

## BRCA1 MUTATIONS IN WOMEN ATTENDING CLINICS THAT EVALUATE THE RISK OF BREAST CANCER

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## ABSTRACT

**Background** To define the incidence of *BRCA1* mutations among patients seen in clinics that evaluate the risk of breast cancer, we analyzed DNA samples from women seen in this setting and constructed probability tables to provide estimates of the likelihood of finding a *BRCA1* mutation in individual families.

**Methods** Clinical information, family histories, and blood for DNA analysis were obtained from 263 women with breast cancer. Conformation-sensitive gel electrophoresis and DNA sequencing were used to identify *BRCA1* mutations.

**Results** *BRCA1* mutations were identified in 16 percent of women with a family history of breast cancer. Only 7 percent of women from families with a history of breast cancer but not ovarian cancer had *BRCA1* mutations. The rates were higher among women from families with a history of both breast and ovarian cancer. Among family members, an average age of less than 55 years at the diagnosis of breast cancer, the presence of ovarian cancer, the presence of breast and ovarian cancer in the same woman, and Ashkenazi Jewish ancestry were all associated with an increased risk of detecting a *BRCA1* mutation. No association was found between the presence of bilateral breast cancer or the number of breast cancers in a family and the detection of a *BRCA1* mutation, or between the position of the mutation in the *BRCA1* gene and the presence of ovarian cancer in a family.

**Conclusions** Among women with breast cancer and a family history of the disease, the percentage with *BRCA1* coding-region mutations is less than the 45 percent predicted by genetic-linkage analysis. These results suggest that even in a referral clinic specializing in screening women from high-risk families, the majority of tests for *BRCA1* mutations will be negative and therefore uninformative. (N Engl J Med 1997;336:1409-15.)

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19 percent of all cases of breast cancer.<sup>1,4</sup> Recently, two genes related to breast cancer (*BRCA1* and *BRCA2*) were identified.<sup>5-7</sup> Genetic-linkage studies of families with multiple members with breast or ovarian cancer, or both, suggest that a mutation in *BRCA1* accounts for 45 percent of hereditary cases of breast cancer and 80 to 90 percent of hereditary cases of combined breast and ovarian cancer.<sup>3,8</sup> These studies also suggest that women in families selected for linkage analysis who carry a *BRCA1* mutation have an 87 percent lifetime risk of breast cancer and a 44 percent lifetime risk of ovarian cancer.<sup>9</sup> Linkage studies suggest that 35 percent of high-risk families may have *BRCA2* mutations.<sup>10</sup>

*BRCA1* is a tumor-suppressor gene postulated to be important in regulating the growth of breast epithelial cells.<sup>11</sup> A study of 256 *BRCA1* mutations showed that the mutations were spread evenly across the entire gene.<sup>12</sup> The most commonly detected mutations are a deletion of adenine and guanine (185delAG) and an insertion of cytosine (5382insC), which have a cumulative frequency of 1.4 percent in the general Ashkenazi Jewish population.<sup>13,14</sup> Approximately 20 percent of Ashkenazi women with breast cancer who are under the age of 40 carry the 185delAG mutation.<sup>15</sup> In comparison, 10 percent of women with breast cancer who are under the age of 35 and who are not selected for testing on the basis of ethnic group or family history carry *BRCA1* mutations.<sup>16</sup> Mutations in noncoding regions of the gene may account for up to 20 percent of *BRCA1* mutations, but they are undetectable by any commercially available test.

Most studies have focused on women who were deliberately selected because they were members of large families with multiple members with breast and ovarian cancer. But such women represent only a fraction of the spectrum of patients who seek ad-

**F**AMILY history is a significant risk factor for the development of breast cancer. The relative lifetime risk of breast cancer ranges from 1.4 for a woman whose mother was given a diagnosis of breast cancer after the age of 60 to 15.0 for a woman with an inherited mutant *BRCA1* gene.<sup>1-3</sup> Breast cancer attributed to a family history of the disease has been reported to account for 6 to

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vice at clinics that evaluate the risk of breast cancer. We currently have no estimate of the incidence of detectable *BRCA1* mutations among women from families with few affected members. To address this issue, we analyzed DNA samples from 263 women with breast cancer who were seen in several breast-cancer clinics. Using these data, we calculated the probability of detecting a *BRCA1* coding-region mutation on the basis of the average age at diagnosis in the family, the presence of ovarian cancer and combined breast and ovarian cancer in a family member, and ethnic group.

