Tamoxifen and Breast Cancer Incidence Among Women With Inherited Mutations in \textit{BRCA1} and \textit{BRCA2}

National Surgical Adjuvant Breast and Bowel Project (NSABP-P1) Breast Cancer Prevention Trial

Mary-Claire King, PhD
Sam Wieand, PhD
Kathryn Hale, BS
Ming Lee, PhD
Tom Walsh, PhD
Kelly Owens, PhD
Jonathan Tait, MD, PhD
Leslie Ford, MD
Barbara K. Dunn, MD, PhD
Joseph Costantino, DrPH
Lawrence Wickerham, MD
Norman Wolmark, MD
Bernard Fisher, MD

The randomized, double-blind, Breast Cancer Prevention Trial (BCPT) was conducted between 1992 and 1998 by the National Surgical Adjuvant Breast and Bowel Project (NSABP). The purpose of the BCPT was to determine whether tamoxifen use by cancer-free, high-risk women significantly altered incidence of invasive breast cancer.\(^1\) The result of the BCPT, which involved 13,388 women, was that the incidence of invasive breast cancer among those randomized to tamoxifen compared with those randomized to placebo (risk ratio [RR]) was 0.51.

The reduction in breast cancer due to tamoxifen revealed by the BCPT trial was consistent for women with no family history of breast cancer (RR, 0.46) and for women with mothers, sisters, and/or daughters with breast cancer (RR, 0.53). This observation led to the suggestion that tamoxifen might be of use in reducing breast cancer risk among women with inherited mutations in the breast cancer predisposing genes \textit{BRCA1} or \textit{BRCA2}.\(^2-5\) Because the risk of breast cancer is much higher among women with \textit{BRCA1} or \textit{BRCA2} mutations compared with women overall, an RR of 0.51 among

Context Among cancer-free women aged 35 years or older, tamoxifen reduced the incidence of estrogen receptor (ER)–positive but not ER-negative breast cancer. The effect of tamoxifen on breast cancer incidence among women at extremely high risk due to inherited \textit{BRCA1} or \textit{BRCA2} mutations is unknown.

Objective To evaluate the effect of tamoxifen on incidence of breast cancer among cancer-free women with inherited \textit{BRCA1} or \textit{BRCA2} mutations.

Design, Setting, and Participants Genomic analysis of \textit{BRCA1} and \textit{BRCA2} for 288 women who developed breast cancer after entry into the randomized, double-blind Breast Cancer Prevention Trial of the National Surgical Adjuvant Breast and Bowel Project (between April 1, 1992, and September 30, 1999).

Main Outcome Measure Among women with \textit{BRCA1} or \textit{BRCA2} mutations, incidence of breast cancer among those who were receiving tamoxifen vs incidence of breast cancer among those receiving placebo.

Results Of the 288 breast cancer cases, 19 (6.6\%) inherited disease-predisposing \textit{BRCA1} or \textit{BRCA2} mutations. Of 8 patients with \textit{BRCA1} mutations, 5 received tamoxifen and 3 received placebo (risk ratio, 1.67; 95\% confidence interval, 0.32-10.70). Of 11 patients with \textit{BRCA2} mutations, 3 received tamoxifen and 8 received placebo (risk ratio, 0.38; 95\% confidence interval, 0.06-1.56). From 10 studies, including this one, 83\% of \textit{BRCA1} breast tumors were ER-negative, whereas 76\% of \textit{BRCA2} breast tumors were ER-positive.

Conclusion Tamoxifen reduced breast cancer incidence among healthy \textit{BRCA2} carriers by 62\%, similar to the reduction in incidence of ER-positive breast cancer among all women in the Breast Cancer Prevention Trial. In contrast, tamoxifen use beginning at age 35 years or older did not reduce breast cancer incidence among healthy women with inherited \textit{BRCA1} mutations. Whether tamoxifen use at a younger age would reduce breast cancer incidence among healthy women with \textit{BRCA1} mutations remains unknown.

