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*[1]

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Abstract

The average breast cancer risk for carriers of a germ-line mutation in *BRCA1* or *BRCA2* (penetrance) has been estimated from the multiple-case families collected by the Breast Cancer Linkage Consortium (BCLC) to be ~80% to age 70. However, women now being tested for these mutations do not necessarily have the intense family history of the BCLC families. Testing for protein-truncating mutations in exons 2, 11, and 20 of *BRCA1* and exons 10 and 11 of *BRCA2* was conducted in a population-based sample of 388 Australian women with breast cancer diagnosed before age 40. Onset of breast cancer was analyzed in the known and potential mutation-carrying first- and second-degree female relatives of cases found to carry a mutation. Of the 18 mutation-carrying cases (9 *BRCA1* and 9 *BRCA2*), only 5 (1 *BRCA1* and 4 *BRCA2*) had at least one affected relative, so family history of breast cancer was not a strong predictor of mutation status in this setting. The risk in mutation carriers was, on average, 9 times the population risk [95% confidence interval (CI), 4–23; $P < 0.001$]. Penetrance to age 70 was 40% (95% CI, 15–65%), about half that estimated from BCLC families. By extrapolation, ~6% (95% CI, 2–20%) of breast cancer before age 40 may be caused by protein-truncating mutations in *BRCA1* or *BRCA2*. Breast cancer risk in *BRCA1* or *BRCA2* mutation carriers may be modified by other genetic or environmental factors. Genetic counselors may need to take into account the family history of the consultand.

Introduction

It is important to know the age-specific risk of breast cancer (penetrance) for women who have inherited a deleterious mutation in either of the recently discovered genes *BRCA1* or *BRCA2*. Testing for mutations in these genes has become widespread, both in the clinical and research settings, and women are being counseled on preventive and management options. Counseling has been based mostly on information derived from analyses of multiple-case families of the BCLC[3] (1, 2). To date, the best estimates of breast cancer risk to age 70 in mutation carriers from this source are 71% (95% CI, 53–82%) for *BRCA1* (3) and 80% (95% CI, 29–98%) or 84% (95% CI, 43–95%) for *BRCA2* (4, 5). However, women now being tested for mutations in *BRCA1* or *BRCA2*, even if seen in a cancer family clinic, do not necessarily have the same intensity of family history as do those in the BCLC families. It may not be appropriate to infer that the BCLC estimates apply to them.

In 1993, upon obtaining the first estimate of penetrance for *BRCA1* mutations from a linkage analysis of the BCLC families, Easton *et al*. (1) raised the possibility that there are mutations "which confer much lower cancer risks," and noted that "such mutations would not, in general, give rise to heavily loaded families." That is, penetrance may differ between mutations or within carriers of a given mutation, according to their genetic or environmental profile.

Here, we present a new risk estimate derived from a population-based sample of breast cancer cases, as distinct from "high-risk families." We have estimated risk for a defined set of mutations, averaged over their observed frequency in women recently diagnosed with breast cancer before the age of 40, systematically sampled from the cancer registries of Victoria and New South Wales, Australia. Estimation is based on the incidence of breast cancer in relatives of cases found to carry a germ-line protein-truncating mutation in one of the tested exons, which cover about two-thirds of the coding regions of *BRCA1* and *BRCA2*. The mutation status of some but not all relatives was known from DNA testing, and this was taken into account in the analysis.

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[3] The abbreviations used are: BCLC, Breast Cancer Linkage Consortium; CI, confidence interval; ABCFS, Australian Breast Cancer Family Study; PTT, protein truncation test; nt, nucleotide(s).

Materials and Methods

Subjects. A population-based case-control family study of early-onset breast cancer, the ABCFS, was carried out in Melbourne and Sydney from 1992 to 1995 (6, 7). Cases were women aged under 40 years at diagnosis of an incident, histologically confirmed, first primary invasive breast cancer identified through the cancer registries of Victoria and New South Wales, Australia. These are population-based registries for which notification is compulsory under state legislation, and registration is considered to be virtually complete. Cases were interviewed face to face, and a detailed cancer history was recorded for all adult first- and second-degree relatives and subsequently checked with relatives at interview, usually by telephone. Verification of every cancer reported by cases or relatives was sought through cancer registries, pathology reports, hospital records, treating clinicians, and death certificates (7). A relative was considered to be unaffected with a particular cancer if he or she was reported not to have had that cancer or if a reported cancer was subsequently shown not to be cancer. Blood samples were sought from cases, sisters, and selected relatives. Blood was not collected from all relatives, especially in families without a history of breast cancer. A total of 467 cases (response rate, 73%) were interviewed (7), and blood samples were obtained from 388 (90% of interviewed cases with and 82% without an affected first-degree relative; 60% of all eligible cases). There were no differences between cases from whom blood was or was not collected in terms of age, country of birth, or any of the measured risk factors (8). Ethical approval for the study was obtained from the Institutional Ethics Committees of The University of Melbourne, The Anti-Cancer Council of Victoria, and The New South Wales Cancer Council.

