

## Pretest Prediction of BRCA1 or BRCA2 Mutation by Risk Counselors and the Computer Model BRCAPRO

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**Background:** Because BRCA gene mutation testing is costly, occasionally uninformative, and frequently associated with ethical and legal issues, careful patient selection is required prior to testing. Estimation of BRCA gene mutation probability is an important component of pretest counseling, but the accuracy of these estimates is currently unknown. We measured the performance of eight cancer risk counselors and of a computer model, BRCAPRO, at identifying families likely to carry a BRCA gene mutation. **Methods:** Eight cancer risk counselors and the computer model BRCAPRO estimated BRCA gene mutation probabilities for 148 pedigrees selected from an initial sample of 272 pedigrees. The final sample was limited to pedigrees with a proband affected by breast or ovarian cancer and BRCA1 and BRCA2 gene sequencing results unequivocally reported as negative or positive for a deleterious mutation. Sensitivity, specificity, negative predictive value, positive predictive value, and areas under receiver operator characteristics (ROC) curves were calculated for each risk counselor and for BRCAPRO. All statistical tests were two sided. **Results:** Using a greater-than-10% BRCA gene mutation probability threshold, the median sensitivity for identifying mutation carriers was 94% (range = 81% to 98%) for the eight risk counselors and 92% (range = 91% to 92%) for BRCAPRO. Median specificity at this threshold was 16% (range = 6% to 34%) for the risk counselors and 32% (range = 30% to 34%) for BRCAPRO ( $P = .04$ ). Median area under the ROC curves was 0.671 for the risk counselors (range = 0.620 to 0.717) and 0.712 (range = 0.706 to 0.720) for BRCAPRO ( $P = .04$ ). There was a slight,

but not statistically significant, improvement in all counselor performance measures when BRCAPRO-assigned gene mutation probability information was included with the pedigrees. **Conclusions:** Sensitivity for identifying BRCA gene mutation carriers is similar for experienced risk counselors and the computer model BRCAPRO. Because the computer model consistently demonstrated superior specificity, overall discrimination between BRCA gene mutation carriers and BRCA gene mutation noncarriers was slightly better for BRCAPRO. [J Natl Cancer Inst 2002;94:844-51]

Women who inherit a BRCA1 or BRCA2 gene with a deleterious mutation face a 35%–85% lifetime risk of developing breast cancer (1–6) and a 16%–57% lifetime risk of developing

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ovarian cancer (2,7–11). Surgical interventions may reduce these risks, with oophorectomy reducing the risk of breast and ovarian cancer by 47% (12) and 98% (13), respectively, and bilateral prophylactic mastectomy reducing the risk of breast cancer by more than 90% (14,15). Cancer susceptibility related to mutated BRCA genes is transmitted in an autosomal dominant fashion so, on average, only half the individuals in a given generation will be at increased risk for the associated cancers. Genetic testing can identify the individuals most likely to benefit from these risk-reducing interventions and spare unaffected relatives the risks associated with them.

Cancer susceptibility gene testing is not appropriate for everyone. BRCA gene mutation testing, in particular, is costly; is sometimes complicated by social, legal, or insurance issues; and can yield results with uncertain clinical significance. For these reasons, pretest counseling performed by professionals knowledgeable about the interpretation and limitations of genetic tests is required for anyone considering BRCA gene testing. One important component of pretest counseling is estimation of the probability that a family carries a BRCA1 or BRCA2 gene mutation. In a recent policy statement, the American Society of Clinical Oncology has suggested that consideration of BRCA gene mutation testing should be limited to individuals whose probability of carrying a mutation exceeds 10% (16). There are currently no validated methods for estimating BRCA gene mutation probabilities. Traditionally, cancer risk counselors familiar with Mendelian genetics and cancer patterns in families with an inherited predisposition (i.e., early age at onset and multiple or bilateral cancers) collect family history information and subjectively estimate an individual's pretest gene mutation probability.

As data sets describing the cancer family histories of BRCA gene mutation carriers have accumulated, investigators have attempted to develop mathematical models for objectively estimating pretest mutation probabilities on the basis of one or more attributes of families with an inherited breast and/or ovarian cancer predisposition (17–22). A recently described computer model that incorporates information for all family members (affected and unaffected with breast or ovarian cancer) is BRCAPRO. This model uses Bayes' theorem to calculate the probability of an individual carrying a BRCA gene mutation, given a specific family history (23,24). The model is based on age-specific and cumulative breast and ovarian cancer incidence rates for BRCA gene mutation carriers (1,25) as compared with the same rates for BRCA gene mutation noncarriers (26). There are factors in the model calculations to account for Ashkenazi Jewish heritage and male breast cancer.

