Breast Cancer Risk for Noncarriers of Family-Specific BRCA1 and BRCA2 Mutations: Findings From the Breast Cancer Family Registry

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ABSTRACT

Purpose

Women with germline BRCA1 and BRCA2 mutations have five- to 20-fold increased risks of developing breast and ovarian cancer. A recent study claimed that women testing negative for their family-specific BRCA1 or BRCA2 mutation (noncarriers) have a five-fold increased risk of breast cancer. We estimated breast cancer risks for noncarriers by using a population-based sample of patients with breast cancer and their female first-degree relatives (FDRs).

Patients and Methods

Patients were women with breast cancer and their FDRs enrolled in the population-based component of the Breast Cancer Family Registry; patients with breast cancer were tested for BRCA1 and BRCA2 mutations, as were FDRs of identified mutation carriers. We used segregation analysis to fit a model that accommodates familial correlation in breast cancer risk due to unobserved shared risk factors.

Results

We studied 3,047 families; 160 had BRCA1 and 132 had BRCA2 mutations. There was no evidence of increased breast cancer risk for noncarriers of identified mutations compared with FDRs from families without BRCA1 or BRCA2 mutations: relative risk was 0.39 (95% CI, 0.04 to 3.81). Residual breast cancer correlation within families was strong, suggesting substantial risk heterogeneity in women without BRCA1 or BRCA2 mutations, with some 3.4% of them accounting for roughly one third of breast cancer cases.

Conclusion

These results support the practice of advising noncarriers that they do not have any increase in breast cancer risk attributable to the family-specific BRCA1 or BRCA2 mutation.

INTRODUCTION

Women who inherit mutations in the BRCA1 and BRCA2 genes have five- to 20-fold increased risks of developing breast or ovarian cancer, with lifetime probabilities of 45% to 65% for breast and 11% to 40% for ovarian cancer.1,2 Once an index patient’s cancer diagnosis prompts clinical genetic testing and identification of a BRCA1 or BRCA2 mutation, unaffected relatives are offered testing for the family-specific mutation to guide management of their cancer risks. Cancer-free BRCA1 and BRCA2 mutation carriers are advised to escalate strategies for early cancer detection and prevention, including magnetic resonance imaging-based breast screening starting at age 25 years, risk-reducing bilateral salpingo-oophorectomy by age 40 years, and risk-reducing mastectomy. Women who are tested and found not to carry the family-specific mutation (hereafter called noncarriers) are advised that their cancer risks are the same as those of average women in the population and are recommended to follow general population cancer screening guidelines.3

A recent study cast doubt on this standard of care for noncarriers: by studying women referred for clinical testing because of strong history of cancer in their family, it estimated that noncarriers of a family-specific mutation had a two- to five-fold increased risk of developing breast cancer. The
investigators proposed that shared genetic or environmental risk modifiers might explain the familial clustering of breast cancer, which often prompts evaluation by cancer genetics clinics; they concluded that noncarriers should consider ongoing breast cancer surveillance according to recommendations for women at increased risk. Some subsequent studies of families having multiple or young breast cancer cases reported a two-fold increased risk for noncarriers, although the risk was not statistically significant in some studies; other studies reported no such risk increase. The results of prior studies reporting increased breast cancer risk for noncarriers may have been influenced, however, by their comparison of families referred clinically for strong history of breast cancer, who undergo more frequent screening and consequently breast cancer diagnosis, to women in the general population; such comparisons might spuriously elevate estimates of breast cancer risks for noncarriers. In addition, the families with multiple cases of early-onset breast cancer who are referred to cancer genetics clinics may possess risk modifiers that are not universal among noncarriers. To clarify the breast cancer risks for women who test negative for a family-specific \( \text{BRCA1} \) or \( \text{BRCA2} \) mutation, we compared such noncarriers with the first-degree relatives (FDRs) of patients with breast cancer who tested negative for \( \text{BRCA1} \) and \( \text{BRCA2} \) mutations, all accrued from population-based case families of the Breast Cancer Family Registry (BCFR).