PTT. Blood was collected, buffy coat-prepared, and DNA-extracted as described previously (8). The PTT approach was a modification of that described by Roest *et al.* (9), using 10 ng of DNA as template. Briefly, exon 11 of *BRCA1* and exon 11 of *BRCA2* were amplified in three segments, and exon 10 of *BRCA2* was PCR-amplified in one fragment. Oligonucleotide primer sets were as follows: *BRCA1* exon 11 (789–2219 nt), 5′-GGA TCC TAA TAC GAC TCA CTA TAG GGA GAC CAC CAT GGC TGC TTG TGA ATT TTC TGA G (Seg 2 T7) and 5′-CAG CTC TGG GAA AGT ATC GCT G (Seg 2 reverse; Ref. 10); *BRCA1* exon 11 (1921–3662 nt), 5′-GCT AAT ACG ACT CAC TAT AGG AAC AGA CCA CCA TGG ACA ATT CAA AAG CAC CTA AAA AG (Seg 3 T7) and 5′-AC GCT TTT GCT AAA AAC AGC AG (Seg 3 reverse; Ref. 11); *BRCA1* exon 11 (3061–4215 nt), 5′-GCT AAT ACG ACT CAC TAT AGG AAC AGA CCA CCA TGG CAC CAC TTT TTC CCA TCA AGT C-3′ (Seg 4 T7) and 5′-GTG CTC CCA AAA GCA TAA A-3′ (Seg 4 reverse; Ref. 11); *BRCA2* exon 10, 5′-TCC TAA TAC GAC TCA CTA TAG GAA CAG ACC ACC ATG GGA TTT GGA AAA ACA TCA GGG (T7) and 5′-AAA CAC AGA AGG AAT CGT CAT C (reverse); *BRCA2* exon 11 (2139–3600 nt), 5′-ATC CTA ATA CGA CTC ACT ATA GGA ACA GAC CAC CAT GTT ATT GCA TTC TTC TGT GAA AAG A (Seg 2 T7) and 5′-CTG ACT TCC TGA TTC TTC TAA T (Seg 2 reverse); *BRCA2* exon 11 (3491–5332 nt), 5′-TCC TAA TAC GAC TCA CTA TAG GAA CAG ACC ACC ATG GCT CAG ATG TTA TTT TCC AAG CA (Seg 3 T7) and 5′-GTT GAC CAT CAA ATA TTC CTT C (Seg 3 reverse); and *BRCA2* exon 11 (5230–7268 nt), 5′-TCC TAA TAC GAC TCA CTA TAG GAA CAG ACC ACC ATG GCC TTA GCT TTT TAC ACA AGT TG (Seg 4 T7) and 5′ CAC TAA GAT AAG GGG CTC T (Seg 4 reverse).

PCR fragments were *in vitro* transcribed and translated using a TNT kit (Promega, Madison, WI). A luciferase control and a negative control that did not contain a truncating mutation in any of the test exons (as assessed by sequencing) were routinely used. Reactions were analyzed via 14% SDS-PAGE on a mini-protean II apparatus (Bio-Rad, Hercules, CA). Dried gels were exposed to overnight autoradiography, enhanced by Amplify fluorographic reagents (Amersham, Buckinghamshire, United Kingdom). Exons 2 and 20 of *BRCA1* were PCR amplified using primers described by Simard *et al.* (10). Heteroduplex analysis was used to screen for sequence variants. Samples displaying truncated protein products or heteroduplex variants were cycle-sequenced (Perkin-Elmer Corp., Norwalk, CT) to confirm mutations (12).

