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An estrogen receptor genetic polymorphism and a history of spontaneous abortion — Correlation in women with estrogen receptor positive breast cancer but not in women with estrogen receptor negative breast cancer or in women without cancer

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Summary

We previously identified a polymorphism in the human estrogen receptor gene. In a preliminary study on women with estrogen receptor positive (ER^+) breast tumors, we found that the presence of the rarer of the two alleles, the B' allele, is correlated with a history of spontaneous abortion. Because that study evaluated only women with estrogen receptor positive (ER^+) breast cancer, it was unknown whether the observed correlation was restricted to the cancer group or was independent of breast cancer. We have now extended our analysis to include not only additional women with ER^+ breast cancer, but also those with estrogen receptor negative (ER^-) breast cancer and women without cancer. Results of the current study continue to show an association between the B' allele and a history of spontaneous abortion in the ER^+ breast cancer group. There was no such correlation either in the ER^- breast cancer group or in the group without cancer. Also, we continue to observe, in the ER^+ breast cancer group, a significantly higher concentration of ER protein in tumors from homozygous wild type women (genotype BB), than in the tumors from women who are heterozygous for the rarer allele (genotype BB'). We conclude that the combination of spontaneous abortion and the BB' ER genotype may be a marker for breast cancer susceptibility.

Introduction

A genetic basis for breast cancer development is probable in a small subset of women, namely those whom the disease strikes at an early age or who have many relatives with breast cancer [1]. There is far less evidence for a genetic basis for breast cancer in women who are afflicted later in life or who do not have a family history. In this latter group, predisposition may involve specific

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alleles of more than one gene, and may be influenced by the woman's life history and environment.

We have identified one genetic marker that is correlated with estrogen receptor positive breast cancer in middle aged and older women, but only in combination with a specific physiological marker. The genetic marker in question is a polymorphism in the estrogen receptor gene; the physiological marker is a history of spontaneous abortion.

We originally found the polymorphism by RNase protection studies of mRNA isolated from estrogen receptor positive (ER^+) human breast tumors [2]. In that study, we found that the newly identified polymorphism was a genetic allele rather than a somatic mutation in the tumors because the same polymorphism was detected in mRNA from noncancerous uterine tissue. The polymorphism was localized to the exon of the gene encoding the B domain of the protein [2]; therefore, the newly identified allele is hereafter referred to as the B' allele to distinguish it from the more common allele (the B allele).

ER^+ tumors from BB' heterozygotes had, on average, lower levels of measured estrogen receptor protein than tumors from ER^+ BB women [2]. Clinical data obtained from the women with ER^+ breast cancer showed that those with the BB' genotype were far more likely to have had a history of spontaneous abortion than those who were BB homozygotes [3].

To study a broader spectrum of women, we made use of an assay that analyzed genomic DNA rather than RNA. We compared the frequency of the B' allele in women with: 1) ER^+ breast cancer, 2) ER^- breast cancer, or 3) no cancer. In all groups, approximately 12% of the women were BB' heterozygotes, indicating that the B' allele alone is not a marker for breast cancer susceptibility [4].

We have now examined the ER B genotype and the reproductive histories of a larger number of women with ER^+ and ER^- breast cancer and women without cancer to determine whether the previously noted correlation of the BB' genotype and a history of spontaneous abortion is restricted to women with ER^+ breast cancer or is seen in BB' women in general.

Methods

Subjects

Women with breast cancer in this study had been patients in New York City at Mount Sinai Hospital between 1986 and 1991 or at Beth Israel Hospital between 1989 and 1992. Clinical and reproductive histories were obtained mainly from medical records at the hospital or, when necessary, by telephone interview by one of us who had no knowledge of the ER genotype of that individual.

Control subjects did not have breast cancer; they were women who had come to Long Island Jewish Hospital for routine gynecologic examination or for minor outpatient surgery in Dermatology or Plastic Surgery. Since women in the breast cancer groups were at least 30 years old, all cancer-free subjects were at least that old. All control subjects, after agreeing to participate in the study, filled out the questionnaire provided by the interviewing physician or nurse, and provided a blood sample for later DNA analysis.

All women in the study had been pregnant at least once. Pregnancy and spontaneous abortion include only those reported by the subjects; early, unrecognized pregnancy loss was not considered in this study. None of the women had had breast cancer during pregnancy.

The sample sizes of the breast cancer groups do not reflect the true relative proportion of women in each group; rather, they represent the minimum number of samples needed to reliably assess incidence of spontaneous abortion in each of the groups.

The incidence of spontaneous abortion in the following six groups was compared: BB' heterozygous women who had 1) ER^+ breast cancer, 2)

ER^- breast cancer, or 3) no breast cancer; BB homozygous women who had 4) ER^+ breast cancer, 5) ER^- breast cancer, or 6) no breast cancer.

Hospital records, the source of most of our data on reproductive history of breast cancer patients, did not include information on the order of full term and aborted pregnancies or on the patient's age at first spontaneous abortion. Therefore, this information was not sought from control subjects.