The best method for estimating pretest mutation probability will ideally have a high sensitivity (i.e., will not miss many mutation carriers), a high specificity (i.e., will not recommend testing for nearly everyone), and a high negative predictive value (i.e., a low probability can provide a measure of reassurance that there really is no mutation). It is currently not known how well cancer risk counselors and the BRCAPRO model measure up to this standard. In addition, it is not known whether BRCA gene mutation probability information generated by BRCAPRO improves or confounds the subjective assessment of cancer risk counselors. In this study, we measured the performance of cancer risk counselors for estimating pretest BRCA gene mutation probability and evaluated the influence of BRCAPRO model probability information on this performance.

## METHODS

### General Approach

Eight experienced cancer risk counselors assigned BRCA gene mutation probabilities to a series of 148 pedigrees from families who had obtained BRCA gene mutation testing through several different university-based clinical cancer genetics programs. Mutation probability assignments were compared with results of complete BRCA1 and BRCA2 gene sequencing. No identifying information from the families was included with the pedigrees. This research was reviewed and approved by the Institutional Review Board at The University of Texas Southwestern Medical Center at Dallas.

To measure the influence of BRCAPRO-assigned mutation probability information on the performance of the cancer risk counselors, the counselors were also supplied with a second set of pedigrees on which the BRCAPRO information was printed. The pedigrees that included BRCAPRO information were admixed with pedigrees that did not include this information, and the risk counselors were not told that they would be seeing each pedigree twice.

### Risk Counselors

All eight cancer risk counselors were from university-based cancer genetics clinics that employ interdisciplinary teams for identifying and managing people at high risk for cancer. Each of these clinics provides patient education, risk assessment, pre- and post-test counseling, and intervention planning and execution to reduce risk. Each of the eight risk counselors indicated that 95% or more of their practice was devoted to clinical cancer genetics, with six of the eight indicating that 90% or more of their practice was devoted specifically to breast-ovarian cancer susceptibility counseling. Three of the clinics counsel more than 30 breast or ovarian cancer families each month, three counsel 11–30 families each month, and two counsel 6–10 families each month. All eight risk counselors have a master's degree, and four are certified by the American Board of Genetic Counselors.

### Ascertainment

Pedigrees and BRCA gene mutation testing results were submitted for 272 families by the eight cancer genetics clinics. To avoid the uncertainty that is introduced when no mutation is detected using a limited genetic test (e.g., the Ashkenazi three-mutation panel), we did not accept pedigrees for any families that had not undergone complete BRCA1 and BRCA2 gene sequencing, regardless of whether a mutation had been identified ( $n = 64$ ). To make it easier for the risk counselors to assign mutation probability estimates, we also rejected pedigrees from families in which the proband was not affected by either breast or ovarian cancer ( $n = 44$ ). Pedigrees from families with mutations of uncertain clinical significance were also rejected ( $n = 8$ ). In addition, six families that were ascertained through a mutation screening research project rather than through a clinical counseling setting were rejected, as were two pedigrees that were exact duplicates of pedigrees submitted by another institution. Three of the cancer genetics clinics submitted pedigrees for all families they had tested to date ( $n = 64$ ), two clinics submitted a recent consecutive series of tested families ( $n = 62$ ), and three clinics submitted a convenience sample of pedigrees that were not necessarily representative of all tested families

from those clinics ( $n = 22$ ). The proportion of BRCA gene mutation-positive families in the third group was judged by the submitting risk counselors to be higher than that of all families that had been tested in those clinics. Inclusion of the convenience sample did not bias ascertainment, however, as the proportion of pedigrees with a BRCA gene mutation was 41% (95% confidence interval [CI] = 33% to 50%) for the 126 consecutive families compared with 43% (95% CI = 35% to 51%) for the entire series of 148 pedigrees. Women with non-deleterious polymorphisms identified by complete gene sequencing were included in the “no mutation” group. The final sample consisted of 148 pedigrees from women affected with either breast or ovarian cancer who had been ascertained through a cancer genetics clinic and had undergone complete BRCA1 and BRCA2 gene sequencing by Myriad Genetics, Inc. (Salt Lake City, UT).

Among the 148 families whose pedigrees were included in the sample, complete BRCA1 and BRCA2 gene sequencing had shown that 42 families carried deleterious BRCA1 mutations, 21 carried deleterious BRCA2 mutations, and 85 had no deleterious BRCA gene mutations. Fifty-two different mutations were recorded among the 63 BRCA mutation-positive families (the spectrum of mutations is available on request to the author or online as supplemental data [available at <http://jncicancerspectrum.oupjournals.org/jnci/content/vol94/issue11/index.shtml>]). The only mutations that occurred in more than one family (pedigree) were in BRCA1: 185delAG (six), 5382insC (four), and 1832delE5 (two); in BRCA2: IVS 17-1 G>C (two) and 5950delCT (two).

