	60.9 ± 14.7	186.0 ± 143.5
Case patient 40–49 y at diagnosis					
Sisters	481	1.3 ± 1.3	21 512	44.7 ± 7.5	59.4 ± 56.5
Mothers	362	1.0 ± 0.0	25 330	70.0 ± 9.6	70.0 ± 9.6
Aunts	1179	3.3 ± 2.4	77 862	66.0 ± 18.2	215.1 ± 163.9
Case patient 50–59 y at diagnosis					
Sisters	427	1.2 ± 1.3	23 112	54.1 ± 8.5	65.1 ± 73.2
Mothers	355	1.0 ± 0.0	26 757	75.4 ± 11.9	75.4 ± 11.9
Aunts	1258	3.5 ± 2.4	79 346	63.1 ± 24.5	223.5 ± 159.7

*Number of relatives per case patient and number of person-years per relative and per case patient are the mean ± standard deviation.

of mothers, 1% of sisters, and 20% of aunts, and age at breast cancer diagnosis was known for all mothers and sisters and unknown for 11% of aunts.

Molecular Analysis

For 788 of the 856 case patients who were younger than 40 years at diagnosis, DNA was extracted from stored buffy-coat cells as described previously *(10)* for those recruited before 1995 and from peripheral blood cells by the use of spin columns (Mini blood spin columns; Qiagen, Hilden, Germany) for those recruited from 1995 onward.

All DNA samples were screened with a protein truncation test covering exon 11 of BRCA1 and exons 10, 11, and 27 of BRCA2. Exon 11 of BRCA1 and exon 11 of BRCA2 were amplified in three overlapping fragments, and exons 10 and 27 of BRCA2 were each amplified in one fragment. These fragments were subjected to transcription with T7 RNA polymerase, followed by translation incorporating [^{35}S]methionine (Amersham Pharmacia Biotech UK Limited, Little Chalfont, U.K.). Reaction products were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis in 14% gels and visualized by autoradiography overnight. Putative protein truncating mutations were confirmed by manual sequencing, as described previously *(11)*.

Sufficient DNA was available for more extensive analysis on samples from 72 of the 85 case patients with two or more first- or second-degree relatives with breast or ovarian cancer. These samples were screened for BRCA1 and BRCA2 germline mutations by manual sequencing of all coding and flanking intronic regions [excluding exon 11 of BRCA1 and exons 10, 11, and 27 of BRCA2, which were screened with the protein truncation test as described above *(12,13)*]. In addition, DNA samples from a randomly selected group of 91 case patients (44 without a family history and 47 with at least one first- or second-degree relative with breast cancer) had the entire BRCA1 coding region (including exon 11) manually sequenced *(12)*.

All DNA samples from the 788 case patients were tested for the three Ashkenazi founder mutations, by the protein truncation test for 6174delT in BRCA2 or by manual sequencing, as described previously *(14)* for 185delT and 5238insC in BRCA1. Testing for the duplication exon 13 protein-truncating mutation in BRCA1 was carried out for 641 subjects as described previously *(15)*. Briefly, specific oligonucleotides that amplify a 1.1-kb product from DNA carrying the duplication were used in a multiplex polymerase chain reaction that included a set of primers that amplified a 1.2-kb fragment of exon 11 of BRCA1 to control for DNA integrity *(14)*. Testing of case patients younger than 40 years at diagnosis for mutations in BRCA1 and BRCA2 identified 42 carriers.

Statistical Analysis

Standardized morbidity ratios (SMRs) were calculated to compare the number of observed cases of breast cancer in sisters, mothers, and aunts with the number expected from applying age- and sex-specific breast cancer incidence rates for the Australian population *(16)*.

Age-specific cumulative incidence in sets of relatives was estimated by Kaplan–Meier product-limit survival functions where failure time is age at onset of breast cancer. In relatives with a reported diagnosis of breast cancer, the relative's age at diagnosis was the time of failure. Relatives without a reported diagnosis of breast cancer were censored at their age at completion of the family history questionnaire if alive, age at death if deceased, or age last known to be alive if vital status was unknown. Cumulative incidence was defined as the complement of the Kaplan–Meier survival function. Confidence intervals (CIs) were computed as the complement of the survival curve confidence envelope by standard methods *(17)*. Population breast cancer cumulative incidence was based on Australian cancer registry data *(16)*.

The risk of breast cancer in the next 10 years, given that a woman was alive and unaffected at a specific age, was estimated by cumulative hazards. A lowess smoothing algorithm was used to obtain a smooth cumulative incidence curve, evaluated at each of the event time points of the step function with a span parameter of 1 *(17)*. A numeric derivative of the smoothed cumulative incidence curve was calculated by dividing the differences in y values by the differences in x values, which approximates the hazard function corresponding to the smoothed CI curve.

Estimates of hazard ratios (HRs) were obtained by use of Cox proportional hazards models. Because, for sisters and aunts, multiple members of a family could belong to the cohort of relatives, robust estimates of standard errors were presented. All statistical analyses were performed with STATA software *(18)*. All statistical tests were two-sided.

