

Estrogen receptor polymorphism, spontaneous abortion, and breast cancer risk

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Abstract. In 1988 we identified a polymorphism in the estrogen receptor (ER) gene. The newly identified allele, called B', contains a silent mutation in codon 87, part of the receptor's B domain. Because of the association we previously reported of the B' allele, spontaneous abortion, and estrogen receptor positive breast cancer, we have now performed a preliminary case-control study to estimate the risk of breast cancer in women with the B' allele. Among BB' heterozygotes with ER-positive breast cancers (23 cases, 27 controls), the risk of this type of cancer was strongly related to a history of spontaneous abortion; the age-adjusted odds ratios were 4.1 (95% confidence limits, 0.95-18) after one spontaneous abortion and 9.7 (1.6-61) after two or more spontaneous abortions. No such association was seen for the risk of ER-negative breast cancer in BB' heterozygotes (N=18). Moreover, among BB homozygotes (137 cancer patients, 235 controls), spontaneous abortion was not related to an increased risk of breast cancer, either ER-positive or ER-negative. In BB' patients with ER-positive tumors, receptor concentrations were significantly lower in those who had a history of spontaneous abortion than in those without previous spontaneous abortions. These findings suggest involvement of a functional mutation associated with the B polymorphism, which is either elsewhere in the ER gene region or in a closely linked gene.

Introduction

In 1988 we identified a variant allele of the estrogen receptor (ER) gene (1). The variant differs from the wild type allele within the coding sequence for the B domain of the receptor, and is therefore referred to as the B' allele. About 12% of the general population carry the B' allele (2).

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Clinically, we noticed an association between the B' allele and a history of spontaneous abortion in women with estrogen receptor (ER)-positive breast cancer (3). In contrast, we saw no such association for women with ER-negative tumors. Nor did there seem to be any relationship between the carrier state for the allele and the risk of breast cancer in women without a history of spontaneous abortion (4).

We have now collected additional data on breast cancer patients and controls. These data enable us to make an estimate of the risk of breast cancer in women carrying the B' allele in a preliminary case-control study. We selected four groups of patients with breast cancer: BB' heterozygotes and BB homozygotes, with and without ER in their tumors, as well as a healthy control group. Our findings suggest a high risk of estrogen receptor positive breast cancer in the subset of women who carry the B' allele and have a history of spontaneous abortion.

Materials and methods

With the intent of performing a case control study, we used data from 178 breast cancer patients who had been pregnant at least once; we selected only women who had been pregnant because we were specifically interested in the incidence of spontaneous abortion. The women had been seen in New York City at Mount Sinai Hospital between 1986 and 1991 or at Beth Israel Hospital between 1989 and 1992. We further selected the patients according to whether their tumors were classified as ER-positive (N=119) or ER-negative (N=59) and whether they were carriers of the variant allele for the estrogen receptor gene (BB', N=41) or wild-type homozygotes (BB, N=137). We then surveyed their medical records to obtain clinical and reproductive histories, completing this information, when necessary, by telephone interview with the patient. The surveyor (interviewer) had no knowledge of the ER genotype or estrogen receptor status of the individual. We included only information on spontaneous abortion recognized by the patient; no attempt was made to gather information on unrecognized spontaneous abortion (5).

We also surveyed the reproductive histories and ER gene alleles of a group of control women (without cancer) of

Table I. Effect of past history of spontaneous abortion on the risk of breast cancer for four selected groups of tumors.

Genotype	Tumor type	Previous spontaneous abortion	N cases	N controls	Age adjusted odds ratio	95% confidence limits
BB'	ER pos	0	8	19	1.0	—
		1	8	6	4.1	0.95-18
		2+	7	2	9.7	1.6-61
	ER neg	0	16	19	1.0	—
		1	0	6	—	—
		2+	2	2	1.2	0.15-9.3
BB	ER pos	0	64	162	1.0	—
		1	22	47	1.2	0.67-2.2
		2+	10	26	1.0	0.46-2.2
	ER neg	0	28	162	1.0	—
		1	9	47	1.1	0.47-2.4
		2+	4	26	0.95	0.31-3.0

similar age who were patients at a nearby medical center (Long Island Jewish Hospital). These women were visiting gynecologists for routine examinations or undergoing minor outpatient surgery in Dermatology or Plastic Surgery, and had also been pregnant at least once.

The B' allele of the estrogen receptor gene was identified by one of two methods described in detail elsewhere (1,2). Briefly, initial studies on breast cancer patients used solution hybridization/RNase protection of tumor RNA (1,3). 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 (2,4). 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 (2).

Breast tumor ER concentrations were obtained from the hospital clinical pathology laboratories. In all cases the values had been determined by a multi-point hormone binding assay using a commercially available kit (Rianen Assay System, DuPont Company, N. Billerica, MA). A tumor was considered to be ER-positive if it contained at least 10 fmol ER/mg protein. This definition of ER-positive was used because tumors with ER concentrations below 10 fmol/mg are generally considered negative or weakly positive (6).

Odds ratios for the risk of breast cancer were calculated using unconditional logistic regression, by SPSS (7), adjusting for age as a continuous variable. Significance tests for trend were carried out using the method of Mantel and Haenszel (8).

Results

Of the 178 breast cancer patients surveyed, the ages ranged from 31 to 99 (mean 59.0), while the controls were aged 31

to 85 (mean 56.4). Approximately 15% were Black, 4% Hispanic and 1% Asian, with no significant differences in the distribution of race between the different groups of patients or between patients and controls. The average numbers of pregnancies in cases and controls respectively were 3.3 ± 1.8 (mean \pm SD) and 3.2 ± 2.5 . The proportions of spontaneous abortions per pregnancy were 0.17 ± 0.27 and 0.13 ± 0.24 . There was a family history of breast cancer in approximately 29% of the cases versus 20% of the controls. There was no significant association between ER genotype and family history of breast cancer ($p=0.22$).