#### Calculation of BRCA Gene Mutation Probabilities by the Computer Model BRCAPRO

BRCAPRO uses Bayes' theorem to calculate the probability that an individual carries a mutation in the BRCA1 or BRCA2 gene on the basis of his or her family history of breast and/or ovarian cancer. An intermediate step in the calculation of this probability is the calculation of a likelihood ratio for each individual in the family. These likelihood ratios are based on the probability that a specific cancer history (i.e., breast and/or ovarian cancer or no cancer at a given age) would be observed whether the individual were a mutation carrier or not. The final probability is calculated using the allelic frequency of BRCA gene mutations in the relevant population (Ashkenazi Jewish or non-Ashkenazi Jewish) and breast and ovarian cancer incidence rates for BRCA gene mutation carriers and noncarriers. The calculation of the final probability incorporates age and cancer information for all first- and second-degree relatives on the basis of Mendelian inheritance of an autosomal dominant gene. A detailed description of this model has been published (23,24). The calculations described are too complex to complete by hand for more than one or two relatives in a given family. BRCAPRO is a DOS-based computer model that automates these probability calculations. Additional information about the BRCAPRO computer model can be found at <http://biosun01.biostat.jhsph.edu/~gparmigi/brcapro.html>.

Family history information, including Ashkenazi Jewish heritage, was entered into the BRCAPRO interface, *CancerGene*, to generate pedigrees for all 148 families that included information for four generations (proband, siblings, offspring, parents, aunts, uncles, and grandparents). *CancerGene* is a pedigree drawing utility that automatically generates the input text file required by

the BRCAPRO model, runs the BRCAPRO model in the background, and then archives family history and mutation probability information to a relational database. Additional information about *CancerGene* can be obtained at <http://www3.utsouthwestern.edu/cancergene/>.

#### BRCA Gene Mutation Probabilities Assigned by Risk Counselors

Risk counselors from each of the eight cancer genetics clinics all came to The University of Texas Southwestern Medical Center at Dallas, where they evaluated each pedigree in a supervised environment. A supervised environment was used to ensure that counselor-generated probabilities were not contaminated by the use of BRCAPRO or other computer models. The pedigrees indicated whether a family was of Ashkenazi Jewish heritage. The risk counselors were asked to estimate the probability of BRCA gene mutation for each pedigree by using a five-point scale (1,  $\leq 10\%$ ; 2, 11%–30%; 3, 31%–70%; 4, 71%–94%; and 5,  $\geq 95\%$ ). These cutoffs distributed all 148 pedigrees equally into five groups based on BRCAPRO-calculated mutation probabilities. The risk counselors evaluated the pedigrees in groups of 20. The first group of 20 pedigrees included the pedigree diagram only. Subsequent groups of pedigrees were admixed with pedigrees the counselor had previously evaluated that, the second time around, had the BRCAPRO probabilities printed on the diagram. The risk counselors were also asked to indicate whether they recognized the pedigree as coming from their own clinic; such pedigrees were excluded from the calculations for that individual risk counselor.

#### Statistical Methods

Receiver operator characteristics (ROC) curves are commonly used to determine which threshold value for a clinical test provides the best discrimination between “normal” and “abnormal.” ROC curves are constructed by plotting the sensitivity of a particular threshold value for detecting abnormal individuals (in this case, BRCA gene mutation carriers) on the y-axis against 1 minus specificity for that threshold value on the x-axis. The area under the ROC curve is a measure of the overall discrimination that a given test can provide between individuals with the condition of interest (BRCA gene mutation, in this instance) and those without the condition. In our study, the area under the ROC curve corresponds to the probability that a family with a BRCA gene mutation will have a greater mutation probability score than a family without a BRCA gene mutation chosen at random. For instance, if the area under the ROC curve were 0.5, a mutation carrier would have a greater mutation probability score than a noncarrier only 50% of the time, a result that is no better than chance. BRCA gene mutation probabilities (categories 1–5) assigned by the risk counselors were used as the threshold values. Sensitivity and specificity for recognizing mutation carriers at each threshold were calculated for each risk counselor, and ROC curves were plotted. A similar ROC curve, using the same five probability thresholds, was generated for mutation probabilities as calculated by the BRCAPRO model. Because pedigrees recognized as coming from the counselor's own clinic were excluded for that counselor, they were also excluded from BRCAPRO calculations that were compared with that counselor. Areas under the ROC curves were compared using the method of DeLong et al. (27).

Performance measures, such as sensitivity, specificity, positive predictive value, and negative predictive value for individual risk counselors blinded to BRCPARO mutation probability information and then provided with this information were analyzed in a pair-wise fashion and compared by using the binomial distribution. Performance measures for the entire group of eight risk counselors were expressed as medians and then compared with BRCPARO mutation probabilities by using the Wilcoxon signed rank test. All tests of significance were two-tailed. When means were reported, they were compared by using two-tailed *t* tests. Proportions were compared by using chi-square tests.