## METHODS

### Patient Population

DNA samples from blood obtained from 263 unrelated women with breast cancer were analyzed for coding-region mutations in *BRCA1*. Of these, 169 women had been referred to breast-cancer clinics because of a familial risk factor for breast cancer. The remaining 94 women were identified in general-oncology practices because of the diagnosis of breast cancer before the age of 40; some of these women also had a family history of breast cancer. The 169 women with a family history reported 1 to 11 cases of breast cancer per family. Twenty-five families reported Ashkenazi Jewish ancestry. The women seen in the clinics had been excluded from linkage analysis because they had too few living affected relatives for the analysis to be informative for that purpose. Samples were consecutively collected between 1993 and 1995, with no specific recruitment or advertising strategy. The patients were either self-referred or referred by a physician, usually because of a concern about genetic risk factors. No patient refused to participate in the study.

All the women were informed that their DNA samples would be analyzed for *BRCA1* mutations; they were offered the opportunity to receive the results and asked to sign a second consent form if they chose to learn the results. They were informed of the possibility that testing could lead to loss of insurance, loss of employment, psychological distress, and family disruption, but that it could also identify those at risk, thus warranting increased surveillance or preventive options that might result in improved health care. All patients were told that the results would be kept in locked, coded research files and would not become part of their clinical records. Not all women chose to learn the test results, but all results were included in this analysis. To clarify the family histories of the women, we requested pathology reports for each affected member of each family, with the written consent of the family member.

### Mutation Analysis

We amplified the entire coding sequence and intron-exon boundaries of the *BRCA1* gene from each of the 263 DNA samples using the polymerase chain reaction (PCR) with 32 primer pairs. Primer sets for exons 2 to 24, excluding exon 4, and PCR mixtures have been previously described.<sup>17</sup> Exon 4 represents a variant exon not seen in the normal *BRCA1* messenger RNA and was not screened for mutations. All PCR assays were performed at an annealing temperature of 55°C. The PCR products of exon 11 ranged from 400 to 600 bp in length and overlapped by a minimum of 50 bp to ensure the detection of all sequence variants.

For conformation-sensitive gel electrophoresis, each PCR product was denatured by heating to 98°C for five minutes, followed by incubation at 68°C for one hour to generate heteroduplexes. Then 2  $\mu$ l of gel loading buffer (30 percent glycerol, 0.25 percent xylene cyanol, and bromophenol blue) was added to 4 to 8  $\mu$ l of each product, and the samples were loaded on acrylamide gels.

The 1-mm-thick 10 percent polyacrylamide gel contained acrylamide and 1,4-bis(acryloyl)piperazine (Fluka) cross-linker in a ratio of 99:1, 10 percent ethylene glycol, and 15 percent formamide in 0.5 $\times$  TRIS-aurine-EDTA buffer (1 $\times$  TRIS-aurine-EDTA contains 89 mM TRIS, 28.5 mM taurine, and 0.2 mM EDTA; pH 9.0).<sup>18</sup> The samples were subjected to electrophoresis at 400 V for 16 hours, and the gels were stained with ethidium bromide for 10 minutes. The PCR products were visualized by ultraviolet light and photographed.

### DNA Sequencing

Each variant exon was reamplified from the original genomic DNA to avoid the possibility of errors. Amplified exons were purified with PCR Select II (5' 3') purification columns and manually sequenced with a PCR sequencing kit (fmol, Promega) according to the manufacturer's instructions. Each fragment was sequenced in both directions with the original PCR primers.