Results

SMRs

Table 2 shows that the SMRs for each of the sisters, mothers, and aunts of case patients, based on comparisons with population incidences, increased substantially as the age at diagnosis of the case patient decreased. The SMR for sisters of case patients diagnosed when younger than 40 years (5.2, 95% CI = 3.5 to 7.7) was more than twice that for sisters of case patients diagnosed when 40–49 years old (2.3, 95% CI = 1.3 to 4.0; $P = .01$) and four times that for sisters of case patients diagnosed when 50–59 years old (1.3, 95% CI = 0.7 to 2.2; $P<.001$). Similarly, the SMR for mothers of case patients diagnosed when younger than 40 years (2.5, 95% CI = 2.0 to 3.1) was greater than that for mothers of case patients diagnosed when 40–49 years old (1.8, 95% CI = 1.3 to 2.5; $P = .2$) or 50–59 years old (1.4, 95% CI = 1.0 to 2.0; $P = .02$). For aunts, an elevated SMR was evident only for case patients diagnosed when younger than 40 years (1.4, 95% CI = 1.2 to 1.6), and this SMR was greater than that for aunts of case patients diagnosed when 40–49 years old (1.1, 95% CI = 0.9 to 1.4; $P = .1$) or 50–59 years old (1.0, 95% CI = 0.8 to 1.2; $P = .01$).

For case patients diagnosed when younger than 40 years, across relatives, the SMR for sisters (5.2, 95% CI = 3.5 to 7.7) was twice that for mothers (2.5, 95% CI = 2.0 to 3.1; $P<.001$), which in turn was almost twice that for aunts (1.4, 95% CI = 1.2 to 1.6; $P = .001$). After excluding relatives of case patients known to carry a BRCA1 or BRCA2 mutation, the SMRs for sisters (4.2, 95% CI = 2.6 to 6.5), mothers (2.2, 95% CI = 1.7 to 2.8), and aunts (1.3, 95% CI = 1.1 to 1.6) remained elevated ($P = .001$, .001, and .006, respectively) and different from one another ($P<.001$ for sisters versus mothers or aunts, and $P = .005$ for mothers versus aunts).

For control subjects, the SMRs were 0.96 (95% CI = 0.53 to 1.74) for sisters, 1.00 (95% CI = 0.74 to 1.34) for mothers, and 0.90 (95% CI = 0.75 to 1.08) for aunts, giving a pooled

Table 2. Observed and expected numbers of breast cancers among sisters, mothers, and aunts of case patients with breast cancer, with standardized morbidity ratios (SMRs) and 95% confidence intervals (CIs) by age group of and relationship to the case patient

Relationship to case patient	No. of breast cancers		SMR (95% CI)
	Observed	Expected	
Case patient younger than 40 y at diagnosis			
All			
Sisters	25	4.8	5.2 (3.5 to 7.7)
Mothers	81	32.2	2.5 (2.0 to 3.1)
Aunts	139	101.3	1.4 (1.2 to 1.6)
Excluding relatives of case patients carrying a BRCA1 or BRCA2 mutation			
Sisters	19	4.6	4.2 (2.6 to 6.5)
Mothers	67	30.7	2.2 (1.7 to 2.8)
Aunts	127	96.6	1.3 (1.1 to 1.6)
Case patient 40–49 y at diagnosis			
Sisters	12	5.3	2.3 (1.3 to 4.0)
Mothers	35	19.8	1.8 (1.3 to 2.5)
Aunts	67	61.0	1.1 (0.9 to 1.4)
Case patient 50–59 y at diagnosis			
Sisters	13	10.3	1.3 (0.7 to 2.2)
Mothers	34	24.2	1.4 (1.0 to 2.0)
Aunts	64	65.5	1.0 (0.8 to 1.2)

SMR of 0.93 (95% CI = 0.80 to 1.08). When divided by age at recruitment of the control subject, the SMR was 1.11 (95% CI = 0.91 to 1.36) for those younger than 40 years, 0.77 (95% CI = 0.55 to 1.08) for those 40–49 years old, and 0.77 (95% CI = 0.58 to 1.03) for those 50–59 years old. That is, there was no evidence that, overall, the observed number of cases of breast cancer in relatives deviated from that expected under population incidence rates, although there was a suggestion that there may have been some under-reporting for the relatives of older control subjects.

Table 2 also shows that, for case patients diagnosed with breast cancer when younger than 40 years, 25 breast cancers were observed in sisters. If all relatives were independent and at population risk (i.e. if there was no familial aggregation), 4.8 cases would have been expected, so there were 21.2 more cases than expected in sisters because of familial aggregation. Six were in sisters of a case patient who carried a BRCA1 or BRCA2 mutation (and hence had a high posterior probability of carrying the same mutation themselves), leaving 14.2 cases "unexplained." For mothers, 81 breast cancers were observed, of which 32.2 were expected if there was no familial aggregation, and 14 had a high probability of being caused by BRCA1 or BRCA2 mutations, leaving at least 34.8 unexplained. For aunts, 139 breast cancers were observed, of which 101.3 were expected if there was no familial aggregation, and a maximum of 12 were caused by BRCA1 or BRCA2 mutations, leaving at least 25.7 unexplained. That is, of the 245 breast cancers observed in sisters, mothers, and aunts of case patients with early-onset disease, at most 32 (13%) have been explained by our testing for mutations in BRCA1 and BRCA2. More than twice as many, 74.7 (30%), remain unexplained. This ratio is approximately the same across sisters (14.2 versus 6), mothers (34.8 versus 14), and aunts (25.7 versus 12). Therefore, fewer than one-third of the excess of breast cancers in relatives of the early-onset case patients that are attributed to familial factors are BRCA1- or BRCA2-related.