## RESULTS

### Characteristics of the Sample

The final sample of 148 families included 15 with Ashkenazi Jewish ancestry. Other characteristics of the study sample are listed in Table 1. The mean numbers of relatives with breast or ovarian cancer per family in the 85 families with no deleterious BRCA gene mutations were 2.5 (95% CI = 2.3 to 2.8) and 0.45 (95% CI = 0.28 to 0.62) respectively, compared with 2.7 (95% CI = 2.3 to 3.0) and 0.71 (95% CI = 0.50 to 0.93) for the 63 families with deleterious BRCA gene mutations. The difference in mean values was statistically significant for ovarian cancer cases only ( $P = .05$ ). Although the proportion of families with breast cancer was similar between the mutation-carrying and noncarrying families (97%; 95% CI = 92% to 100% versus 95%; 95% CI = 91% to 100%;  $P = .96$ ), the mean age at breast cancer diagnosis among women from mutation-carrying families (43.2 years; 95% CI = 41.4 years to 45.1 years) was lower than the age at diagnosis for women from noncarrying families (49.1 years; 95% CI = 47.4 years to 50.8 years,  $P < .001$ ). Conversely, 31 (49%; 95% CI = 37% to 62%) of the 63 families with a BRCA gene mutation had at least one member with ovarian cancer compared with only 26 (31%; 95% CI = 21% to 40%) of 85 families without a BRCA gene mutation ( $P = .03$ ). However, the mean age at ovarian cancer diagnosis was similar for BRCA mutation-carrying families and noncarrying families (52.0 years; 95% CI = 47.4 years to 56.5 years versus

54.4 years; 95% CI = 49.0 years to 59.7 years, respectively,  $P = .37$ ).

### Discrimination of BRCA Mutation Carriers from Noncarriers

Each risk counselor assigned a BRCA gene mutation probability to each of the 148 pedigrees using a five-point scale. The sensitivity and specificity for identifying mutation carriers was then calculated at each of the five probability levels, and ROC curves were plotted (Fig. 1). The median area under the ROC curves for the eight risk counselors was 0.671 (range = 0.620 to 0.717) compared with 0.712 (range = 0.706 to 0.720) for BRCPARO ( $P = .04$ ; Table 2). The difference between the areas under the ROC curves for the risk counselors and BRCPARO was statistically significant for only one of the counselors ( $P = .03$ ). When BRCPARO mutation probability information was printed on the pedigrees, the areas under the ROC curves increased for six of the eight risk counselors, but this increase was statistically significant for only one counselor ( $P = .05$ ; Table 2). The median area under the ROC curves for the risk counselors blinded to BRCPARO gene mutation probability information was 0.671 (range = 0.620 to 0.717) compared with 0.698 (range = 0.638 to 0.723) when BRCPARO information was provided ( $P = .10$ ).

### The Greater-Than-10% Mutation Probability Threshold

Genetic testing is commonly recommended for individuals whose pretest mutation probability exceeds 10%. Consequently, differentiating pedigrees with a 31%–70% mutation probability from those with a 10%–30% probability may not be clinically relevant. It may be more useful to measure the discrimination between mutation carriers and noncarriers that can be achieved by recommending genetic testing only for those families that exceed the 10% mutation probability threshold. On the basis of the greater-than-10% mutation probability threshold, the median sensitivity for identifying BRCA gene mutation carriers was 94% (range = 81% to 98%) for the eight risk counselors blinded to BRCPARO mutation probability information compared with 96% (range = 83% to 98%) when this information was provided ( $P = .31$ ; Table 2). When BRCPARO mutation probabil-

Table 1. Characteristics of the sample

Characteristic	Mutation negative	Mutation positive*	<i>P</i>
No. of pedigrees	85	63	
Mean No. of relatives per pedigree (95% CI)†	14.2 (11.1 to 17.4)	13.2 (10.1 to 16.3)	.18‡
No. (%) of pedigrees with:			
Any breast cancer	81 (95.3)	61 (96.8)	.96§
Any ovarian cancer	26 (30.6)	31 (49.2)	.03§
Breast cancer only	59 (69.4)	32 (50.8)	.03§
Ovarian cancer only	4 (4.7)	2 (3.2)	.96§
Breast and ovarian cancer	22 (25.9)	29 (46.0)	.02§
Bilateral breast cancer	24 (28.2)	22 (34.9)	.49§
Breast and ovarian cancer in the same individual	7 (8.2)	14 (22.2)	.03§
Mean No. of relatives with breast cancer per family (95% CI)	2.5 (2.3 to 2.8)	2.7 (2.3 to 3.0)	.52‡
Mean age at breast cancer diagnosis (95% CI)¶	49.1 (47.4 to 50.8)	43.2 (41.4 to 45.1)	<.001‡
Mean No. of relatives with ovarian cancer per family (95% CI)	0.45 (0.28 to 0.62)	0.71 (0.50 to 0.93)	.05‡
Mean age at ovarian cancer diagnosis (95% CI)	54.4 (49.0 to 59.7)	52.0 (47.4 to 56.5)	.37‡

\*42 BRCA1 and 21 BRCA2 families.