Statistical Methods. The average age-specific cumulative risk of breast cancer was estimated from the ages at interview or death and ages at onset of breast cancer, in the known or potential mutation-carrying adult female first- or second-degree relatives of mutation-carrying cases. Relatives shown by DNA testing not to carry the family-specific mutation or not to be genetically related to mutation carriers (*e.g.*, because they were on the side of the family shown by DNA testing not to carry the family-specific mutation) were excluded from analysis, as were those for whom cancer status was not known (*e.g.*, family C502, seven aunts living overseas; family B481, an unknown number of maternal aunts for whom contact was lost during World War II).

The age-specific cumulative risk was estimated using a repeated sampling method and by maximum likelihood. The first method involved enumerating, for each family, all possible combinations of known and potential mutation-carrying relatives. For each such combination of relatives, the probability of occurrence conditional on all observed genotype and phenotype information for that family was calculated, assuming that all affected relatives were carriers (*i.e.*, no phenocopies), the mother in family B770 had breast cancer, and there was no nonpaternity. [We identified one instance of nonpaternity, which led to that family (C951) becoming uninformative.] Random samples were drawn, one from each family, based on the above probabilities, and pooled to form a pseudocohort of mutation carriers. Observation time was defined as age at onset for the affected relatives; otherwise the observation time was censored to age at interview or age at death. A Kaplan-Meier curve for the time to onset of breast cancer was estimated using S-Plus (13) and inverted to give an empirical cumulative risk step-function. Steps in this function were possible only at the observed ages of onset among the relatives. Bootstrap samples of size 100 were taken for each pooled sample to obtain a 95% envelope for the estimated cumulative risk function. This process was repeated 1000 times. Average risk function was estimated as the median of the 100,000 empirical risk functions, and the 95% CI envelope estimated using the 2.5 and 97.5 percentiles.

Maximum likelihood estimation was performed using the statistical package MENDEL (14). In this modified segregation analysis, the joint likelihood of each family was expressed as a function of the observed disease status, age at interview, death or diagnosis, and genotype (known heterozygote or unknown) of family members, conditional on the population-sampled case (proband) being a known heterozygote diagnosed before the age of 40. The relative hazard rate (*i.e.*, the risk of breast cancer in mutation carriers relative to that for women in the Australian population; Ref. 15) was estimated on the logarithmic scale, separately for each of five decades of age, as in Ford *et al.* (5). We also fitted a

Table 1 Protein-truncating mutations in *BRCA1* and *BRCA2* detected in cases and disease and carrier status of female relatives and fathers

Family	Mutation	Age at onset (yr)	Female relatives (Relationship[a]/observation time[b]/carrier status[c])				Carrier status of father
			Affected		Unaffected		
A. *BRCA1*							
C832	1806 C→T stop	28	PG/43/o	M/60/o PA/62/o	MA/61/o PA/64/o	PA/61/o MG/51/o	o
C642	3818 del GTAAA	37	None	M/65/− PG/64/o	S/37/−	PA/66/o	(+)
C413	3273 del A	38	None	M/72/−	S/44/+	PG/82/o	+
C613	2800 del AA	26	None	M/63/−	PG/90/o		+
C521	3415 del C	34	None	M/64/o MA/60/o MG/59/o	S/31/o MA/68/o PG/80/o	MA/58/o PA/66/o	o
C312	4184 del TCAA	35	None	M/70/o MA/69/o	S/45/o MA/59/o	MA/60/o MG/49/o	o
B691	3450 del CAAG	23	None	M/58/o PA/56/o PG/83/o	S/25/o MA/60/o	PA/54/o MG/70/o	o
C951	1876 del C	37	M/48/−		None		(+)
C042	188 del 11	28	?		?		?
B. *BRCA2*							
C271	6503 del T	36	M/65/+ MA/56/(+) MA/38/o	S/50/+[d]	S/42/−	MG/64/o	−
B741	6503 del T	33	M/54/+	S/32/−	MA/37/o	MG/78/o	−
B770	4113 del A	37	MA/53/o M/32/o[e]	MA/49/o	MG/96/o	PG/80/o	o
B481	6174 del T	39	M/59/o	PG/76/o			o
B261	6174 del T	29	None	M/68/o	MG/28/o		o
C401	6174 del T	32	None	M/56/−	S/31/+	PG/90/o	+
C971	4393 del T	36	None	M/58/o MA/61/o MG/76/o	S/34/o MA/63/o PG/83/o	MA/56/o MA/70/o	o
C002	5803 del ATTA	34	None	M/72/o PA/29/o PG/79/o	S/31/− PA/69/o	MA/56/o MG/88/o	o
C502	4856 del A	39	None	M/68/o S/34/− S/40/o PG/70/o	S/29/o S/36/+ S/43/−	S/33/o S/37/+ MG/80/o	o