Age-Specific Cumulative Risk to Relatives

Fig. 1 and Table 3 show different patterns in the cumulative risks of breast cancer in sisters, mothers, and aunts according to the age at diagnosis of the case patient. For case patients younger than 40 years at diagnosis, the cumulative risk to age 50 years was 10.0% (95% CI = 5.7% to 17.4%), 4.9% (95% CI = 3.6% to 6.7%), and 2.9% (95% CI = 2.3% to 3.6%), or 6, 3, and 2 times the population risk of 1.7%, in sisters, mothers, and aunts, respectively ($P<.001$ for all). In contrast, for relatives of case patients who were older at diagnosis, the cumulative risk to age 50 years was less, and sisters were at no higher risk than mothers. As for cumulative risk to age 80 years (population risk = 7.8%), the risk to mothers decreased as the age at diagnosis of the case patient increased and was consistently greater than the risk in aunts ($P = .003$, .01, and .03, respectively).

For relatives of control subjects, the pooled cumulative risks to age 50 years and 80 years were 1.9% (95% CI = 1.5% to 2.4%) and 6.8% (95% CI = 5.7% to 8.1%), respectively (data not shown). These risks closely followed the population risks to at least age 50 years. In relatives of older control subjects, the cumulative risks from age 50 years onward started to diverge below the population risks.

Age-Specific Cumulative Risk in Relatives of Case Patients Diagnosed When Younger Than 40 Years and Without a BRCA1 or BRCA2 Mutation

For case patients diagnosed when younger than 40 years, Table 3 and Fig. 2 show that, by excluding the 42 who carried a BRCA1 or BRCA2 mutation, the cumulative risk of breast cancer to age 50 years was reduced by 20% in sisters (from 10.0 [95% CI = 5.7 to 17.4] to 8.0 [95% CI = 4.1 to 15.6]), by 10% in mothers (from 4.9 [95% CI = 3.6 to 6.7] to 4.4 [95% CI = 3.1 to 6.1]), and by 10% in aunts (from 2.9 [95% CI = 2.3 to 3.6] to 2.6 [95% CI = 2.0 to 3.4]). All three categories of relatives were still at increased risk compared with the population risk ($P<.001$ for all). For relatives with an affected first-degree relative other than the case patient, the cumulative risk to age 50 years was 22.2% (95% CI = 10.0% to 49.7%), or 13 times the population risk for sisters, and 5.7% (95% CI = 2.6% to 12.7%) and 6.9% (95% CI = 4.6% to 10.2%), or approximately four times the population risk, for mothers and aunts, respectively ($P<.001$ for all). Even to age 80 years, these subsets of mothers and aunts were at increased and similar risks. It is