Detection of the ER-B polymorphism

The ER-B polymorphism was identified by two methods.

Method 1. Some frozen breast tumors were genotyped by a solution hybridization/RNase protection assay of total RNA [2]. The radiolabeled antisense RNA probe for these studies was the "ab1" subclone described in Ref. 2, Fig. 1.

Method 2. To extend the range of samples to be studied, we used the polymerase chain reaction to amplify genomic DNA around the polymorphic region of the ER gene, followed by allele specific oligonucleotide hybridization (PCR/ASO). After verification that the two assays gave internally consistent information on the ER B-genotype of several breast tumors, all other samples were analyzed by PCR/ASO only. This analysis used DNA obtained from frozen or paraffin-embedded breast tumors or from blood lymphocytes of women without breast cancer, as we have described [4].

The ASO hybridization assay was based on our reported DNA sequence analysis, describing two nearby point mutations in codons 86 and 87 [5]. The synthetic oligonucleotides used for hybridization were tctgaggCggcGgcgttcggc (wild type; B allele) and tctgaggTggcGgcgttcggc (variant: B' allele). We [6] and others [7] have now reported a corrected sequence for the B' allele, with only a point mutation in codon 87.

We have continued to use the B' oligonucleotide described above. Hybrids formed between it and the wild type B allele sequence can be readily destabilized because they differ by 2 of 21 nucleotides, thus facilitating identification of putative B'B' homozygotes.

All women with the ER B' allele in this study were heterozygous for it. In other ongoing studies we have identified at least two B'B' homozygotes (B. Schachter and S. Lehrer, unpublished observation).

Estrogen receptor values

Hormone receptor values of tumors were obtained from the Departments of Pathology at Mount Sinai and Beth Israel Hospitals. At both institutions, breast tumor ER concentrations were determined by the dextran-coated charcoal method using the commercially available kit (Rianen Assay System, DuPont Company, N. Billerica MA) and Scatchard analysis of the data.

We have scored all tumor samples with >3 fmol receptor/mg protein to be receptor positive. Using a slightly higher cutoff for receptor positivity (e.g. 10 fmol/mg protein) did not significantly alter the results, since very few samples fell in that range.

One BB' breast cancer patient included in this study had a tumor whose receptor status was assessed by immunocytochemistry, which is now the only method used in the Department of Pathology at Mount Sinai Hospital. This woman is considered only in the evaluation of reproductive history as a function of genotype. She could not be included in the assessment of receptor levels as a function of genotype.

Results

The incidence of spontaneous abortion was significantly greater in the BB' ER^+ breast cancer group than in the other five groups ($p = 0.0035$, Kruskal-Wallis one way ANOVA corrected for

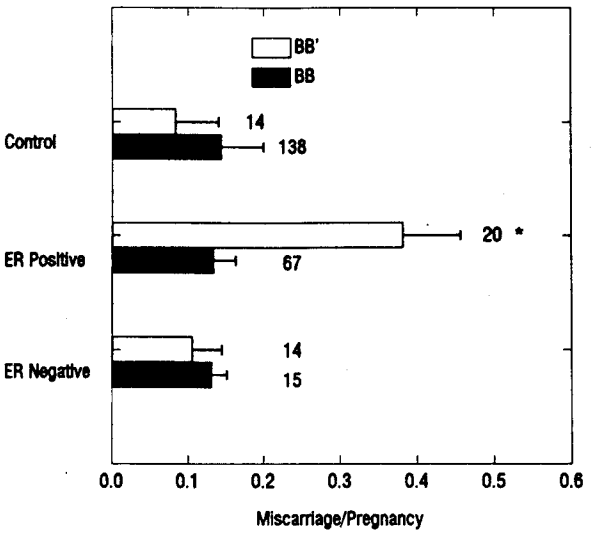

Figure 1. Ratio of spontaneous abortion (miscarriage) to pregnancy as a function of ER B genotype (BB' or BB) in breast cancer patients and in control women without breast cancer who had been pregnant at least once (mean ± SEM). Numbers to the right of each error bar are the number of women in each group. The miscarriage/pregnancy ratio is highest in BB' women with ER positive tumors. ($p = 0.0035$ by Kruskal Wallis one way ANOVA corrected for ties; * indicates that this group is significantly different from the other 5 groups by the Tukey B multiple range test at the 0.05 level).