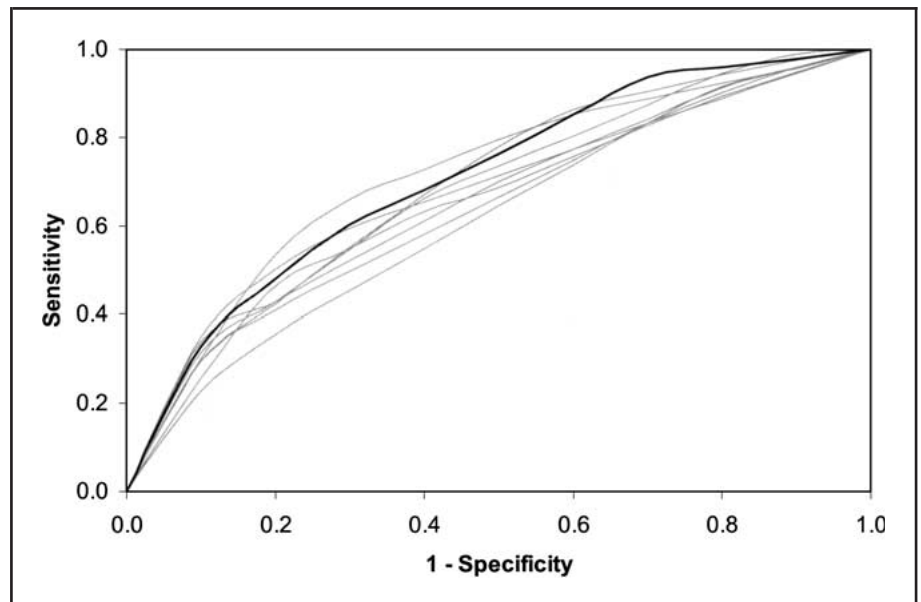
†CI = confidence interval.

‡Two-tailed *t* test.

§Chi-square test.

¶For women with bilateral breast cancer, only the age at diagnosis of the first breast cancer is included in the calculation.

**Fig. 1.** Receiver operator characteristics (ROC) curves for the computer model, BRCAPRO (thick line), and for eight cancer risk counselors (thin lines) in a study of pretest prediction of BRCA1 and BRCA2 gene mutations. The sensitivity (y-axis) for identifying a BRCA gene mutation-carrying family is plotted against 1 minus specificity (x-axis) for identifying a mutation-carrying family for each of the five possible mutation probability assignments. The area under the ROC curve is a measure of the discrimination between gene mutation-carrying and noncarrying families.



**Table 2.** Performance measures for eight risk counselors and the computer model BRCAPRO

Performance measures	Counselor								Median
	1	2	3	4	5	6	7	8	
No. of pedigrees*	143	144	140	146	141	148	148	148	
ROC curve area									
Counselor blinded to BRCAPRO probabilities	0.645	0.690	0.696	0.717	0.666	0.620	0.650	0.681	0.671
Counselor aware of BRCAPRO probabilities	0.638	0.710	0.723	0.709	0.701	0.690	0.708	0.705	0.698
<i>P</i> value†	.80	.40	.41	.68	.32	.05	.06	.21	.10‡
BRCAPRO alone§	0.709	0.713	0.720	0.706	0.711	0.712	0.712	0.712	0.712
<i>P</i> value†	.19	.66	.58	.73	.19	.03	.07	.44	.04‡
Sensitivity at the >10% threshold									
Counselor blinded to BRCAPRO probabilities	0.984	0.984	0.967	0.952	0.931	0.905	0.889	0.810	0.942
Counselor aware of BRCAPRO probabilities	0.984	0.967	0.967	0.968	0.931	0.952	0.937	0.825	0.960
<i>P</i> value†	1.00	1.00	1.00	1.00	1.00	.25	.38	1.00	.31‡
BRCAPRO alone§	0.918	0.921	0.917	0.921	0.914	0.921	0.921	0.921	0.921
<i>P</i> value†	.13	.13	.25	0.38	1.00	1.00	.50	.07	.55‡
Specificity at the >10% threshold									
Counselor blinded to BRCAPRO probabilities	0.061	0.145	0.138	0.120	0.169	0.224	0.212	0.341	0.157
Counselor aware of BRCAPRO probabilities	0.061	0.120	0.163	0.133	0.169	0.259	0.282	0.365	0.166
<i>P</i> value†	1.00	.69	.69	1.00	1.00	.55	.18	.73	.09‡
BRCAPRO alone§	0.317	0.313	0.338	0.301	0.313	0.318	0.318	0.318	0.317
<i>P</i> value†	.001	.007	.001	.001	.01	.10	.004	.85	.04‡

\*Pedigrees recognized as coming from the counselor's own institution were excluded for that counselor and from the BRCAPRO calculations used for comparison with that counselor.