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mutation carriers would convey a substantial absolute benefit. However, the estrogen-receptor (ER) profiles of BRCA1 and BRCA2 breast tumors introduced a complexity into these considerations. In the breast, tamoxifen is an antiestrogen that targets the ER. Therefore, precancerous changes in the breast that have lost the cytoplasmic receptor (ie, tissues that are ER-negative) are not affected by tamoxifen. In the BCPT, tamoxifen reduced the incidence of ER-positive tumors, but had no effect on the incidence of ER-negative tumors. Tumors with mutations in BRCA1 and BRCA2 differ in ER status and other biological features, in that BRCA1 tumors lack estrogen and progesterone receptors and overexpress P53 more frequently, and also exhibit higher nuclear grade and proliferative rates than do BRCA2 tumors or breast tumors generally.6-10 These differences raised the possibility that tamoxifen might be effective in reducing breast cancer risk among women with BRCA2 mutations, but not among women with BRCA1 mutations.

To address these questions, we evaluated the effect of tamoxifen among participants in the NSABP/BCPT with inherited BRCA1 or BRCA2 mutations. Women entered the BCPT trial without knowledge of their inherited BRCA1 or BRCA2 genotype since the genes had not been cloned when the trial began. Participants provided peripheral blood samples at entry to the BCPT trial, from which constitutional DNA could now be extracted and BRCA1 and BRCA2 sequenced. This report presents the genetic analysis of BRCA1 and BRCA2 for the participants who developed invasive breast cancer during the BCPT trial. Based on these invasive breast cancer cases, we estimate RRs for breast cancer among women with BRCA1 or BRCA2 mutations associated with use of tamoxifen vs placebo.

METHODS

Eligibility
The complete eligibility requirements for participants in the BCPT trial have been described previously.1 Briefly, eligibility required that participants be 35 years or older with a 1.66% or greater risk of breast cancer over the next 5 years, estimated by the model by Gail et al11; have had lobular carcinoma in situ; or be 60 years or older.

Case Definition
For our current analysis, cases were defined as all incident invasive breast cancers occurring before the assigned treatment of BCPT participants was revealed on April 1, 1998, and all cases reported to the NSABP headquarters between April 1, 1998, and September 30, 1999. Because BCPT participants who were randomized to placebo were offered the opportunity to use tamoxifen if they chose to do so after April 1, 1998, this choice was recorded for each case diagnosed after that date. By September 30, 1999, follow-up was available for 13,195 participants. Complete 3-year follow-up was available for 80% of participants, complete 5-year follow-up was available for 65% of participants. Median follow-up was 5.7 years.

Anonymization
When this study was initiated, the NSABP Biostatistical Center created a data file that contained key clinical outcome and demographic information for all participants who developed breast cancer. At that time, the NSABP identification number of each patient was provided to the Northwest Lipid Research Laboratory (Seattle, Wash) whereuffy coat samples were stored, and to the Molecular Diagnostics Laboratory (University of Washington, Seattle). Buffy coats for each identified patient were provided to the Molecular Diagnostics Laboratory, where DNA was extracted. Three aliquots (15 µg each) were prepared and labeled with NSABP identification number. After the DNA was extracted, the independent statistician took both identification numbers to the Molecular Diagnostics Laboratory and replaced the sample labels containing NSABP identification numbers with new labels. Immediately thereafter, the data set was returned to NSABP, and the link between the 2 sets of identification numbers was destroyed. Subsequently, 2 aliquots of DNA for each subject were provided to the King Laboratory (University of Washington, Seattle) for genomic analysis, and the third aliquot was held in the Molecular Diagnostics Laboratory. Thus, once mutation analysis began, there was only 1 identification number for both the DNA and the clinical data, and there was no longer any link between those data and the original patient identification number.

Genomic Analysis
Blood samples were obtained from BCPT participants at entry to the initial study. At that time, buffy coats were isolated from the 10 mL of whole blood and frozen at −70°. For the BRCA1/BRCA2 sequencing study, DNA was extracted using Purgene DNA isolation kits (Gentra Systems, Minneapolis, Minn) and re-suspended in 10 mmol buffer to a final concentration level of 20 µg/mL. DNA quality was assessed by spectrophotometry and gel electrophoresis to monitor purity and molecular weight.