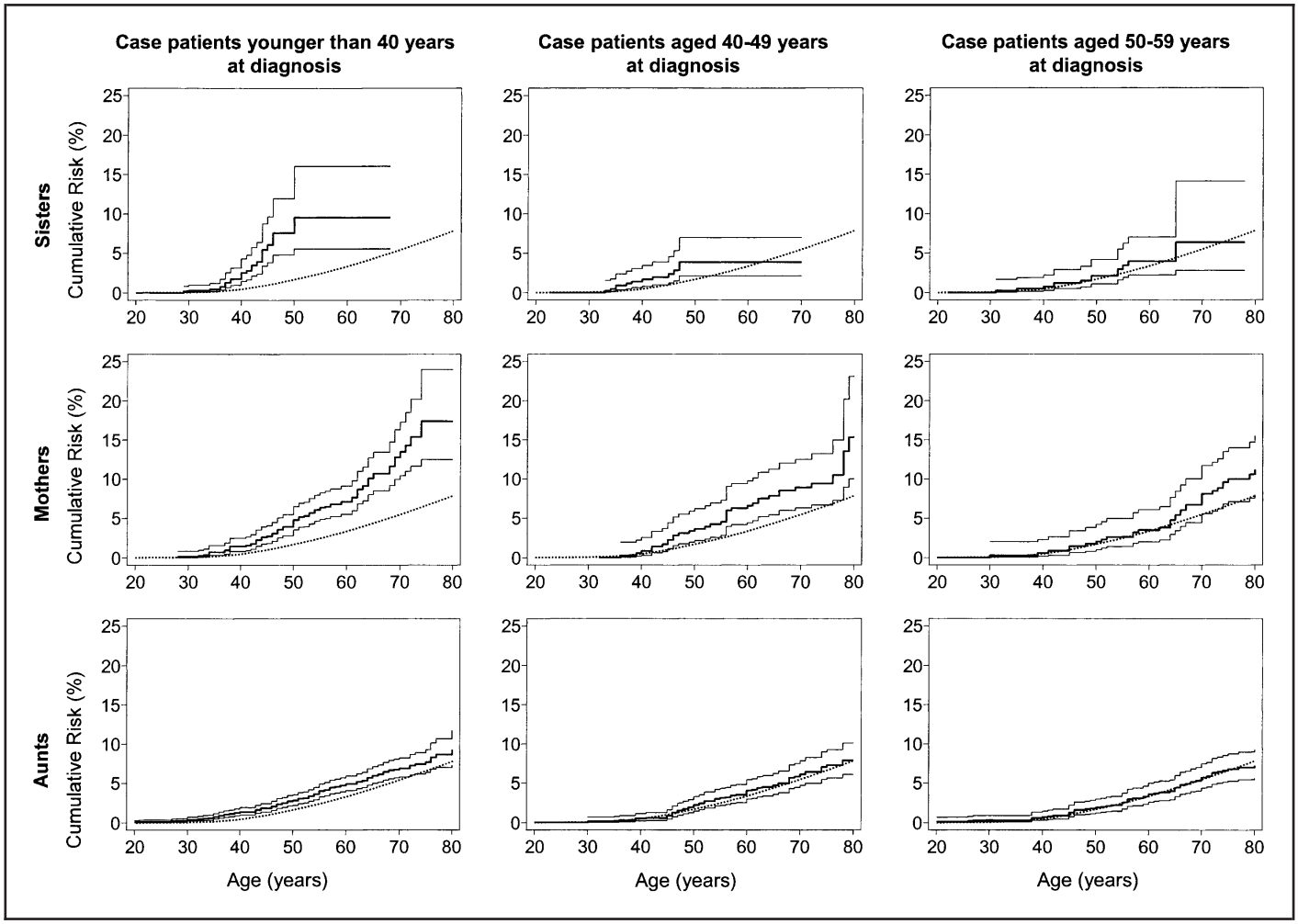

Fig. 1. Cumulative risk (%) and 95% confidence intervals (CIs) of breast cancer in sisters, mothers, and aunts of case patients diagnosed with breast cancer at younger than 40 years, 40–49 years, and 50–59 years. **Bold line** = cumulative risk; **thin line** = 95% CIs; **dotted line** = cumulative risk in the population.

noteworthy that, compared with the complementary subsets, in which there were no other affected first-degree relatives, aunts were at twice the risk if they had other affected first-degree relatives (18.1% [95% CI = 12.5% to 26.2%] versus 7.9% [95% CI = 5.7% to 11.1%]; $P = .002$), whereas there was little difference in risk between the two subgroups of mothers (20.9% [95% CI = 10.6% to 41.3%] versus 16.4% [95% CI = 10.2% to 26.5%]; $P = .6$).

Ten-Year Cumulative Risks in Unaffected Relatives

Table 4 shows that, for case patients diagnosed when younger than 40 years, the cumulative risk of breast cancer in unaffected relatives over the next 10 years was as high in 40-year-old sisters (7.4%, 95% CI = 3.6% to 15.3%) as it was in 60-year-old mothers (7.1%, 95% CI = 4.4% to 11.4%). Even after excluding families in which the case patient was known to carry a BRCA1 or BRCA2 mutation, the cumulative risk was 5.7% (95% CI = 2.3% to 14.1%) and 6.5% (95% CI = 3.8% to 11.1%), respectively.

Cox Proportional Hazards Multiple Regression Modeling of Risk in Relatives

The results of modeling shown in Table 5 suggest that the sisters of case patients diagnosed at younger than 40 years were at a substantially greater risk if they had at least one other affected first-degree relative (in addition to the case patient). The magnitude of this increased risk is shown by an HR of 13.4 (95% CI = 6.2 to 29.0) if the (earliest) age at diagnosis of the other affected relative(s) was 40 years; this value increased or decreased by 7% (95% CI = 2% to 12%) for each year that the age of diagnosis differed from 40 years (less than or more than, respectively). When sisters of case patients with a known BRCA1 or BRCA2 mutation were excluded, the increased risk was reduced (HR = 10.7, 95% CI = 4.2 to 26.8), and the effect of age at diagnosis of another first-degree relative was unchanged.

In contrast, the mothers of case patients diagnosed at younger than 40 years were at a modest increased risk (HR = 2.1, 95% CI = 1.2 to 3.9) through having at least one affected first-degree relative other than the case patient. This increase in risk was independent of the age at diagnosis of that relative and was not evident when mothers of case patients with a known BRCA1 or BRCA2 mutation were excluded.

The aunts of case patients diagnosed at younger than 40 years were at an increased risk, as shown by an HR of 5.0 (95% CI = 2.8 to 8.7) if they had any affected first-degree relative, and this increase was further increased or decreased by 4% (95% CI = 1% to 7%) for each year that the age at diagnosis differed from 40 years (less than or more than, respectively). Excluding aunts of case patients with a known BRCA1 or BRCA2 mutation reduced the increased risk (HR = 4.2, 95% CI = 2.2 to 8.1).

Table 3. Age-specific cumulative risk (%) to relatives of case patients with breast cancer and 95% confidence intervals (CIs)

Relationship to case patient	Age-specific cumulative risk, % (95% CI)	
	To age 50 y	To age 80 y*
Case patient younger than 40 y at diagnosis		
All		
Sisters	10.0 (5.7 to 17.4)	—
Mothers	4.9 (3.6 to 6.7)	19.0 (13.3 to 27.2)
Aunts	2.9 (2.3 to 3.6)	9.8 (7.6 to 12.5)
Excluding relatives of case patients carrying BRCA1 or BRCA2 mutation		
Sisters	8.0 (4.1 to 15.6)	—
Mothers	4.4 (3.1 to 6.1)	16.8 (11.3 to 24.9)
Aunts	2.6 (2.0 to 3.4)	9.6 (7.5 to 12.4)
Excluding relatives of case patients carrying a BRCA1 or BRCA2 mutation, those with another affected first-degree relative		
Sisters	22.2 (10.0 to 49.7)	—
Mothers	5.7 (2.6 to 12.7)	20.9 (10.6 to 41.3)
Aunts	6.9 (4.6 to 10.2)	18.1 (12.5 to 26.2)
Excluding relatives of case patients carrying a BRCA1 or BRCA2 mutation, those without another affected first-degree relative		
Sisters	6.3 (2.6 to 14.9)	—
Mothers	4.2 (2.9 to 6.1)	16.4 (10.2 to 26.5)
Aunts	1.8 (1.3 to 2.6)	7.9 (5.7 to 11.1)
Case patient 40–49 y at diagnosis		
Sisters	3.9 (2.2 to 7.2)	—
Mothers	3.7 (2.2 to 6.4)	16.5 (10.5 to 26.0)
Aunts	2.2 (1.5 to 3.3)	8.2 (6.3 to 10.7)
Case patient 50–59 y at diagnosis		
Sisters	2.1 (1.1 to 4.3)	6.5 (2.8 to 15.0)
Mothers	2.0 (1.0 to 4.3)	11.8 (8.3 to 16.8)
Aunts	1.9 (1.2 to 3.0)	7.6 (5.8 to 9.8)

*— = insufficient information to estimate risk to this age.