[a] S, sister; M, mother; MA, maternal aunt; PA, paternal aunt; MG, maternal grandmother; PG, paternal grandmother.
[b] Observation time = age at onset, if affected, or age or age at death if unaffected.
[c] Carrier status: +, proven carrier, (+), obligate carrier, −, proven non-carrier, o, unknown carrier status.
[d] This woman later developed breast and ovarian cancer; see text.
[e] This woman was reported to have had cancer, but the site was not known, and the cancer was not verified. In all analyses, it has been assumed that she had breast cancer.

model in which the above relative risk was assumed to be a constant over all ages, and the likelihood ratio criterion was used to assess whether this provided a more parsimonious fit. The allele frequency was assumed to be 0.003, following the estimate of Claus *et al.* (16). (It was also assumed to be 0.010 and 0.001, and the estimates of both relative and absolute risk in carriers were shown to be insensitive to the allele frequency in this range.) Note that the selection of relatives for genetic typing based on family history does not invalidate the consistency of the maximum likelihood estimators (5).

Comparisons were made with published estimates of breast cancer risk in *BRCA1* and *BRCA2* carriers by transforming all penetrance estimates to the logistic scale (the CIs were reasonably symmetric on that scale, at least for ages of >50). An inverse variance weighted average and SE of the published *BRCA1* and *BRCA2* estimates was calculated and compared with our estimates of penetrance (in which there were equal numbers of *BRCA1* and *BRCA2* mutation carriers).

The proportion of breast cancer attributable to mutations was calculated as $(RR - 1) \times f/RR$ (17), where RR is the maximum likelihood estimated average hazard rate in carriers and f is the proportion of carriers among cases. A CI was calculated using the delta method (18). Exact CIs for estimates of the odds ratio of being a carrier for different family histories were calculated following the method of Mehta (19), and the significance for being different to unity based on Fisher's exact test. All CIs were computed at the 95% level.

Results

Table 1 shows that 15 different mutations were detected in 18 cases, 9 of which had been previously reported to the Breast Cancer Information Core.[4] Three cases, two of known Jewish

[4] http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/bic/index.html.

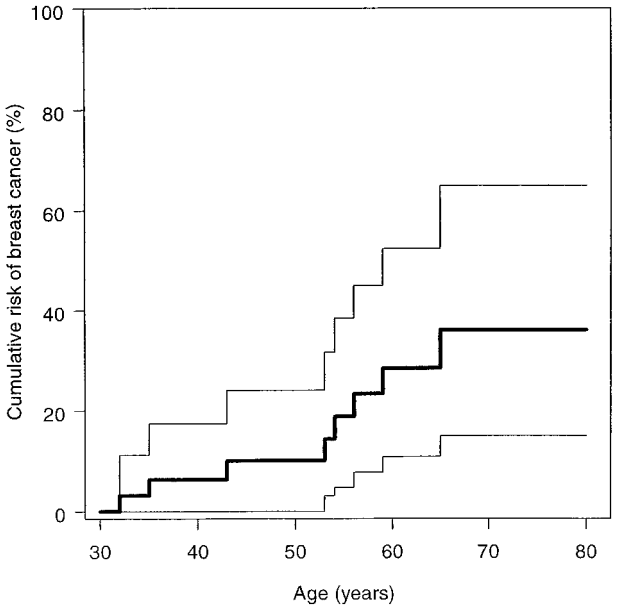

Fig. 1. Average age-specific cumulative risk of breast cancer (*bold line*), with 95% confidence limits (*thin lines*), for female carriers of a protein-truncating mutation in exon 2, 11, or 20 of *BRCA1* or exon 10 or 11 of *BRCA2*, estimated by repeated sampling.

descent, carried the 6174 del T *BRCA2* mutation. Mutation carriers did not differ from noncarriers in age (mean ± SE: 35 ± 0.2 years in noncarriers; 33 ± 5 in mutation carriers; and 32 ± 2 and 35 ± 1 for *BRCA1* and *BRCA2*, respectively; $P > 0.1$) or any factors shown to be associated with breast cancer in this study (7).