†All *P* values are two-tailed and were based on the binomial distribution for paired proportions, unless otherwise indicated.

‡Wilcoxon signed rank test.

§Because pedigrees recognized as coming from the counselor's own institution were excluded for that counselor, BRCAPRO probabilities were generated only for those pedigrees that were included in the counselor's calculation.

||Compared with counselor blinded to BRCAPRO probabilities.

ity information was printed on the pedigree, sensitivity for identifying mutation carriers increased for four of the eight counselors, but these increases were not statistically significant. The corresponding median sensitivity for BRCAPRO alone at the greater-than-10% mutation probability threshold was 92% (range = 91% to 92%) compared with 94% for risk counselors blinded to BRCAPRO mutation probability information (range = 81% to 98%; *P* = .55).

Median specificity for identifying mutation carriers based on the greater-than-10% mutation probability threshold for the eight risk counselors blinded to BRCAPRO gene mutation probability information was 16% (range = 6% to 34%) compared

with 32% (range = 30% to 34%) for BRCAPRO alone (*P* = .04; Table 2). Differences between specificities calculated for the counselors and the corresponding specificities calculated for the BRCAPRO model were statistically significant for six of the eight counselors. When BRCAPRO mutation probability information was provided, the median specificity increased to 17% (range = 6% to 37%; *P* = .09).

The negative predictive value of a mutation probability assignment of 10% or less ranged from 71% to 92% for the risk counselors and was 84% for BRCAPRO (based on all 148 pedigrees). That is, 8% to 29% of families assigned a mutation probability of 10% or less by the risk counselors actually carried

a BRCA gene mutation, whereas 16% of families assigned a mutation probability of 10% or less by BRCAPRO were found to carry a BRCA gene mutation.

### Positive Predictive Value for the 95%-or-Greater Mutation Probability Threshold

Measures of sensitivity and negative predictive value for the greater-than-10% mutation probability threshold provide information about the number of mutation-positive families that would be missed if this threshold were widely adopted. By contrast, evaluation of the positive predictive value for the 95%-or-greater mutation probability threshold provides information about the sensitivity of the genetic test for detecting an inherited predisposition to breast and ovarian cancer. The study sample included 31 pedigrees to which BRCAPRO assigned a mutation probability of 95% or greater. BRCAPRO, therefore, had a positive predictive value (i.e., the proportion of families that actually carry a mutation that are scored as positive on the basis of the probability of mutation) of 74% when the 95%-or-greater mutation probability threshold was used. In other words, complete BRCA1 and BRCA2 gene sequencing failed to identify a BRCA gene mutation in 26% of families with a BRCAPRO probability of 95% or greater. Similarly, the positive predictive value for the risk counselors blinded to BRCAPRO probability information, based on a 95%-or-greater mutation probability threshold, ranged from 65% to 100% (median = 75%). That is, approximately 25% of the families assigned a mutation probability of 95% or greater by an experienced cancer risk counselor had no BRCA1 or BRCA2 gene mutation detected by complete sequencing. Results for each of the performance measures are summarized in Fig. 2.