Table I shows the age-adjusted odds ratios for ER-positive and ER-negative breast cancers in women having the BB and BB' genotypes, according to the number of spontaneous abortions. The reference group in each case is women having no history of spontaneous abortions. Heterozygote (BB') women had a four-fold increase in the risk of ER-positive breast cancer (odds ratio=4.1, 95% confidence interval (CI)=0.95-18) if they gave a history of one spontaneous abortion. After two or more spontaneous abortions, BB' women had more than a nine-fold increase in the risk of these tumors. (OR=9.7, 95%CI=1.6-61). The findings are unlikely to be due to chance (p for trend=0.008).

Also as indicated in Table I, there was no significant association between ER-negative breast cancer and a history of spontaneous abortion in BB' heterozygote women. However, the numbers available for study are small, so that an effect cannot be excluded. In BB (wild-type) homozygotes the risk of breast cancer did not vary according to a history of spontaneous abortion, either for ER-positive or ER-negative tumors.

In the BB' ER-positive breast tumor group, measures of ER protein concentration in the tumors varied according to the history of spontaneous abortion. The tumors of women who had had no spontaneous abortions had ER concentration of 210 ± 198 fmol/mg (mean \pm SD); the tumors of women who had had one spontaneous abortion had ER concentration of 65 ± 28 fmol/mg; and the tumors of women with two or more spontaneous abortions had ER concentration of 42 ± 26

fmol/mg. This variability was significant ($p=0.02$, Kruskal-Wallis one way ANOVA) and the tumor ER concentrations of the latter two groups were significantly lower than the former ($p<0.05$, Tukey B multiple range test). A relationship between tumor ER concentration and history of spontaneous abortion was not present in the ER BB breast cancer patients ($p=0.34$), nor has such a relationship been demonstrated in other studies (9).

The average age of the patients with BB' ER-positive breast cancer was 58 ± 12 , and was not significantly different from the ages of the other breast cancer patients or controls. The average number of pregnancies in BB' women with ER-positive breast cancer was 3.8 ± 2.3 , which is not significantly different from the average number of pregnancies of the other cancer patients or controls. There was no difference in the mean number of full-term pregnancies in any of the groups ($p=0.9$, one way ANOVA).

Discussion

Several studies have investigated a possible role for spontaneous abortion as a risk factor for breast cancer. Some reports have indicated a small but statistically significant effect (10-13) whereas others have found no such effect (14-16).

Addressing a separate issue, many studies have asked whether specific risk factors are associated with ranges of tumor estrogen receptor levels. For example, both patient age at tumor diagnosis (17,18) and age at first full term pregnancy (19) may be inversely related to the level of ER in the tumors. Factors such as race (6) or percentage of calorie intake represented by fat versus carbohydrate (20) may contribute to the tumor ER level. One study, which analyzed the prevalence of genotypes of a diallelic locus in the first intron of the estrogen receptor gene, found an association of one homozygous genotype with ER-positive tumors and the other homozygous state with ER-negative tumors (21). However, a separate analysis of the same locus showed no such association with tumor ER status (22). Also, a subgroup of ER-positive tumors was found to be associated with gene amplification on chromosome band 11q13 (23); whether this amplification is a cause or a consequence of tumor ER-positivity is not known.

Results of the current study provide evidence both to support the notion that a history of miscarriage may be a risk factor for breast cancer, and that a specific set of risk factors may selectively predispose certain individuals to ER-positive but not ER-negative breast cancer. Specifically, our results suggest that spontaneous abortion is a significant risk factor for breast cancer, but only in a subgroup of women, namely those who carry the ER B' allele. Moreover, the risk in this genetically defined group who have had miscarriages is only for estrogen receptor positive breast cancer, and indeed, only for tumors that contain low-to-moderate, but not high, ER levels.

One possible limitation of our study is that DNA from different sources - frozen and paraffin-embedded breast tumor tissue, and blood lymphocytes - was used for identifying the B' allele. However, when we had occasion to genotype DNA from the same individual from tumor and

blood, the results were identical. A second limitation is that our sample of BB' heterozygotes was too small to properly evaluate other breast cancer risk factors. However, the relative risks for these factors - family history of breast cancer, early menarche, late age at first birth, etc. - are quite small. For example, the factor with highest relative risk, for mother and sister with breast cancer, is 2.5; for a sister alone, the relative risk is 1.84 (24). For menarche earlier than age twelve, relative risk is 1.72 (24). For age at first birth over thirty, relative risk is 1.86 (24). Nevertheless, because of their magnitude, these risk factors would not be expected to greatly alter the estimate of estrogen receptor positive breast cancer risk in women with the B' allele and a history of spontaneous abortion (Table I).

The B' allele described in this study results from a single base change in the third position of codon 87 (25,26). The change is a silent mutation since both the B allele triplet GCG and the B' allele triplet GCC code for the same amino acid, alanine. Hence, the B' allele itself seems unlikely to be the functional mutation increasing the risk of breast cancer among women who miscarry.

We postulate that a functional mutation segregating with the B' allele may cause miscarriage and increased risk for ER-positive breast cancer. This second mutation might lie within the ER gene itself or within one of the genes nearby. Our data showing a difference in tumor ER levels in women in the specific risk group suggest that the postulated mutation is indeed elsewhere in the ER gene. For example, there could be non-random segregation of the B' allele and a mutation in a regulatory region of the ER gene that results in relatively low levels of ER transcription, or a mutation in the coding sequence that causes reduced stability of the ER protein. Further studies may clarify this matter.

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