**PATIENTS AND METHODS**

**Patients**

Women with incident breast cancer (hereafter called probands) were ascertained through population-based cancer registries and recruited to the BCFR sites in Melbourne and Sydney, Australia (\( n = 799 \)); Ontario, Canada (\( n = 1,034 \)); and Northern California, United States (\( n = 1,214 \)). Most probands were enrolled between 1996 and 2000; from 2001 to 2009, contributing BCFR sites recruited families with specific criteria of interest, including oversampling of racial and ethnic minorities. The Northern California site enrolled population-based probands diagnosed with breast cancer at age younger than 65 years through the Greater Bay Area Cancer Registry. The Ontario site recruited population-based probands diagnosed at age younger than 70 years through the Ontario Cancer Registry. Probands at these BCFR sites were enrolled through a two-stage sampling strategy that included all patients diagnosed between ages 18 and 34 years or having a family history of cancer suggestive of increased genetic susceptibility, and a random sampling of patients without such features. This two-stage sampling design provides unbiased risk estimates that have greater precision than those obtained from random sampling of all incident breast cancer cases. The Australian site enrolled population-based probands according to age at diagnosis and without selection for family history of cancer, including all women diagnosed from age 18 to 39 years and random samples of women diagnosed from age 40 to 59 years, through the Victorian and New South Wales Cancer Registries. Written informed consent was obtained from all patients; the institutional review boards of all participating institutions approved the study. Probands, and their female FDRs, including mothers and full sisters (and excluding half-sisters), constitute the study participants.

**BRCA1 and BRCA2 Mutation Testing**

Probands from all three contributing BCFR sites were tested for mutations in both \( \text{BRCA1} \) and \( \text{BRCA2} \). For the Northern California site, most probands were tested for \( \text{BRCA1} \) and \( \text{BRCA2} \) alterations by using exon grouping analysis (EGAN) or capillary exon grouping analysis (cEGAN). EGAN is based on conformation-specific gel electrophoresis on ABI377 instruments, and cEGAN is based on conformation-specific capillary electrophoresis on ABI-3730XL instruments (Applied Biosystems, Foster City, CA). All coding exons and surrounding intronic sequences were amplified and analyzed. Polymerase chain reaction fragments with aberrant mobility were sequenced. These methods permit detection of mutations and polymorphisms in \( \text{BRCA1} \) and \( \text{BRCA2} \) coding regions as well as splice-site mutations, as previously described. For the Ontario and Northern California sites, testing methods for germline mutations in \( \text{BRCA1} \) and \( \text{BRCA2} \) also included an RNA/DNA-based protein truncation test with complementary 5' sequencing or complete gene sequencing by Myriad Genetics (Salt Lake City, Utah), as previously described. For the Australia site, testing methods included exon and flanking intron sequencing, protein truncation, two-dimensional gel scanning, site-specific testing for founder mutations, multiplex ligand-dependent probe amplification, and BRACAnalysis (consisting of full sequencing of \( \text{BRCA1} \) and \( \text{BRCA2} \), with testing for five large rearrangements in \( \text{BRCA1} \) by Myriad Genetics, as previously described. These testing methods have previously been compared and have generally shown similar sensitivity to full DNA sequencing. At all BCFR sites, all mutations were confirmed by DNA sequencing. Mutations were classified as deleterious if they were protein-truncating, missense, or splice-site mutations as defined by the Breast Cancer Information Core. If a proband tested positive for a mutation in \( \text{BRCA1} \) or \( \text{BRCA2} \), her FDRs who had provided DNA samples were tested for the same mutation. Her untested FDRs were assigned probabilities of mutation carriage, conditional on the known genotypes in the family. FDRs of probands who tested negative for \( \text{BRCA1} \) and \( \text{BRCA2} \) mutations were themselves considered negative for mutations in both genes.

**Statistical Methods**

We used segregation analysis to fit a model that accommodates familial correlation in breast cancer risk that is not due to \( \text{BRCA1} \) or \( \text{BRCA2} \) mutations, as has been widely discussed in the literature. It seems increasingly likely that many genes influence risk, but it is not feasible to create a model that could incorporate all of these genes. Instead, like Tyer et al., we assumed that risk depends on carrier status of \( \text{BRCA1} \) and \( \text{BRCA2} \) mutations, as well as carrier status of an unobserved, dominantly acting gene that represents all shared genetic and nongenetic factors (hereafter called the latent gene), which segregates within families. We modeled the breast cancer incidence rate for a patient from study site \( k \), given her carrier status for \( \text{BRCA1} \) and \( \text{BRCA2} \) mutations and for the risk allele of the latent gene, as:

\[
\begin{align*}
\hat{h}(t) & = h_1(t) R_{12} R_{12}^c R_{12}^R R_{12}^c R_{12}^R ; \\
\end{align*}
\]