Table 4. Ten-year cumulative risk (%) with 95% confidence intervals (CIs) of breast cancer among relatives of case patients with breast cancer from age 40, 50, and 60 years by relationship to and age group of the case patient

Relationship to case patient	10-y cumulative risk, % (95% CI)		
	From age 40 y	From age 50 y*	From age 60 y*
Case patient younger than 40 y at diagnosis			
All			
Sister	7.4 (3.6 to 15.3)	—	—
Mother	3.5 (2.4 to 5.1)	2.4 (1.5 to 3.9)	7.1 (4.4 to 11.4)
Aunt	1.5 (1.1 to 2.1)	2.2 (1.6 to 3.0)	2.1 (1.4 to 3.2)
Excluding relatives of case patients carrying a BRCA1 or BRCA2 mutation			
Sister	5.7 (2.3 to 14.1)	—	—
Mother	3.1 (2.1 to 4.7)	2.1 (1.2 to 3.6)	6.5 (3.8 to 11.1)
Aunt	1.5 (1.0 to 2.1)	2.1 (1.5 to 2.9)	2.1 (1.5 to 3.3)
Case patient 40–49 y at diagnosis			
Sister	2.3 (0.9 to 5.6)	—	—
Mother	2.9 (1.6 to 5.4)	3.1 (1.7 to 5.7)	2.5 (1.2 to 5.3)
Aunt	1.8 (1.1 to 2.8)	1.9 (1.2 to 3.0)	2.2 (1.3 to 3.6)
Case patient 50–59 y at diagnosis			
Sister	1.4 (0.6 to 3.4)	1.9 (0.7 to 5.1)	2.5 (0.4 to 17.8)
Mother	1.5 (0.6 to 3.5)	1.5 (0.6 to 3.7)	4.9 (2.9 to 8.2)
Aunt	1.2 (0.7 to 2.2)	1.8 (1.1 to 2.9)	2.2 (1.4 to 3.6)

*— = insufficient information to estimate risk from this age.

DISCUSSION

We have confirmed that the breast cancer risk in a female relative of a woman recently diagnosed with breast cancer is considerably higher if that woman was diagnosed when she was younger, especially if another first-degree relative was affected. This risk was strikingly increased if the woman was diagnosed when younger than 40 years, where it was greater in sisters than in mothers and also evident in aunts. We found that only a small amount of this familial risk associated with early-onset breast cancer was explained by currently detectable mutations in BRCA1 and BRCA2, despite their popular description as early-onset breast cancer genes. An earlier statistical modeling analysis of self-reported data (from the Cancer and Steroid Hormone Study) of women who were diagnosed when they were younger than 55 years of age, also concluded that relatives of "pre-

Table 5. Estimated hazard ratios (HRs) with 95% confidence intervals (CIs) from Cox proportional hazards multiple regression modeling of risk of breast cancer for relatives of case patients younger than 40 years at diagnosis

	HR (95% CI)	
	All	Excluding relatives of BRCA1 and BRCA2 mutation carriers
Sisters		
One or more other affected first-degree relatives (yes/no)*	13.4 (6.2 to 29.0)	10.7 (4.2 to 26.8)
Youngest age at diagnosis of any affected first-degree relative(s)†	0.93 (0.88 to 0.98)	0.92 (0.85 to 1.01)
Mothers		
One or more other affected first-degree relatives (yes/no)*	2.1 (1.2 to 3.9)	1.1 (0.5 to 2.6)
Youngest age at diagnosis of any affected first-degree relative(s)†	0.99 (0.95 to 1.02)	1.01 (0.98 to 1.05)
Aunts		
At least one affected first-degree relative (yes/no)*	5.0 (2.8 to 8.7)	4.2 (2.2 to 8.1)
Youngest age at diagnosis of any affected first-degree relative(s)†	0.96 (0.93 to 0.99)	0.96 (0.93 to 1.00)

*Effect of having at least one affected first-degree relative, other than the case patient.

†Effect for each year of age at diagnosis minus 40 years, for the youngest affected first-degree relative, other than the case patient.

dicted" noncarriers were at increased risk *(19).* In that study, no mutation testing was carried out on any subjects with mutation status "predicted" by family history of breast and ovarian cancer.

There could be many reasons for the unexplained familial risks associated with early-onset breast cancer. Our study found higher risks of breast cancer for sisters than for mothers of early-onset case patients. This observation would be anticipated if there were recessively inherited risk factors segregating within these families *(20).* Indeed, our segregation analyses of these data supported a model that invoked a very highly penetrant, recessively inherited genetic effect, with virtually complete pen-