### Statistical Analysis

We used univariate and multivariate analyses to examine possible associations between specific familial characteristics (phenotype) and the presence of a *BRCA1* mutation (genotype). We examined the following variables: unilateral breast cancer, bilateral breast cancer, ovarian cancer, combined breast and ovarian cancer, the number of women at risk in a family (those over 20 years of age), the average age at diagnosis of breast cancer, the average age at diagnosis of ovarian cancer, and Ashkenazi Jewish ancestry. All variables were analyzed both as ordinal or continuous variables and as categorical variables. Analyses for the number of unilateral breast cancers were initially performed by dichotomizing this variable at several points. The lowest point at which dichotomization of the number of familial cases of breast cancers was found to be statistically significantly associated with the presence of a *BRCA1* mutation was seven or more cases (vs. six or fewer); thus, this was the category entered into the multivariate analysis. The median number of bilateral breast cancers, ovarian cancers, and women with both breast and ovarian cancer, which was less than one for each of these variables (vs. one or more), was used for multivariate analysis. The average age at onset of breast cancer in each family was calculated by dividing this variable into 5-year age categories (<35, 35 to 39, 40 to 44, 45 to 49, 50 to 54, 55 to 59, and >59). In all cases, the lowest category of each variable was used as the reference range. In the construction of the multivariate model, all variables except the number of women at risk were treated as categorical to facilitate interpretation of the results in the clinical setting.

For univariate analyses, the Kruskal-Wallis chi-square approximation was chosen as the nonparametric measure of association; parametric analyses were performed by logistic regression. We first constructed a logistic model for unadjusted (univariate) associations between familial characteristics and *BRCA1* mutations, followed by a multivariate model, using a stepwise selection procedure. Variables for which the univariate chi-square approximation achieved a P value of 0.05 or less or that changed the results of univariate analyses for other variables from significant to non-significant were added to this model. The variables were added sequentially, with the variable associated with the largest chi-square approximation added first. With the addition of each new variable, variables were removed whose adjusted chi-square approximation achieved a P value exceeding 0.05. This method was used to identify the best-fitting model.

Predicted probability estimates were constructed with the regression coefficient estimates from the best-fitting logistic-regression model based on the stepwise selection criteria. These predicted probabilities, as well as confidence intervals, were computed for all permutations of the predictor variables. These calculations were performed with SAS statistical analysis software. In strata with no data points, regression coefficients were used to calculate the predicted probability estimates. No confidence intervals were available for these strata.

## RESULTS

## Patient Population

Of the 263 women in this study, 169 attended our clinics because of a familial risk factor for breast cancer. The remaining 94 women were seen primarily because they had been given a diagnosis of breast cancer before the age of 40 and were omitted from the analysis of familial breast cancer. There was an average of 4.0 breast cancers per family (median, 4.6; total, 660) among the 169 families with a history of breast cancer, and an average of 1.5 cases of ovarian cancer per family (median, 0.8; total, 68) in the 45 families with a history of both breast and ovarian cancer. Women with both breast and ovarian cancer were identified in 15 families. Bilateral breast cancer was reported in at least one woman in 57 families,

**TABLE 1.** DISTRIBUTION OF *BRCA1* MUTATIONS IN 169 FAMILIES WITH BREAST CANCER.

CASES OF CANCER	<i>BRCA1</i> MUTATIONS		
	NO. OF FAMILIES (N = 169)	NO. OF MUTATIONS	PERCENT OF TOTAL MUTATIONS*
<b>Breast</b>			
1	4	1	3.7
2	35	5	18.5
3	48	5	18.5
4	27	5	18.5
5	25	3	11.1
6	17	3	11.1
7	4	0	0
8	5	2	7.4
9	1	1	3.7
10	1	0	0
11	2	2	7.4
Total		27	
<b>Breast and ovarian</b>			
0	124	8	29.6
1	31	11	40.7
2	8	4	14.8
3	4	2	7.4
4	1	1	3.7
5	1	1	3.7
Total		27	
<b>Breast and ovarian in a single member</b>			
0	154	17	63.0
1	13	8	29.6
2	1	1	3.7
3	1	1	3.7
Total		27	
<b>Bilateral breast</b>			
0	112	17	63.0
1	43	9	33.3
2	9	0	0
3	3	1	3.7
4	2	0	0
Total		27	

\*Because of rounding, not all categories total 100 percent.

and the average age at diagnosis of breast cancer was 48 years in all 169 families. Table 1 gives the frequency of these diseases, and Table 2 shows the average age at diagnosis of breast cancer in families.