For two cases, one of whom was adopted (family C042), no potential mutation-carrying relatives were available. In family C951, analysis of DNA from both parents and a sister showed that the affected mother did not carry the mutation, and the biological father was not known (13). Of the remaining 16 cases with an informative family history (7 *BRCA1* and 9 *BRCA2*), only 5 (1 *BRCA1* and 4 *BRCA2*) had a known or potential mutation-carrying first- or second-degree relative with breast cancer; *i.e.*, ~30% (95% CI, 10–50%). In family C613, the maternal grandmother was affected, but the mutation was carried by the father and not evident in the maternal line.

In family C271, a deceased affected maternal aunt was an obligate carrier (two of her children were carriers). Two maternal uncles were diagnosed with bowel cancer in their late 50s and 60s, but no DNA was available. In family B770, the mother was reported to have died of an unknown cancer diagnosed in her early 30s, and a maternal aunt was reported to have had breast cancer. Neither cancer could be verified. In family B741, the mother was affected and shown to carry the same mutation. No cases of ovarian cancer were observed in any female relatives.

The nonbreast cancers in potential mutation-carrying relatives were: six bowel; two each of lung and stomach; one each of uterus, prostate, larynx, kidney, bile duct, and bone; and one non-Hodgkin's lymphoma.

Fig. 1 and Table 2 show that, using repeated sampling and assuming that all of the women in these families who had breast cancer were carriers (*i.e.*, no phenocopies) and that the mother in family B770 with unknown cancer had breast cancer, the estimated average risk of breast cancer in carriers reached a maximum of 36% (95% CI, 15–65%) at age 65.

Maximum likelihood analysis estimated the relative hazard rate in carriers compared with the population to be independent of age ($\chi_4^2 = 5.2$; $P > 0.2$). Under this model, the risk in mutation carriers was, on average, 9 (95% CI, 4–22) times that in the Australian population ($P < 0.001$), and the absolute risk to age 70 was estimated to be 40% (95% CI, 27–53%). If the relative hazard rate was allowed to vary by decade of age, the estimate was still 40%, and the 95% CI was 16–64%, very similar to that obtained from the repeated sampling analysis. If it was assumed the mother in B770 was not affected, the maximum likelihood estimated risk to age 70 became 34%.

Table 2 also shows that the risk estimates for *BRCA1* and *BRCA2* based on the BCLC multiple-case families were similar to one another. The weighted average of these risks were: 14% (95% CI, 4–37%) to age 40, 41% (28–56%) to age 50, 58% (44–71%) to age 60, and 73% (60–84%) to age 70. Therefore, our population-based estimates of penetrance for protein-truncating mutations in the tested exons were lower at ages 50, 60, and 70 ($P < 0.05$).

From Table 1, again assuming no phenocopies and that the mother in family B770 was an affected carrier, we anticipate that, on average, 7.5 of the mothers would be mutation carriers, and the average observation time, weighted by probability of being a carrier, was 61 years. The observed number affected was 4. For aunts, there were 10.25 carriers expected (average observation time = 57 years), of whom 3 were affected. For grandmothers, there were 7.5 carriers expected (average observation time = 73 years), of whom 1 was affected. For sisters, there were 8.5 carriers expected, including 5 known carriers (average observation time = 37 years), of whom none were affected. Overall, therefore, there were 33.75 carriers expected among the female relatives of case carriers, and of these, we observed 8 affected carriers. This is 5 times the 1.5 affected carriers expected under the population risks of 0.8% in sisters, 5% in mothers and aunts, and 7.5% in grandmothers ($P < 0.001$). If, as suggested by the BCLC estimates, the corresponding breast cancer risks were 10, 60, and 75%, respectively, we would have expected 17.1 affected carriers, more than twice as many as we observed.

Assuming that protein-truncating mutations are uniformly distributed across the coding regions and given that the exons examined cover about two-thirds of the coding regions of *BRCA1* and *BRCA2*, we estimate that ~7% (95% CI, 4–10%) of breast cancer in Australian women before the age of 40 is in a mutation carrier, and therefore, ~6% (95% CI, 2–20%) of cases can be attributed to protein-truncating mutations in *BRCA1* or *BRCA2*.