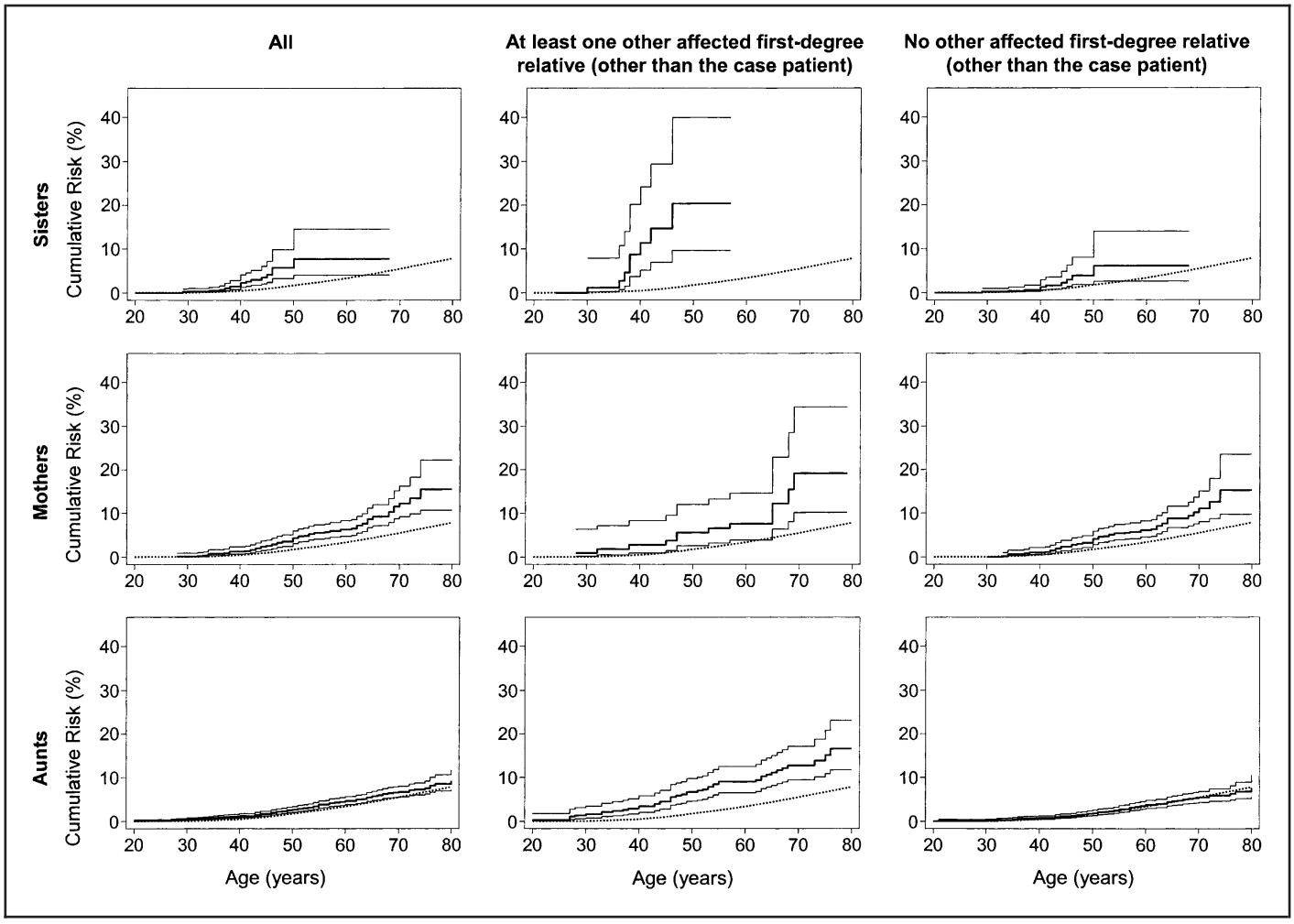

Fig. 2. Cumulative risk (%) and 95% confidence intervals (CIs) of breast cancer in sisters, mother, and aunts of case patients who were diagnosed with breast cancer when younger than 40 years and were not identified as carriers of a BRCA1 or BRCA2 mutation for all, for those with another affected first-degree relative (other than the case patient), and for those without an affected first-degree relative (other than the case patient). **Bold line** = cumulative risk; **thin line** = 95% CIs; **dotted line** = cumulative risk in the population.

etrance by age 50 years, as well as dominantly inherited genetic effects other than BRCA1 and BRCA2 mutations *(21).* Other models are plausible, and even our two-loci segregation analyses of three-generation data cannot definitively differentiate between all alternatives. For example, we could not rule out polygenic effects on a background of major gene effects that, if they existed, would be more plausible if recessively inherited than if dominantly inherited. A similar segregation analysis of population-based U.K. families also provided evidence for a recessively inherited genetic effect but of lower penetrance *(22).* Those families were ascertained through case patients younger than 55 years at diagnosis, with only a small proportion diagnosed at younger than 40 years. The different results for recessive inheritance, therefore, may be explained by the rapid attenuation we observed in the increased risk to sisters with increasing age at diagnosis of the case patient. Other explanations are polygenic effects, as proposed by Pharoah et al. *(23),* or combinations of synergistic genes influencing age at diagnosis, as proposed by Peto and Mack *(24).* The effects of environmental or lifestyle factors correlated in relatives is not likely to explain much familial aggregation *(4),* especially not the very high risks we found in multiple-case early-onset families.

A strength of our study is the quality of its family history data. Unlike case–control studies that have relied on unverified self-reports of cancers in primarily first-degree relatives, we have tried to systematically approach all living parents, siblings, aunts, and grandparents of both case patients and control subjects to obtain cancer histories for them and their relatives *(6–8).* For each relative, even if deceased or unavailable, we obtained one or more reports from close relatives. Reports on breast cancer in first-degree relatives have been shown to be reliable *(25).* We have sought to verify all reports of any cancer in relatives by use of state cancer registries and death certificates *(7).* We also found that the risk to relatives of control subjects compared well with the recent population cumulative incidence, especially for the relatives of the younger control subjects and across age groups where these population incidences may be appropriate. Under-reporting may have existed for relatives of control subjects aged 60 years or older when the control subject was 40 years or older. This may have been because 40% of the mothers and aunts of the latter group of control subjects were deceased compared with less than 15% of the mothers and aunts of the younger control subjects in the former group (data not shown). This under-reporting may not apply to relatives of case patients, because they might be more aware of any family history of breast cancer, but if it did, we would have underestimated the absolute risk and, therefore, the increase in familial risks at the older ages.