## Mutation Analysis

All testing for *BRCA1* germ-line mutations was performed in a single affected family member. However, since no new mutations in *BRCA1* have been identified to date, the probability analyses were based on the assumption that all family members with breast or ovarian cancer carried the mutation identified in the proband.

Of the 169 women with breast cancer and a familial risk factor, 27 (16 percent) had a *BRCA1* mutation (Fig. 1). We found no association between mutations at the 5' end of the gene and ovarian cancer, as has been previously suggested.<sup>11,19</sup> Mutations were identified in 12 of 94 women (13 percent) in whom breast cancer was diagnosed before the age of 40.

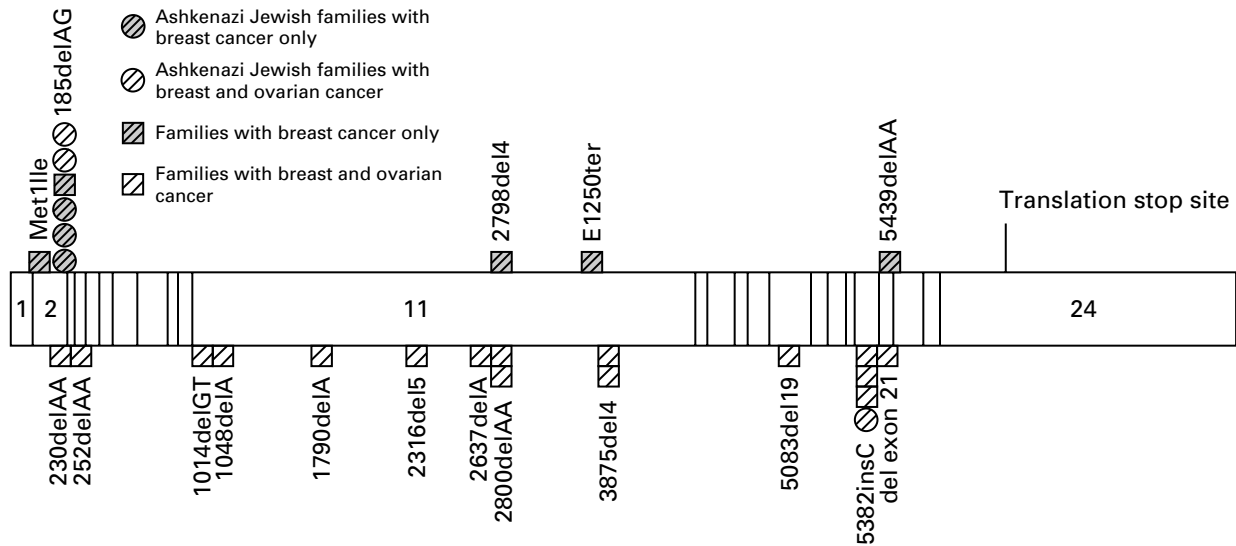
*BRCA1* mutations were identified in 9 of 124 families (7 percent) with members with breast cancer without ovarian cancer, 10 of 57 families (18 percent) with members with bilateral breast cancer, 18 of 45 families (40 percent) with members with both breast and ovarian cancer, and 10 of 15 families (67 percent) with a single member with both breast and ovarian cancer. The frequency of *BRCA1* mutations among Ashkenazi Jewish women was 26 percent; all mutations identified in this subgroup were either 185delAG or 5382insC. The median age at diagnosis of breast cancer was 41.0 years in families