Discussion

This population-based study has found that the risk breast cancer for the mutations in *BRCA1* and *BRCA2* that are causing early-onset breast cancer in Australia is about one-half that for the mutations in these genes that are causing breast cancer in extended North American and Western European kinships with multiple cases of the disease. Our finding is consistent with other recently reported population-based studies, for the mutations that are causing breast cancer in young women in the United Kingdom (20), a founder mutation in *BRCA2* in Iceland (21), and the three Ashkenazi founder mutations (22). The "average risk" we estimated was weighted according to how often each mutation was observed and by the expected number of mutation-carrying relatives. Not only were the mutations

Table 2 Age-specific cumulative risk of breast cancer for the Australian population and estimated average risk for carriers of a *BRCA1* or *BRCA2* mutation

	Age (yr)			
	40	50	60	70
Australian population (Ref. 15)	0.8	2.5	4.9	7.5
ABCFS (*BRCA1* or *BRCA2*)				
Repeated sampling	6 (0–17)[a]	10 (0–24)	28 (11–52)	36 (15–65)
Maximum likelihood	8 (0–20)	10 (0–24)	31 (7–56)	40 (16–64)
BCLC families				
BRCA1 (Ref. 3)	18 (0–35)	49 (28–64)	64 (43–77)	71 (53–82)
BRCA2 (Ref. 5)	12 (0–24)	28 (9–44)	48 (22–65)	84 (43–95)

[a] Values are percentage risks; values in parentheses are 95% CIs.

protein-truncating and, therefore, presumed to be deleterious, but the cases were of early onset. Therefore, the mutations most likely to be in this set would be those with the highest risk at younger ages, and/or those more common in the population.

We found that a woman who carries a protein-truncating mutation in the tested exons of *BRCA1* or *BRCA2* is clearly at an increased risk of breast cancer, by a factor on the order of 10-fold. There was no evidence that this relative hazard rate was greater at younger ages, as suggested by the earliest estimate of risk in carriers (1), but this may be due to lack of statistical power. Under the best-fitting model, the probabilities that affected female relatives of unknown carrier status were carriers, as assumed in the repeated sampling analysis, were >0.9 and 0.8 for first- and second-degree relatives of a carrier.

The lifetime risk for a woman who carries a protein-truncating mutation in the tested exons of *BRCA1* or *BRCA2*, estimated from this study, was about one-half that estimated for the *BRCA1* and *BRCA2* mutations detected in the BCLC families. The distribution of the mutations we detected, in terms of type and position, is very similar to that reported in the multiple-case families. Although there is some evidence of different risks in mutations toward the 3′ end of *BRCA1* (23), this appears to related more to a greater risk of ovarian cancer rather than any difference in breast cancer risk.

What Explanations Are There for Our Population-based Estimates of Average Risk Being Less than Those Derived from Multiple-Case Families? The maximization of LOD score (or MOD) method, used for BCLC analyses of *BRCA1* and *BRCA2* (1–3, 5), is known to give consistent estimates of penetrance parameters provided the model is correctly specified. It does not, however, allow for sources of familial aggregation of breast cancer in the families other than the segregating disease alleles, and this might result in inflated genetic risk estimates. Both the methods used in this paper and the maximum LOD (MOD) score method used in the BCLC analyses give consistent estimates of penetrance, assuming that a single penetrance function applies to all carriers. Therefore, assuming that the difference in penetrance is not simply due to chance, there must be some variation in penetrance among carriers. In principle, this could be due to allelic heterogeneity in risk, with the more common mutations having, on average, a lower risk. One-third (6 of 18) of the carrier cases had mutations not previously reported to the Breast Cancer Information Core, and only one of these had a family history possibly associated with that mutation (family B770). On the other hand, of the cases carrying a mutation that had been observed previously, only one-third (4 of 12) had affected relatives who were known or potential carriers. Therefore, even for mutations reported previously, when detected in a population-based sample, the majority of case-carriers did not have a family history of breast cancer in their first- or second-degree relatives. Furthermore, the average risk estimated from these families alone was just 45% to age 70.

Table 3 Percentage of cases with a family history, by different definitions, comparing cases with and without a detected germ-line protein-truncating mutation in exon 2, 11, or 20 of *BRCA1* or exon 10 or 11 of *BRCA2*

Affected relative(s)	Mutation-carrier status of case		Odds ratio (95% CI)
	Carrier (n = 18)	Noncarrier (n = 370)	
Mother	28% (5)[a]	11% (41)	3.1 (0.8–9.8)
Sister	0% (0)	4% (14)	
Mother or sister	28% (5)	13% (48)	2.6 (0.7–8.1)
Second-degree only	6% (1)	19% (69)	0.3 (0.0–1.7)
First- or second-degree	33% (6)	31% (114)	1.1 (0.3–3.3)
More than one first- or second-degree on same side of family	11% (2)	5% (19)	2.3 (0.2–11)

[a] Values in parentheses represent n.