Here \( c_1 \) and \( c_2 \) are indicators for carriage of \( \text{BRCA1} \) and \( \text{BRCA2} \) mutations, respectively; \( g \) is an indicator for carriage of the latent gene risk allele, and \( z \) is an indicator assuming the value one if the patient’s family segregates a \( \text{BRCA1} \) or \( \text{BRCA2} \) mutation but she herself is mutation negative, and zero otherwise. Thus, \( R \) is the hazard ratio associated with carriage of the risk allele of the unobserved gene, and \( R_{12} \) is the hazard ratio for members of \( \text{BRCA1} \) or \( \text{BRCA2} \) segregating families who themselves test negative. In addition, \( R_{12}^c \) and \( R_{12}^R \) are age-specific relative risks of developing breast cancer for carriers of mutations of \( \text{BRCA1} \) and \( \text{BRCA2} \), respectively, which we obtained from a meta-analysis. Finally, \( h_1(t) \) is the age-specific baseline breast cancer incidence rate for women from study site \( k \) who carry the low-risk genotypes for \( \text{BRCA1} \), \( \text{BRCA2} \), and the latent gene. These baseline incidence rates were estimated by combining carrier prevalence estimates with population-based breast cancer incidence rates, specific for each proband’s country of residence, and for probands from the Northern California BCFR (which oversampled racial and ethnic minorities) for race/ethnicity, by using categories of African American, Asian-American, Hispanic, and non-Hispanic white.

Our assumption of an unobserved, dominantly acting gene to handle the familial disease correlation that remains after accommodating known breast cancer risk factors follows the approach used to develop the International Breast Cancer Intervention Study (IBIS [Tyrer-Cuzick]) breast cancer risk models. Like these investigators, we chose this model for several reasons: (1) it agrees with the observed similarity of relative risks for mothers and sisters of patients with breast cancer, whereas a recessive model would cause the relative risk for a sister to be much higher, and a nongenetic model would not adequately account for either risk; (2) it does not overestimate the risks associated with having two affected FDRs; (3) it is simple to implement compared with more complex polygenic models; and (4) simulations have shown that it yields...
unbiased estimates for other parameters of interest, even when the familial correlation is due to other mechanisms.\textsuperscript{22} For uncommon variants with few homozygous carriers, the predictions of the dominant model agree well with those of an additive genetic model.

As given by equation (1), the model involves three unknown parameters: the hazard ratio \( R_{\text{car}} \) for noncarriers in \( \text{BRCA1} \) or \( \text{BRCA2} \)-segregating families (the parameter of primary interest), the hazard ratio \( R \) corresponding to the unobserved gene, and the prevalence \( p \) of carriers of the high-risk allele of the unobserved gene. These parameters were estimated by maximizing a Horvitz-Thompson pseudo-likelihood function that accommodates the two-stage sampling of probands in Ontario and Northern California.\textsuperscript{12,24} When the noncarrier hazard ratio is one, the model predicts that the fraction \( p \) of women at high risk for currently unexplained reasons accounts for 100\% \( p(1 + p(R - 1)) \) percent of all breast cancer cases.

Results

Data in Table 1 show the distribution of family characteristics by BCFR enrollment site and by the proband’s \( \text{BRCA1} \) or \( \text{BRCA2} \) mutation status. Non-white families are more heavily represented in Northern California than in the other two countries. However, families from the three countries show similar prevalences of \( \text{BRCA1} \) and \( \text{BRCA2} \) mutations, breast and ovarian cancers, and similar mean ages at onset for these cancers.

We first considered a model that accommodated country-specific carrier prevalences of the risk genotype for the assumed latent gene. The estimated prevalences did not differ significantly across countries, so we assumed a parsimonious model that included a common prevalence estimated at 0.034 (95\% CI, 0.01 to 0.16) or 3.4\%. The estimated relative risk of developing breast cancer for noncarriers of family-specific \( \text{BRCA1} \) or \( \text{BRCA2} \) mutations compared with that for members of families without identified \( \text{BRCA1} \) or \( \text{BRCA2} \) mutations, was 0.39 (95\% CI, 0.04 to 3.81). This relative risk is not significant and is nearly identical with the one obtained from the model that included country-specific carrier prevalences.