**TABLE 2.** FREQUENCY OF *BRCA1* MUTATIONS ACCORDING TO THE AVERAGE AGE AT DIAGNOSIS OF BREAST CANCER.

AVERAGE AGE AT DIAGNOSIS OF BREAST CANCER (YR)*	<i>BRCA1</i> MUTATIONS		
	NO. OF FAMILIES	NO. OF MUTATIONS	PERCENT OF TOTAL MUTATIONS†
<35	5	1	3.7
35-39	27	7	25.9
40-44	32	5	18.5
45-49	24	5	18.5
50-54	34	4	14.8
55-59	24	1	3.7
>59	23	4	14.8
Total	169	27	99.9

\*The median age at diagnosis was 41.0 years in families with a *BRCA1* mutation and 50.7 years in families without a *BRCA1* mutation ( $P < 0.001$ ).

†Because of rounding, the total does not equal 100 percent.



**Figure 1.** Location and Tumor Specificity of the 27 *BRCA1* Mutations Identified in Families with Breast Cancer.

Exon 1 and the coding region of *BRCA1* are depicted, with exons 1, 2, 11, and 24 included for reference. The translation start site is located at the mutation Met11le.

with *BRCA1* mutations and 50.7 years in families without *BRCA1* mutations ( $P < 0.001$ ).

**Associations with *BRCA1* Mutations**

We evaluated specific factors in family members that have been associated with *BRCA1* mutations in previous studies. In both univariate and multivariate analyses, the diagnosis of breast cancer before the age of 55 ( $P = 0.004$ ), ovarian cancer ( $P < 0.001$ ), and breast and ovarian cancer in a single family member ( $P < 0.001$ ) significantly predicted the presence of a *BRCA1* mutation. In the univariate analysis Ashkenazi Jewish ancestry was not significantly associated with *BRCA1* mutations ( $P = 0.20$ ), but when age, ovarian cancer, breast and ovarian cancer in a single family member, and ethnic origin were all added to the same model, ethnic origin achieved statistical significance ( $P = 0.03$ ). When the analysis was adjusted for the number of women in the family who were over 20 years of age (a measure of family size), there was no significant association between the presence of a *BRCA1* mutation and the number of breast cancers in a family ( $P = 0.20$ ). Neither bilateral breast cancer ( $P = 0.90$ ) nor the average age at diagnosis of ovarian cancer ( $P = 0.10$ ) significantly predicted the presence of a *BRCA1* mutation.

**Predicted Probabilities**

We developed a model of predicted probability estimates based on the best-fit multivariate logistic regression, using the variables that were predictive of *BRCA1* mutations in the univariate analysis. The results of these analyses are presented in Table 3.

The optimal use of this table requires a detailed family history and knowledge of all cases of breast and ovarian cancer in the family. The predicted probabilities in this table are for families as a whole. The predicted probability of a *BRCA1* mutation in a woman with breast or ovarian cancer is equal to the probability for the family. For example, a woman with breast cancer who is from a family in which the average age at diagnosis of breast cancer was 40 to 44 years has a predicted probability of 7.7 percent (95 percent confidence interval, 3.6 to 15.6 percent) of having a detectable *BRCA1* mutation (Table 3). For an unaffected family member, the predicted probability is determined by the relationship to the affected family member. In the case of an unaffected sibling or child of a woman with breast or ovarian cancer, the predicted probability of a *BRCA1* mutation is half the probability for the family. For an unaffected grandchild, the predicted probability is 25 percent of the probability for the family.

**DISCUSSION**

Data based on genetic-linkage analysis of families suggest that 45 percent of all hereditary cases of breast cancer are associated with *BRCA1* mutations. However, in our series, only 16 percent of women with breast cancer and a family history of breast or ovarian cancer or both had detectable *BRCA1* mutations. This proportion is far lower than that predicted on the basis of these previously published data.<sup>3</sup> By virtue of its ability to detect known substitutions of single base pairs, we estimate that the method of identifying mutations that we used (con-