### DISCUSSION

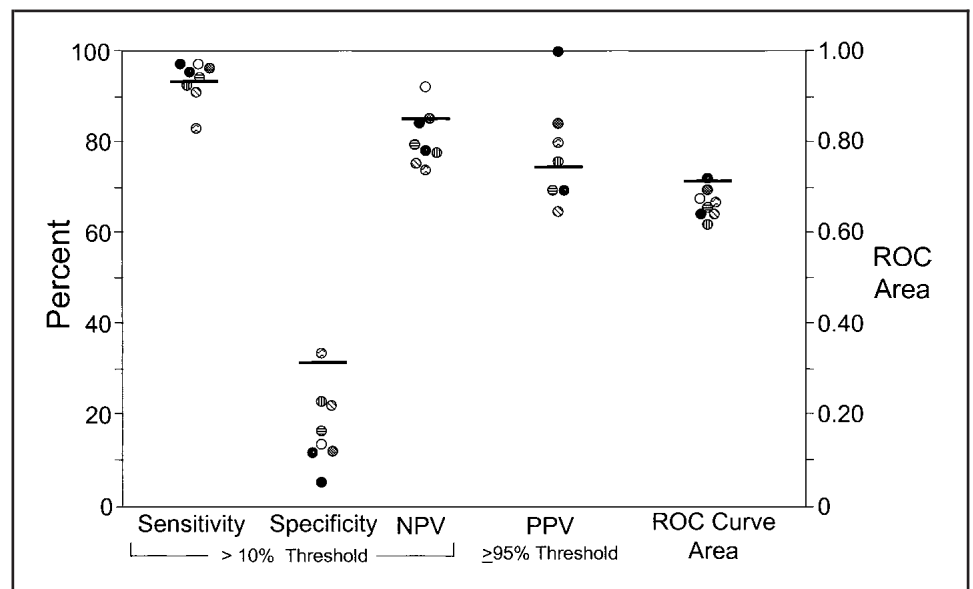
We measured the performance of eight cancer risk counselors and the computer model BRCAPRO for assigning pretest BRCA gene mutation probabilities. The sensitivity for identifying mutation carriers, based on a greater-than-10% mutation probability threshold, was greater than 90% for most risk counselors and for the computer model BRCAPRO. BRCAPRO consistently demonstrated superior specificity over the risk counselors for BRCA

gene mutation prediction; consequently, BRCAPRO was slightly better at overall discrimination between BRCA mutation carriers and noncarriers. In addition, there was a trend toward better discrimination for all performance measures when the risk counselors were provided with BRCAPRO mutation probability information on the pedigrees.

The family histories evaluated in this study provided a particularly stringent challenge for the risk counselors and the BRCAPRO computer model. These pedigrees were ascertained from a highly prescreened sample of women attending cancer genetics clinics who had already been selected for complete BRCA gene sequencing on the basis of cancer family history information suggestive of an inherited breast and ovarian cancer predisposition (early age at onset, bilateral breast cancer, and both breast and ovarian cancer in the family). The mean age at breast cancer diagnosis of 49.1 years in the women with no BRCA mutation detected by gene sequencing attests to the unique nature of this sample. The preponderance of relatives diagnosed with breast cancer at an early age in both the BRCA mutation-positive and BRCA mutation-negative groups may account for the rather flat ROC curves observed for the risk counselors and BRCAPRO (Fig. 1).

Because we excluded pedigrees from this study if the proband was not affected by breast or ovarian cancer, the pretest BRCA gene mutation probabilities estimated for the proband were also applicable to the family as a whole. Therefore, this allowed valid comparisons between the BRCA gene mutation probability estimates assigned by the risk counselors and those assigned by BRCAPRO using the entire sample. Similarly, because we also excluded pedigrees from this study if the initial genetic test was not complete BRCA1 and BRCA2 gene sequencing, we were able to estimate the sensitivity of the genetic test for identifying an inherited breast and ovarian cancer predisposition using the entire sample. In clinical practice, it is generally preferable to test individuals who are affected by breast or ovarian cancer before testing unaffected relatives; however, this is frequently not possible. Consequently, in most clinical populations, the proportion of families found to carry a deleterious BRCA gene mutation is lower than the 43% observed in our study. Furthermore, many cancer genetics clinics use limited mutation panels

**Fig. 2.** Summary of performance measures for eight cancer risk counselors (circles) and median values for the computer model BRCAPRO (horizontal lines). NPV = negative predictive value; PPV = positive predictive value; ROC curve area = receiver operator characteristics curve area. Sensitivity, specificity, and NPV were all calculated based on a greater-than-10% probability that a pedigree represented a BRCA gene mutation-carrying family. PPV was calculated based on a 95%-or-greater probability that a pedigree represented a BRCA gene mutation-carrying family. One counselor (open circle) did not score any pedigrees as having a mutation probability of 95% or greater, so no PPV could be calculated for that counselor. Sensitivity, specificity, NPV, and PPV were all measured as a percentage (left-hand side y-axis) and ROC curve area was measured as the area under the curve (right-hand side y-axis).



(e.g., the Ashkenazi three-mutation panel) for initial genetic testing rather than complete BRCA1 and BRCA2 gene sequencing, which was a requirement for inclusion of families in our study. Therefore, it may not be possible to generalize our results to every cancer genetics clinic.