DNA was gridded in 96-well format using a Hydra robot (Robbins Scientific, Sunnyvale, Calif). DNA samples were amplified by polymerase chain reaction (PCR) using 78 pairs of M13-tagged PCR primers. Each pair of primers was designed to amplify an exon and flanking intron sequence encompassing splice sites. Larger exons were amplified in overlapping amplicons. When possible, primers were chosen to avoid Alu sequences and regions that produced compressions or slippage during cycle sequencing. Optimal PCR conditions allowing maximal levels of product were determined empirically. A complete list of primers and PCR conditions are available on request.
TAMOXIFEN AND BRCA1 AND BRCA2 MUTATIONS

Sephacryl-purified PCR products were cycle-sequenced using BigDye (Applied Biosystems, Foster City, Calif) primer sequencing chemistry following the manufacturer’s specifications and analyzed on ABI 377 DNA Sequencer (Applied Biosystems). Amplicons of each sample were sequenced in both forward and reverse directions. For each DNA sample, 24,133 nucleotide base pairs of BRCA1 and BRCA2 were evaluated. These included the 5589-base pair coding sequence, and 2752-base pair flanking intronic sequences and regulatory regions of BRCA1, the 10254-base pair coding sequence, and 5538-base pair intronic and regulatory sequences of BRCA2. All cases were fully screened for both genes.

Sequences from study samples were compared with consensus genomic sequences of BRCA1 at GenBank L78833 and of BRCA2 at GenBank Z74739 and GenBank Z73359. Base pairs were identified with the Phred and PolyPhred programs and aligned using the Phrap program. We used a modified version of the SeqHelp program in conjunction with Consed to display multiple aligned forward and reverse sequences and to highlight variants noted by PolyPhred analysis. Variants were confirmed first by sequencing the same exon again from the original DNA sample, then by separating wild-type and mutant alleles by gel electrophoresis, cutting each band from the gel, and sequencing each separately.

To assess the sensitivity and accuracy of the sequencing effort, 26 DNA samples with known BRCA1 or BRCA2 mutations were included in the study. These control samples were obtained from laboratories not affiliated with this project. Control samples were assigned identification numbers at the same time as samples from participants, so that the genetic analysis group were blind to the identities and genotypes of the controls. After samples were evaluated, the identification numbers of the control samples were revealed. The control samples included 10 different single nucleotide substitutions, leading variously to nonsense, missense, or splice mutations, and 17 insertions or deletions of between 1 and 11 base pairs. (One control carried 2 different mutations.) All control mutations were successfully identified.

For the study participants, all mutations definitely predisposing to breast cancer were included in this analysis. These were defined as protein-terminating mutations anywhere in BRCA1 and in exons 2 through 26 of BRCA2, and missense mutations in the canonical cysteine residues of the BRCA1 ring finger. Protein-terminating mutations were defined as insertions or deletions leading to frameshifts, nonsense mutations, and mutations in splice sites known to lead to frameshifts. Proton-terminating mutations in BRCA2 exon 27 and missense mutations and potential splice variants of uncertain significance in either gene were identified but were not included in this analysis.

Statistical Methods

Randomization ensured that a woman with a BRCA1 or BRCA2 mutation was equally likely to receive tamoxifen or placebo. Hence, if tamoxifen had no protective (or harmful) effect on cancer incidence in women with a BRCA1 or BRCA2 mutation, then a woman with a mutation who developed cancer was equally likely to have received tamoxifen or placebo. To test the hypothesis that tamoxifen did not alter the incidence of breast cancer, it was only necessary to find the mutation status of women who developed cancer and test the null hypothesis that the proportion of these women who received tamoxifen (\(p_T\)) was consistent with the hypothesis (\(p_T=0.50\)). If the observed proportion \(p_T\) was less than 0.50, this was evidence that tamoxifen was beneficial in these women. For example, under the null hypothesis, the probability that 3 or fewer of the 11 women with cancer and BRCA2 mutations received tamoxifen could be expressed as the probability of observing \(p_T\) of 3/11 or less when \(p_T\) is equal to 0.50, corresponding to a 1-sided \(P\) value of .11. It would have been possible to condition on the proportion of women with follow-up who received tamoxifen (6591/13195) or on the proportion of person-years of follow-up for women who received tamoxifen (32959/65860). However, these numbers are so close to a \(p_T\) of 0.50 that the 3 approaches lead to identical results, as one would expect in such a large randomized trial.