TABLE 3. PROBABILITY OF DETECTING A BRCA1 MUTATION IN FAMILIES.\*

AVERAGE AGE AT DIAGNOSIS OF BREAST CANCER (YR)	PREDICTED PERCENT PROBABILITY (95% CI)†	AVERAGE AGE AT DIAGNOSIS OF BREAST CANCER (YR)	PREDICTED PERCENT PROBABILITY (95% CI)†
<b>Families with breast cancer only</b>		<b>Ashkenazi Jewish families with breast cancer only</b>	
<35	17.4 (6.5–38.8)	<35	47.9
35–39	11.7 (5.1–24.6)	35–39	36.7 (12.8–69.6)
40–44	7.7 (3.6–15.6)	40–44	26.8 (9.7–55.3)
45–49	5.0 (2.3–10.8)	45–49	18.7 (6.8–42.0)
50–54	3.2 (1.2–8.1)	50–54	12.7 (4.3–31.8)
55–59	2.1 (0.6–6.5)	55–59	8.4 (2.5–24.8)
>59	1.3 (0.3–5.5)	>59	5.5 (1.3–20.0)
<b>Families with breast and ovarian cancer</b>		<b>Ashkenazi Jewish families with breast and ovarian cancer</b>	
<35	55.0 (27.2–80.0)	<35	84.2
35–39	43.5 (22.4–67.2)	35–39	77.1 (40.1–94.4)
40–44	32.7 (17.0–53.5)	40–44	67.9
45–49	23.4 (11.4–42.1)	45–49	57.2 (24.9–84.3)
50–54	16.2 (6.7–34.2)	50–54	45.7
55–59	10.8 (3.5–28.8)	55–59	34.7 (10.8–70.0)
>59	7.1 (1.7–24.8)	>59	25.1
<b>Families with breast and ovarian cancer in a single member</b>		<b>Ashkenazi Jewish families with breast and ovarian cancer in a single member</b>	
<35	77.0	<35	93.6
35–39	67.8 (37.1–88.3)	35–39	90.2
40–44	57.1 (28.4–81.7)	40–44	85.3
45–49	54.5	45–49	78.5
50–54	34.6 (12.1–67.0)	50–54	69.8
55–59	25.0	55–59	59.3
>59	17.3	>59	47.8
<b>Families with breast and ovarian cancer and 1 member with both breast and ovarian cancer</b>		<b>Ashkenazi Jewish families with breast and ovarian cancer and 1 member with both breast and ovarian cancer</b>	
<35	96.6	<35	98.8
35–39	92.4 (72.0–98.3)	35–39	96.8
40–44	88.5 (63.4–97.2)	40–44	98.1
45–49	82.9 (52.0–95.6)	45–49	95.5
50–54	75.4	50–54	93.0
55–59	65.9	55–59	89.4
>59	54.9	>59	81.3

\*The optimal use of this table requires a detailed family history and knowledge of all cases of breast and ovarian cancer in the family. The predicted probabilities are for families as a whole. The predicted probability of a *BRCA1* mutation in a woman with breast or ovarian cancer is equal to the probability for the family. For example, a woman with breast cancer who is from a family in which the average age at diagnosis of breast cancer was 40 to 44 years has a predicted probability of 7.7 percent (95 percent confidence interval, 3.6 to 15.6) of having a detectable *BRCA1* mutation. For an unaffected family member, the predicted probability is determined by the relationship to the affected family member. In the case of an unaffected sibling or child of a woman with breast or ovarian cancer, the predicted probability of a *BRCA1* mutation is half the probability for the family. For an unaffected grandchild, the predicted probability is 25 percent of the probability for the family.

†Confidence intervals (CI) could not be calculated for some entries because of the absence of a data point matching the criteria in the original data set.

formation-sensitive gel electrophoresis) is 95 to 99 percent sensitive. For this reason, the most likely explanation for the difference between our results and those previously reported is that the earlier studies selected large families with several cases of ovarian cancer. These families were actively sought for linkage studies because the presence of ovarian cancer significantly increases the likelihood of finding a *BRCA1* mutation in a family member.<sup>20-22</sup>

The population we studied is more representative of the kinds of patients seen in a referral clinic for the evaluation of the risk of breast cancer. Many families in our population were too small for us to predict the

presence of a *BRCA1* mutation from a pattern of inheritance in the family, and in most families the only relevant neoplasm was breast cancer. The relatively few cases of ovarian cancer in our study made the confidence intervals for some strata wide. Nonetheless, we believe that this model provides physicians who counsel women facing the decision whether to undergo testing with a template estimating the likelihood that a *BRCA1* test will be positive. Since our population consisted almost entirely of white women, the data may not be useful for Asian, black, Native American, or Hispanic women.