Residual breast cancer correlation within families was strong, as measured by a hazard ratio of 13.4 (95\% CI, 8.7 to 22.5) for carriers versus noncarriers of the risk allele of the unobserved gene, which we assumed to represent all unobserved genetic and nongenetic factors. This hazard ratio is remarkably similar to the estimate of 13.0 found by Tyrer et al\textsuperscript{23,25} when fitting the same model to family data from a population-based Swedish study. Tyrer et al found a latent gene carrier prevalence of 11\% for the Swedish population; however, this estimate does not differ significantly from our estimate of 3.4\%.

Our results imply that the 3.4\% of women in these populations who are at high risk for unexplained reasons account for 32\% of all breast cancer cases. Moreover, FDRs of affected women are more likely to belong to this high-risk group. Although these inferences represent crude approximate measures of breast cancer risk heterogeneity, they are in qualitative agreement with findings from other studies.\textsuperscript{26}

Discussion

We studied breast cancer risk for female noncarriers of family-specific \( \text{BRCA1} \) or \( \text{BRCA2} \) mutations in a population-based setting by using data collected by Australian, Canadian, and Northern Californian sites of the BCFR. In contrast to some prior studies,\textsuperscript{4-6,27} we found no evidence of increased breast cancer risk for noncarriers of family-specific mutations compared with relatives of women with breast cancer who tested negative for \( \text{BRCA1} \) and \( \text{BRCA2} \) mutations; our finding of a relative risk less than one was likely due to chance and was not statistically significant. This difference between our results and those of some prior studies may result from our comparing noncarriers of family-specific mutations to a control group accrued identically from the same population. Prior clinic-based studies that reported high risks for noncarriers compared them to general population controls in England, Canada, and Poland,\textsuperscript{4,6} who almost certainly underwent less intensive screening (and thus had less opportunity for breast cancer diagnosis) than do women with multiple affected relatives. However, our findings are consistent with those of two recent US clinic-based studies.\textsuperscript{2,6} One possible explanation for the discrepancy between results of different clinic-based studies is the high rate of breast cancer screening of the US population: consequently, the US control group may have received screening similar to that of clinically accrued US noncarriers of family-specific mutations in contrast to populations in the other countries studied.\textsuperscript{4,6} This study of 292

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Characteristic} & \textbf{Melbourne/Sydney, Australia (n = 799)} & & \textbf{Ontario, Canada (n = 1,034)} & & \textbf{Northern California, United States (n = 1,214)} & & \textbf{All Sites (N = 3,047)} \\
\hline
\textbf{No. of families} & \textbf{BRCA1} & \textbf{BRCA2} & \textbf{Neither} & \textbf{BRCA1} & \textbf{BRCA2} & \textbf{Neither} & \textbf{BRCA1} & \textbf{BRCA2} & \textbf{Neither} & \textbf{BRCA1} & \textbf{BRCA2} & \textbf{Neither} \\
\hline
Non-Hispanic white & 38 & 22 & 714 & 57 & 49 & 844 & 24 & 20 & 97 & 119 & 91 & 1655 \\
Non-Hispanic black & 0 & 0 & 0 & 0 & 0 & 7 & 8 & 10 & 322 & 8 & 10 & 329 \\
Hispanic & 0 & 0 & 0 & 0 & 0 & 1 & 24 & 13 & 293 & 24 & 13 & 294 \\
Asian & 1 & 0 & 18 & 1 & 1 & 21 & 4 & 15 & 380 & 6 & 16 & 419 \\
Other/unsupplied & 1 & 0 & 5 & 2 & 1 & 50 & 0 & 1 & 3 & 3 & 2 & 58 \\
\hline
Total & 40 & 22 & 737 & 60 & 51 & 923 & 60 & 59 & 1095 & 160 & 132 & 2755 \\
\hline
No. of females per family* & 3.33 & 3.23 & 3.36 & 4.08 & 3.72 & 4.05 & 3.77 & 3.69 & 4.38 & 3.87 & 3.68 & 4.13 \\
With breast cancer & 1.48 & 1.59 & 1.13 & 1.45 & 1.36 & 1.20 & 1.43 & 1.35 & 1.14 & 1.44 & 1.37 & 1.18 \\
Age at diagnosis, years & 38.76 & 42.94 & 38.01 & 42.71 & 43.81 & 52.88 & 42.32 & 45.41 & 50.05 & 42.09 & 44.46 & 50.94 \\
With ovarian cancer & 0.13 & 0.05 & 0.01 & 0.15 & 0.04 & 0.02 & 0.13 & 0.04 & 0.02 & 0.14 & 0.04 & 0.02 \\
Age at diagnosis, years & 54.4 & 50 & 47.44 & 51.7 & 48.75 & 50.97 & 57.11 & 54.67 & 47.60 & 53.99 & 51.34 & 49.51 \\
\hline
\end{tabular}
\caption{Family Characteristics, by Breast Cancer Family Registry Site and BRCA Mutation Status}
\end{table}