A limitation of our study could arise if Australian women with a family history of breast cancer were more likely to undergo mammography, as may be true for U.S. women with a family history of breast cancer *(26).* Such screening is more likely to detect small tumors and to detect them earlier. The key subjects of our analyses are the relatives of the newly diagnosed case patients. The subset of sisters at high risk (Fig. 2) had an affected first-degree relative diagnosed before the case patient was diagnosed, so that some portion of their increased incidence of breast cancer may be attributed to a greater rate of screening generated by their pre-existing family history. The same line of reasoning, however, would apply to the subsets of mothers and aunts at high risk (Fig. 2), who by definition also have an additional affected first-degree relative. The effect on incidence of breast cancer attributed to having an additional affected first-degree relative is higher in aunts than in mothers, although both are in the same birth cohort (*see* Table 5). Note also that, within these subsets, the mothers have at least two affected first-degree relatives, but the aunts may only have one (because the case patient is their second-degree relative). That is, the influence on risk of having another affected first-degree relative is not as strong in mothers as it is in aunts (Fig. 2); thus, a pre-existing family history of breast cancer may not be having a strong effect on incidence through any association with increased mammography.

We may have failed to identify some BRCA1 and BRCA2 mutation carriers among the early-onset case patients, even though we subjected the DNA of more than 90% of them to a protein truncation test covering at least two-thirds of the coding region *(11)* and tested for the three Ashkenazi founder mutations and a duplication in exon 13. In addition, 147 DNA samples were manually sequenced to detect coding region mutations in BRCA1 and 72 were manually sequenced to detect coding region mutations in BRCA2, with selection based on family history of cancer. Therefore, another 5–10 mutation carriers may have been found if the full range of testing was extended to all early-onset case patients. However, it is unlikely that many, if any, would be in the subsets of relatives at higher risk (Fig. 2), because these case patient families have at least two other affected first- or second-degree relatives and thus have been subjected to the most extensive mutation testing. Although a high-risk BRCA1 or BRCA2 mutation could be responsible for the family history of breast cancer, yet not be carried by the case patient, this is unlikely given the early onset of disease in the tested subject. Although we may have missed mutation carriers among the case patients who have none or at most one affected relative, which would explain a part of the small residual increased risk evident in Fig. 2 for the sisters, mothers, and aunts whose only affected first-degree relative was the case patient, these missed carrier families would not have explained the large residual increased risk evident in Fig. 2 for the sisters, mothers, and aunts who had another affected first-degree relative in addition to the case patient.

Our findings may appear to contradict those of the Breast Cancer Linkage Consortium (BCLC), in which the vast majority of families with the worst histories of breast and ovarian cancer now appear to carry BRCA1 or BRCA2 mutations *(5).* Although those families were selected for breast cancers diagnosed earlier than average, they do not have a large proportion of members with the early-onset disease on which the key findings of our study are based [e.g., only 43% were younger than 45 years at diagnosis *(27)*]. Furthermore, the selection of these families for study was based on the expectation that the familial clustering of disease was being caused by an underlying dominantly inherited risk. The highly penetrant recessively inherited risk that we estimated from a two-loci segregation model *(21)* predicts that all homozygotes will develop breast cancer by 50 years of age; if that is true, then only a few BCLC families would segregate such a recessively inherited risk. Given our data and the lack of success at finding other dominantly inherited loci *(28),* closer examination of recessive models is now justified, particularly for family data from women with premenopausal breast cancer. We have found a subset of relatives at substantial risk yet unlikely to carry a high-risk germline mutation in BRCA1 or BRCA2: the

mothers, aunts, and especially sisters who have at least one first-degree relative with breast cancer in addition to the case patient. Replication of our results in large population-based series of similar families with one or more cases of early-onset breast cancer, such as those being accrued by other sites involved with the National Institutes of Health-funded Breast Cancer Family Registries, would add impetus to this research.

In summary, familial and genetic aspects of susceptibility for breast cancer include more genes than BRCA1 and BRCA2, especially for disease in women diagnosed when younger than 40 years *(19,24).* Even with extensive mutation testing, we have found that only 5%–10% of breast cancers diagnosed in women younger than 40 years can be attributed to mutations in these genes, similar to reports by others *(29).* Although uncommon, breast cancer in young women is important because of the number of productive adult years of life lost. There may be other, as yet undiscovered, major genes *(22),* and mutations in these genes confer a recessively or dominantly inherited high risk *(21).* Large studies focused on rare families with multiple cases of breast cancer diagnosed in women aged 20–39 years may be necessary to discover these major genes and, to be successful, this effort is likely to require international collaborations.

REFERENCES

(1) Claus EB, Risch NJ, Thompson WD. Age at onset as an indicator of familial risk of breast cancer. Am J Epidemiol 1990;131:961–72.

(2) Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. Lancet 2001;358:1389–99.

(3) Peto J. Genetic predisposition to cancer. In: Cairns J, Lyon JL, Skolnik M, editors. Cancer incidence in defined populations. Banbury Report No. 4. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 1980. p. 203–13.