Similar reasoning applies to our presentation of relative risks. Denoting the number of mutation carriers who received tamoxifen by \(M_T\) and the number of mutation carriers who received placebo by \(M_U\), we assumed that \(M_T/M_U\) is equal to 1 and that the number of cases with a mutation was small relative to the number of participants without a mutation. In that case, the relative risk is approximately equal to \(p_T\) divided by \(1-p_T\) and the 95% confidence interval (CI) for the relative risk is \((p_T/[1-p_T])\) to \(p_T/[1-p_T])\), in which \(p_T\) to \(p_T\), is the 95% CI for \(p_T\) when \(p_T\) is observed. All CIs were computed using exact distributions.

RESULTS

As of September 30, 1999, 320 participants in the BCPT had developed invasive breast cancer. For 13 participants, DNA was not available because the participant either withdrew consent for additional involvement in the BCPT after developing cancer or chose not to have her sample included in this genetics study. For 19 participants, adequate DNA could not be obtained from the stored buffy coat samples. Hence 90% (288/320) of cases are included in this analysis.

Of the 288 breast cancer cases screened for BRCA1 and BRCA2, 19 (6.6%) carried inherited, disease-predisposing mutations (TABLE 1). Sixteen different definite cancer-associated mutations are distributed throughout the BRCA1 and BRCA2 sequences. Carrier status of BRCA1 and BRCA2 was associated with a family history of breast cancer, especially if 2 or more first-degree relatives were affected (TABLE 2). Also as expected, the proportion of patients with mutations was higher for those diagnosed when they were younger than age 50 years

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Table 1. Inherited Pathogenic Mutations in \textit{BRCA1} or \textit{BRCA2} Among Participants in the Breast Cancer Prevention Trial Who Developed Invasive Breast Cancer

<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>185 del AG</td>
<td>Stop 39</td>
</tr>
<tr>
<td>1</td>
<td>2041 ins G</td>
<td>Stop 615</td>
</tr>
<tr>
<td>1</td>
<td>5215 del GTCA</td>
<td>Stop 1668</td>
</tr>
<tr>
<td>1</td>
<td>5273 del G</td>
<td>Stop 1705</td>
</tr>
<tr>
<td>1</td>
<td>5445 del TTTAGT</td>
<td>Stop 1739</td>
</tr>
<tr>
<td>1</td>
<td>5950 del CT</td>
<td>Stop 1909</td>
</tr>
<tr>
<td>1</td>
<td>6503 del TT</td>
<td>Stop 2099</td>
</tr>
<tr>
<td>2</td>
<td>8765 del AG</td>
<td>Stop 2867</td>
</tr>
</tbody>
</table>

\textit{BRCA2}

<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>983 del ACAG</td>
<td>Stop 275</td>
</tr>
<tr>
<td>10</td>
<td>1529 del AAAG</td>
<td>Stop 458</td>
</tr>
<tr>
<td>10</td>
<td>2041 ins A</td>
<td>Stop 615</td>
</tr>
<tr>
<td>11</td>
<td>5215 del GTCA</td>
<td>Stop 1668</td>
</tr>
<tr>
<td>11</td>
<td>5273 del G</td>
<td>Stop 1705</td>
</tr>
<tr>
<td>11</td>
<td>5445 del TTTAGT</td>
<td>Stop 1739</td>
</tr>
<tr>
<td>11</td>
<td>5950 del CT</td>
<td>Stop 1909</td>
</tr>
<tr>
<td>11</td>
<td>6503 del TT</td>
<td>Stop 2099</td>
</tr>
<tr>
<td>11</td>
<td>6763 ins 8</td>
<td>Stop 2193</td>
</tr>
<tr>
<td>20</td>
<td>8765 del AG</td>
<td>Stop 2867</td>
</tr>
</tbody>
</table>