*Including proband and female first-degree relatives, excluding half-sisters.
BRCA1 or BRCA2 mutation-carrying families compared with 2,755 families without BRCA1 or BRCA2 mutations from the same populations represents the largest analysis to date of breast cancer risk to noncarriers of family-specific mutations.

Our analysis assumed that the risk of developing breast cancer depends on a dominantly acting, unobserved latent gene to address residual disease correlation in families after accounting for BRCA1 and BRCA2 mutations. This latent gene assumption is merely a device to facilitate analysis and does not imply the existence of an undiscovered breast cancer gene with penetrance comparable with that of BRCA1 and BRCA2. On average, women in families with multiple cases of breast cancer have a higher risk of breast cancer than the general population, because cancers aggregate within families as a result of shared genetic and nongenetic factors. Consistent with data from prior studies, our data showed a large relative risk for carriers compared with noncarriers of a high-risk latent gene allele, a model variable that encompasses not only shared genes but also shared lifestyle exposures that influence breast cancer risk within a family. Our data suggest that women at high risk because of these factors account for only 3.4% of the female populations of the three countries, but 32% of all their breast cancer cases. Moreover, close relatives of patients with breast cancer are more likely than other women to belong to such a high-risk group, independent of their carrier status for BRCA1 and BRCA2, and thus they warrant clinical attention. The results from genome-wide association studies suggest that such familial breast cancer aggregation is likely due to many genetic variants of low to moderate penetrance. Such observations may inform risk counseling, because models that incorporate multiple genes and non-genetic factors are increasingly used in clinical genetics and oncology practice to guide breast cancer screening and prevention.

Some limitations of this analysis warrant consideration when interpreting the findings. The model given by equation (1) uses breast cancer risk ratios for BRCA1 and BRCA2 mutation carriers from the published literature, and it is possible that they do not pertain to the families in this study. However, misspecification of these risk ratios would seem unlikely to obscure an increased risk ratio for noncarriers. Another potential concern is the validity of the assumptions underlying the model. This model assumes that breast cancer risk depends on a multiplicative interaction between mutations in BRCA1 or BRCA2 and an unobserved, dominantly acting gene or genes, and that this latent gene accommodates the additional breast cancer risks within families selected because a member was diagnosed with breast cancer. Nevertheless, departures from these assumptions in our data are unlikely to bias our findings concerning breast cancer risk for noncarriers in families with BRCA1 or BRCA2 mutations.

An additional limitation is the lack of complete testing of all family members for BRCA1 and BRCA2 mutations. All FDRs of BRCA1 or BRCA2 mutation carriers who themselves provided DNA were tested for the mutation carried by the proband; most importantly, the key noncarrier hazard ratio estimate of interest was based only on FDRs who tested negative for the proband’s mutation. We believe it unlikely that uncertainty in the BRCA status of FDRs without DNA samples available could bias our results, since such FDRs were assigned a probability of carriage of the proband’s mutation based on the known mutation status of all tested family members.

For families of probands negative for BRCA1 and BRCA2 mutations, we assumed that all members did not carry a BRCA1 or BRCA2 mutation. Because the probability that some of these members are themselves BRCA mutation carriers is small, the likelihood of bias toward the null in the noncarrier relative risk estimate is small.

In summary, we found no evidence for an increase in breast cancer risk for noncarriers of a family-specific BRCA1 or BRCA2 mutation in this study of 3,047 population-based families recruited from three countries. These results support the standard clinical practice of advising noncarriers that they do not have any increase in breast cancer risk attributable to the family-specific BRCA mutation and, in the absence of other strong risk factors, should follow general population guidelines for breast cancer screening.
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