Genotype-specific risks may be more relevant for *BRCA2* mutations. There is evidence of a higher ovarian:breast cancer risk ratio for mutations between nt 3034 and 6629, the designated ovarian cancer cluster region (24). About 60% of the region we tested fell within the ovarian cancer cluster region. In addition to a higher risk of ovarian cancer, it is possible that mutations in this region are associated with a lower risk of breast cancer.

No ovarian cancers were observed in the first- or second-degree relatives of the mutation-carrying cases. This is somewhat surprising, given the high risks of ovarian cancer estimated by linkage studies (1, 3) and the prevalence of *BRCA1* mutations reported in unselected cases of ovarian cancer (25). This may be, in part, due to the fact that most ovarian cancers in *BRCA1* and *BRCA2* mutation carriers occur after the age of 50. In fact, it is now known that, since the time of study but prior to our determination of her *BRCA2* carrier status, the unaffected carrier in family C271 was diagnosed with both breast and ovarian cancer in her early 50s. (Recalculation of penetrance assuming she was affected, however, only increased the estimated risk to age 70 to 47%.) There is also the possibility that ovarian cancers may be reported as other malignancies. However, it might also suggest site-specific modification of risk. It is also of interest to note that age-specific ovarian cancer incidence rates are ~30% lower in Australia than in Western Europe or the United States (26).

A more important explanation for the observed differences in penetrance may be family-specific "triggers" that modify risk in mutation carriers. These could be modifier genes or nongenetic factors, some of which may be related to lifestyle. Large

systematic and prospective studies of carrier cohorts, such as those being assembled by the Cooperative Family Registry for Breast Cancer Studies[5] and the BCLC, will be needed to address these issues.

Summary. We have studied the mutations in *BRCA1* and *BRCA2* that cause breast cancer in young women. We have studied cases ascertained through complete population registries and obtained a high response rate. We have interviewed first- and second-degree relatives on both sides of the family. By collecting blood samples from some relatives, we have been better able to estimate probabilistically the mutation status of relatives than similar studies that have been restricted to first-degree relatives without blood samples. We have used an efficient parametric method of statistical analysis based on asymptotic likelihood theory and confirmed our findings by using a repeated sampling method. We have restricted attention to those mutations that, by their biological function, are considered to be deleterious. We have covered a large proportion of the coding region, including exons known to be the site of mutations that cause the extensive breast cancer kindreds of the BCLC and breast cancer in Ashkenazi women.

Given the high penetrance for mutations in *BRCA1* and *BRCA2* derived from the BCLC families, a high risk of breast cancer in our carriers would have been expected. That this does not appear to be the case, as is evident by the upper 95% CI shown in Fig. 1, raises many interesting questions. It suggests that whether a female mutation carrier will develop breast cancer and at what age could depend on other genetic and nongenetic factors. Identification of such factors is now a major issue in the genetic epidemiology of breast cancer, with prevention of breast cancer in mutation carriers the long-term aim.

Furthermore, in the setting of a population-based sample, the extent of family history typically observed does not appear to be a strong predictor of mutation-carrier status and has low sensitivity. Table 3 shows that having a mother with breast cancer only tripled the probability of being a carrier ($P = 0.05$). Because <10% of women with breast cancer carry a mutation in *BRCA1* or *BRCA2* (9, 27–29), only families with an extensive history of breast and/or ovarian cancer (*e.g.*, 3 or more affected relatives) have more than a 1 in 5 chance of carrying *BRCA1* or *BRCA2* mutation (27, 30).

As observed by us and others (20, 28, 29), a substantial proportion of mutation-carrying early-onset population-sampled cases do not have a family history of breast cancer. This suggests that the imprecise expressions “familial” and “sporadic” breast cancer, based on family history alone, yet often used to imply “genetic” and “nongenetic” susceptibility in cases, respectively, need to be reviewed (31). Accurate estimates of penetrance are critical for predictive genetic testing, which is now widespread in many countries. The results of this study suggest that counseling may need to take into account not only the mutation status but also the extent of family history of the consultand.

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[5] http://www-dceg.ims.nci.nih.gov/cfrbcs/.

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