(4) Hopper JL, Carlin JB. Familial aggregation of a disease consequent upon correlation between relatives in a risk factor measured on a continuous scale. Am J Epidemiol 1992;136:1138–47.

(5) Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet 1998;62:676–89.

(6) Hopper JL, Giles GG, McCredie MR, Boyle P. Background, rationale and protocol for a case–control-family study of breast cancer. Breast 1994;3: 79–86.

(7) McCredie MR, Dite GS, Giles GG, Hopper JL. Breast cancer in Australian women under the age of 40. Cancer Causes Control 1998;9:189–98.

(8) Hopper JL, Chenevix-Trench G, Jolley D, Dite GS, Jenkins MA, Venter DJ, et al. Design and analysis issues in a population-based, case–control-family study of the genetic epidemiology of breast cancer and the Cooperative Family Registry for Breast Cancer Studies (CFRBCS). Monogr Natl Cancer Inst 1999;26:95–100.

(9) Giles GG, Farrugia H, Silver B, Staples M. Cancer in Victoria 1982–1987. Melbourne (Australia): Anti-Cancer Council of Victoria; 1992.

(10) Southey MC, Batten LE, McCredie MR, Giles GG, Dite G, Hopper JL, et al. Estrogen receptor polymorphism at codon 325 and risk of breast cancer in women before age 40. J Natl Cancer Inst 1998;90:532–6.

(11) Hopper JL, Southey MC, Dite GS, Jolley DJ, Giles GG, McCredie MR, et al. Population-based estimate of the average age-specific cumulative risk of breast cancer for a defined set of protein truncating mutations in BRCA1 and BRCA2. Cancer Epidemiol Biomarkers Prev 1999;8:741–7.

(12) Southey MC, Tesoriero AA, Andersen CK, Jennings KM, Brown SM, Dite GS, et al. BRCA1 mutations and other sequence variants in a population-based sample of Australian women with breast cancer. Br J Cancer 1999; 79:34–9.

(13) Armes JE, Egan AJ, Southey MC, Dite GS, McCredie MR, Giles GG, et al. The histologic phenotypes of breast carcinoma occurring before age 40 in women with and without BRCA1 or BRCA2 germline mutations: a population-based study. Cancer 1998;83:2335–45.

(14) Leong T, Whitty J, Keilar M, Mifsud S, Venter DJ, McKay M, et al. Mutation analysis of BRCA1 and BRCA2 cancer predisposition genes in radiation hypersensitive cancer patients. Int J Radiat Oncol Biol Phys 2000; 48:959–65.

(15) Puget N, Sinilnkova OM, Stoppa-Lyonnet D, Audoynaud C, Pages S, Lynch HT, et al. An Alu-mediated 6-kb duplication in the BRCA1 gene: a new founder mutation? Am J Hum Genet 1999;64:300–2.

(16) Kricker A, Jelfs P. Breast cancer in Australian women 1921–1994. Cancer Series No. 6. Canberra (Australia): Australian Institute of Health and Welfare; 1996.

(17) Cleveland W. Visualising data. Summit (NJ): Hobart Press; 1993.

(18) STATA statistical software: release 7.0. College Station (TX): Stata Corporation; 2001.

(19) Claus EB, Schildkraut J, Ivesen ES, Berry D, Parmigiani G. Effect of BRCA1 and BRCA2 on the association between breast cancer risk and family history. J Natl Cancer Inst 1998;90:1824–9.

(20) Risch N. Linkage strategies for genetically complex traits. I. Multi-locus models. Am J Hum Genet 1990;46:222–8.

(21) Cui J, Antoniou AC, Dite GS, Southey MC, Venter DJ, Easton DF, et al. After BRCA1 and BRCA2-what next? Multifactorial segregation analyses of three-generation, population-based Australian families affected by female breast cancer. Am J Hum Genet 2001;68:420–31.

(22) Antoniou AC, Pharoah PD, McMullan G, Day NE, Stratton MR, Peto J, et al. A comprehensive model for familial breast cancer incorporating BRCA1, BRCA2 and other genes. Br J Cancer 2002;86:76–83.

(23) Pharoah PD, Antoniou A, Bobrow M, Zimmern R, Easton DF, Ponder BA. Polygenic susceptibility to breast cancer and implications for prevention. Nat Genet 2002;31:33–6.

(24) Peto J, Mack TM. High constant incidence in twins and other relatives of women with breast cancer. Nat Genet 2000;26:411–4.

(25) Theis B, Boyd N, Lockwood G, Tritchler D. Accuracy of family cancer history in breast cancer patients. Eur J Cancer Prev 1994;3:321–7.

(26) Murabito JM, Evans JC, Larson MG, Kreger BE, Splansky GL, Freund KM, et al. Family breast cancer history and mammography: Framingham Offspring Study. Am J Epidemiol 2001;154:916–23.

(27) Easton DF, Bishop DT, Ford D, Crockford GP. Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. The Breast Cancer Linkage Consortium. Am J Hum Genet 1993;52:678–701.

(28) Thompson D, Szabo CI, Mangion J, Oldenburg RA, Odefrey F, Seal S, et al. Evaluation of linkage of breast cancer to the putative BRCA3 locus on chromosome 13q21 in 128 multiple case families from the Breast Cancer Linkage Consortium. Proc Natl Acad Sci U S A 2002;99:827–31.

(29) Peto J, Collins N, Barfoot R, Seal S, Warren W, Rahman N, et al. Prevalence of BRCA1 and BRCA2 gene mutations in patients with early-onset breast cancer. J Natl Cancer Inst 1999;91:943–9.

NOTES

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