Table 2. Number of Invasive Breast Cancer Cases Among Women by \textit{BRCA1} and \textit{BRCA2} Genotype, Family History, and Age at Diagnosis

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of Cases</th>
<th>Proportion With Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>0.05</td>
</tr>
<tr>
<td>≥2</td>
<td>5</td>
<td>0.15</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Table 3. Study Participants Who Developed Breast Cancer

<table>
<thead>
<tr>
<th>BRCA1 mutation</th>
<th>Placebo</th>
<th>Tamoxifen</th>
<th>Risk Ratio (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1.67 (0.32-10.70)</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>2</td>
<td>0.38 (0.06-1.56)</td>
</tr>
<tr>
<td>Wild type</td>
<td>182</td>
<td>87</td>
<td>0.48 (0.37-0.61)</td>
</tr>
<tr>
<td>All participants*</td>
<td>211</td>
<td>109</td>
<td>0.52 (0.41-0.65)</td>
</tr>
</tbody>
</table>

*Includes 288 genotyped cases and 32 cases without DNA available.

Table 4. Estrogen-Receptor (ER) Status of Tumors*

<table>
<thead>
<tr>
<th>ER-Positive</th>
<th>ER-Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>Tamoxifen</td>
</tr>
<tr>
<td>BRCA1 mutation</td>
<td>0</td>
</tr>
<tr>
<td>BRCA2 mutation</td>
<td>4</td>
</tr>
<tr>
<td>Wild type</td>
<td>132</td>
</tr>
</tbody>
</table>

*ER status unknown for 1 \textit{BRCA1} tumor, 2 \textit{BRCA2} tumors, and 28 wild-type tumors.
COMMENT

Healthy women with inherited cancer-predisposing BRCA1 or BRCA2 mutations face high risks of breast and ovarian cancer. Prophylactic mastectomy significantly reduces breast cancer risk among these women. Over 3 years of follow-up, invasive breast cancer occurred in 8 of 63 women with inherited BRCA1 or BRCA2 mutations, who had opted for surveillance alone, but in none of 76 women who underwent prophylactic mastectomy.37 Other data indicates that early prophylactic oophorectomy reduces the risk of subsequent breast cancer among BRCA1 mutation carriers by approximately 50%.38

The critical question for our study was whether chemoprevention, specifically prophylactic use of tamoxifen, would also reduce incidence of invasive breast cancer among cancer-free women with inherited BRCA1 or BRCA2 mutations. Given the sample size of the BCPT, inference from the genetic data alone cannot fully answer this question. The observed risk ratio for BRCA2 of 0.38 would be statistically significant if based on 24 rather than 11 cases, requiring a trial of approximately twice the size. The consistency of genetic and biological evidence favors tamoxifen for cancer-free women with BRCA2 mutations, but not for cancer-free women with BRCA1 mutations.

For women with BRCA1 mutations, an important question remains unanswered. It is possible that early in the course of BRCA1 tumors, tamoxifen might still have a role to play. If tamoxifen and oophorectomy are nearly equivalent, as they are in breast cancer treatment, and if oophorectomy is performed before age 35 years and is effective in reducing breast cancer incidence among women with BRCA1 mutations,38 then tamoxifen might be effective in younger, cancer-free women with BRCA1 mutations. This question could best be addressed by a prospective randomized trial involving a sufficient number of such women.

Finally, it is important to bear in mind that this study addressed the efficacy of tamoxifen in reducing incidence of breast cancer among healthy women with BRCA1 or BRCA2 mutations. The BCPT, and thus this genetics study, did not address treatment with tamoxifen of existing breast cancer. For women with ER-positive breast cancer, including ER-positive tumors of women with BRCA1 or BRCA2 mutations, tamoxifen has been shown to be effective in reducing risk of contralateral breast cancer and recurrence of disease.39-41 In other words, it is well established that for ER-positive breast cancers, tamoxifen is an effective treatment regardless of the patient’s genotype. On the other hand, this genetic analysis of the BCPT reveals that for women who have not yet developed breast cancer, genotype at BRCA1 and BRCA2 has a major impact on the expected effect of tamoxifen in reducing incidence of primary breast cancer.

REFERENCES