As in all previous studies, a young age at diagnosis

of breast cancer was associated with a detectable *BRCA1* mutation. Our findings also support the well-established link between ovarian cancer and *BRCA1* mutations. Previous work identified an association between a young age at diagnosis of ovarian cancer in family members and *BRCA1* mutations, but we did not find such a link.

In contrast to previous work, our study suggests that the presence of breast cancer alone (without ovarian cancer in the family) is infrequently associated with mutations in the coding region of *BRCA1*; only 7 percent of women from such families had detectable mutations. Thus, there remain a large number of families in which breast cancer may be associated with mutations in noncoding regions of *BRCA1* or other susceptibility genes. Mutations in the *BRCA2* gene are thought to account for 35 percent of hereditary cases of breast cancer,<sup>6</sup> but even if we excluded 35 percent of the families in this study with a history of breast cancer alone the majority of families would still not have detectable mutations in either *BRCA1* or *BRCA2*. Given that the proportion of families with *BRCA1* mutations was lower than expected (16 percent, as opposed to 45 percent), it is likely that the percentage of families with *BRCA2* mutations, also derived from linkage studies of large families, will similarly be lower than the currently estimated 35 percent. Clinical manifestations of other inherited breast-cancer syndromes, such as the Li-Fraumeni syndrome (p53 mutations),<sup>23</sup> the Muir-Torre syndrome (mutations in *MLH1* and *MSH2*),<sup>24-26</sup> and Cowden's disease,<sup>27</sup> were not observed in this series. Thus, we estimate that *BRCA1* and *BRCA2* mutations together may account for only 40 to 50 percent of the hereditary cases of breast cancers, not 90 percent, as has been suggested.<sup>10</sup>

Surprisingly, the number of breast cancers in a family, when considered alone, was not predictive of the presence of a *BRCA1* mutation. Since the number of breast cancers in a family may simply be a marker of family size and not a useful single determinant for predicting the presence of a *BRCA1* mutation, the decision not to test women because they have a small number of relatives with breast cancer may miss a substantial number of carriers. Bilateral breast cancer has also been considered a marker of familial predisposition to breast cancer, but in our series the incidence of *BRCA1* mutations in families with and those without members with bilateral breast cancer was virtually identical (18 percent and 15 percent, respectively). Neither parametric nor non-parametric methods of statistical analysis revealed an association between bilateral breast cancer and the presence of detectable *BRCA1* mutations.

Our predicted probability tables provide statistical estimates of the presence of a *BRCA1* mutation in most families with a history of breast cancer. However, because Ashkenazi Jewish ancestry is an inde-

pendent predictor of *BRCA1* mutations and mutation frequencies among Ashkenazi Jews and non-Ashkenazi whites differ, we created separate categories for these groups.

This model was designed to provide likelihood estimates for detecting a *BRCA1* mutation in women with a family or personal history of breast cancer, ovarian cancer, or both. However, our analysis was based on relatively small numbers of subjects and mutations and will undoubtedly require modification as more patients and families are analyzed. Until further information can be obtained about the outcome of screening and preventive interventions for women with *BRCA1* mutations, we believe that clinicians should disclose the uncertainties associated with the testing of and approach to carriers of *BRCA1* mutations and leave the final decision regarding testing to the woman. It is to be expected that a majority of those tested in most settings will not have an identifiable *BRCA1* mutation. These women need to know that in the absence of a known *BRCA1* mutation in the family, negative results are not truly negative, but uninformative in most cases, and they must be cautioned against having a false sense of security.

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