The limitations of complete BRCA gene sequencing for identification of families with an inherited predisposition to breast and ovarian cancer is illustrated by examining the BRCA gene mutation frequency in families with a very high pretest mutation probability. The study sample included 31 pedigrees with mutation probabilities, according to BRCAPRO, of 95% or greater. Complete BRCA gene sequencing failed to identify a BRCA gene mutation in eight (26%) of these families. Because breast and ovarian cancers unrelated to BRCA gene mutations can occur in BRCA families, it is possible that an affected proband in one of these eight families could be BRCA mutation negative while other affected, but untested, relatives could be BRCA mutation carriers. The breast cancers in these eight probands were more consistent with inherited breast/ovarian cancer susceptibility than with sporadic cancer, however, because the age at diagnosis ranged from 31 to 50 years, with a mean of 38 years, and five of the eight women were affected with bilateral breast cancer. It is also conceivable that these eight families carry a mutation in a not-yet-identified breast cancer susceptibility gene or that these families carry deleterious alterations in BRCA1 or BRCA2 that are not detected by sequencing, as it is currently performed. Large genomic deletions in BRCA1, which are difficult to detect by polymerase chain reaction-based (PCR-based) mutation screening, were first described in 1997 (28) and were thought to account for at least 25% of BRCA1 mutations in one Dutch study (29). Because these large genomic deletions are likely to be founder mutations in the Dutch population, it is not possible to estimate the prevalence of large genomic deletions among breast cancer families in the United States on the basis of our data. Because BRCA1 and BRCA2 gene sequencing will miss large genomic deletions, and because there may be additional breast cancer predisposition genes yet to be described, we suggest that women with a high pretest mutation probability (95% or greater) be managed as though they had a mutation, regardless of the test result.

Failure to recognize the limitations of complete BRCA gene sequencing for identification of families with an inherited breast and ovarian cancer predisposition is one pitfall of clinical cancer genetics; another is failure to recommend genetic testing to a mutation carrier because of a low pretest probability. Among the 148 families, 32 had mutation probabilities, according to BRCAPRO, of 10% or less. Of these, five families (16%) were found to have mutations by complete BRCA1 and BRCA2 gene sequencing. Two of these families had a preponderance of male relatives with very few female relatives, one lacked any family history information from the maternal side, and two had a paucity of relatives on the affected side. Most of the risk counselors also assigned low pretest mutation probabilities to three of these five pedigrees. Of the 24 probability assignments made by the eight counselors for these three families, only six (25%) probabilities were assigned as being greater than 10%.

Of note, seven of the eight risk counselors had assigned BRCA gene mutation probabilities of greater than 10% to two of the five mutation-positive families to which BRCAPRO had assigned a probability of 10% or less. In both of these families, the proband was affected with ovarian cancer, and one family

had ovarian cancer only. These family histories highlight an idiosyncrasy of the BRCAPRO model: BRCAPRO uniformly assigned a low mutation probability to non-Ashkenazi families with ovarian cancer only. Based on a sample of 104 families with an average of 3.8 relatives affected with breast or ovarian cancer per family, it was recently reported (30) that the occurrence of ovarian cancer, bilateral breast cancer, or breast cancer diagnosed before the age of 40 showed a significant correlation with the identification of a BRCA1 or BRCA2 gene mutation. These investigators recommended that any family meeting any one of these criteria be referred for genetic counseling. Applying these criteria to the five mutation-positive families in which BRCAPRO assigned a mutation probability of 10% or less would have resulted in four of these five families being referred for genetic counseling and, potentially, genetic testing.

In general, pedigrees that were assigned to the appropriate gene mutation category by the risk counselors (on the basis of the greater-than-10% mutation probability threshold) were also assigned to the appropriate gene mutation category by the BRCAPRO model. However, three pedigrees from probands with BRCA gene mutations (two BRCA1 and one BRCA2) were scored as 10%-or-less mutation probability by two or more risk counselors but as greater-than-10% mutation probability by BRCAPRO. In each of these three families, only the proband had been diagnosed with breast cancer and always before the age of 45. In two of these three families, the proband had bilateral breast cancer.

Recognizing families likely to carry a germline mutation in a cancer predisposition gene is only one of several tasks performed by the cancer risk counselor. Counselors must also educate patients concerning the limitations and interpretation of genetic test results; the potential impact of testing on family, social, and economic interests; and the options for managing cancer risk. Most importantly, the cancer risk counselor serves as a well-informed sounding board for individuals who must make complicated decisions in an emotionally charged environment. Estimation of pretest gene mutation probability is an important initial task for cancer risk counselors, especially for BRCA gene mutation testing, which is expensive, is frequently associated with ethical and legal issues, and occasionally yields a result of uncertain clinical significance. The computer model BRCAPRO is a useful tool for calculating BRCA gene mutation probabilities, but for some mutation-carrying families, BRCAPRO will assign a mutation probability of 10% or less. In our study, misassignment of mutation-carrying families to a low pretest gene mutation probability group was largely a consequence of incomplete or limited family history information, making it essential that health care providers familiar with inherited cancer predisposition syndromes, and with the limited sensitivity of the greater-than-10% mutation probability threshold, be involved in the screening process.

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## NOTES

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