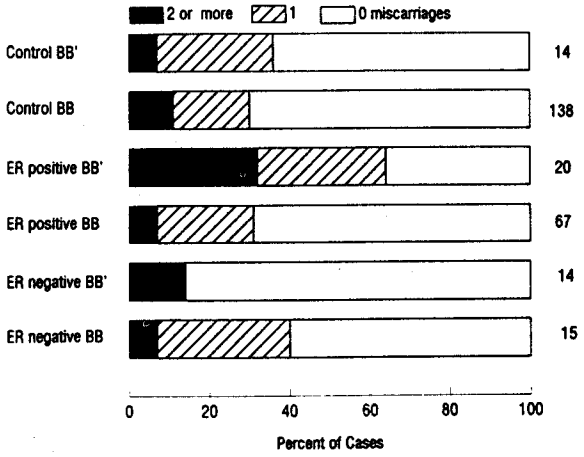

Figure 2. Percentage of women in each of the study groups who had zero, one, or two or more miscarriages. Study groups, indicated on the ordinate, are the same as those in Figure 1. The total number of cases in each group is indicated to the right of the corresponding bar. Women with ER^+ breast tumors and the BB' genotype had the highest incidence of a history of miscarriage (Chi-square = 23, $p = 0.011$).

ties; Figure 1). Whereas the incidence of spontaneous abortion per pregnancy in the BB' ER^+ group was 38%, in the other five groups it was not substantially different from the reported values of 12-16% [8,9].

Moreover, fourteen of the twenty women (70%) in the BB' ER^+ group had a history of at least one spontaneous abortion. In contrast, in the other five groups, the percentage of miscarriers ranged from 14% to 40% (Chi square = 23, $p = 0.011$; see Figure 2). There were no significant differences among the six groups in the number of full term pregnancies per woman, as we had reported in our preliminary study.

We compared the tumor ER protein concentrations from the BB ER^+ and BB' ER^+ women analyzed above; square-root transformation was done on receptor values to normalize the variances of each group. We found that the BB' ER^+ women had significantly lower ER concentrations than the ER^+ BB women ($p = 0.037$, separate variance estimate; Figure 3). Insufficient amounts of frozen tumors from BB' women remained for further analyses to determine the molecular basis for the low measured ER levels.

Discussion

In our previous study, we found that women with ER^+ breast cancer and the ER BB' genotype had a surprisingly high incidence of spontaneous abortion. We have now expanded our analysis, and find that the correlation between the ER BB' genotype and miscarriage is *restricted*'' to the ER^+ breast cancer group. No such association was seen in women without breast cancer. Therefore, the genetic polymorphism is not linked in a simple way with problems of pregnancy maintenance. In addition, association of spontaneous abortion and the BB' allele is not seen in women with ER^- breast cancer,

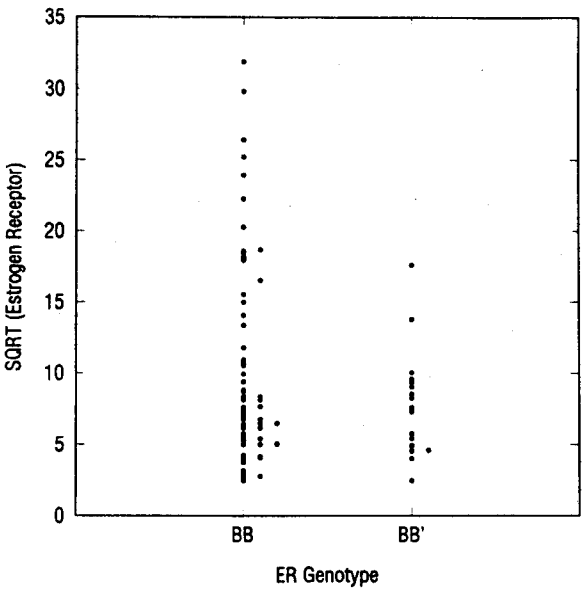

Figure 3. Estrogen receptor concentration in ER^+ breast tumors of women with the ER BB and BB' genotypes. Receptor concentrations, measured by steroid binding, have been transformed to square-root values. The mean of the BB group (10.4; N = 65) was significantly greater than that of the BB' group (7.9; N = 19) (p = 0.037; separate variance estimate).

suggesting that there may be different genetic and life history bases for development of certain ER^+ and ER^- breast cancers.

As was mentioned, the single point mutation of the B' allele does not result in an amino acid change in the ER protein. Therefore, another mutation, segregating with the B' allele, may play a role in the spontaneous abortions of the ER^+ breast cancer patients. Our results showing significantly lower levels of estrogen receptor in BB' than in BB tumors suggest that the linked mutation may indeed lie elsewhere in the estrogen receptor gene.

The combination of the BB' genotype and spontaneous abortion may be a marker for breast cancer susceptibility. Knowing if a woman is susceptible to breast cancer is important because experimental and clinical evidence suggest that treatment with the antiestrogen tamoxifen may be preventive [10-12]. Indeed, in the United Kingdom, women with a high risk of breast cancer are now enrolled in a large double-blind randomized study using tamoxifen or placebo as the chemopreventive agent [13]. A similar, large scale study has recently begun in the United States [14]. Hence, it will be useful to determine whether women with the B' allele and a history of spontaneous abortions have a significantly increased relative risk of developing ER^+ breast cancer, and could benefit from tamoxifen chemoprevention.

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