Oestrogen receptor B-region polymorphism and spontaneous abortion in women with breast cancer

STEVEN LEHRER MERCEDES SANCHEZ HEE KYUNG SONG
JACK DALTON EVA LEVINE PENNY SAVORETTI SWAN N. THUNG
BETH SCHACHTER

To examine whether a variant human oestrogen receptor gene, which differs from the wild-type gene in the B region, was associated with spontaneous abortion, obstetric histories of breast cancer patients with this variant were compared with those of breast cancer patients with the wild-type gene. In women with the B-variant, 50% of pregnancies ended in spontaneous abortion, compared with 10% for women homozygous for the wild-type gene. B-variant women also had a higher proportion of spontaneous abortions and a higher number of spontaneous abortions per woman than did those with the wild-type gene.

Lancet 1990; **335**: 622–24.

Introduction

Most publications on genetic causes of habitual spontaneous abortion deal with chromosomal abnormalities such as translocations, inversions, and mosaicism.[1] There is also speculation about a relation between single-gene defects and spontaneous abortions when there are no discernible chromosomal abnormalities,[2] since chromosomally normal women who habitually abort spontaneously have increased likelihood of a family history of repeated pregnancy loss.[3]

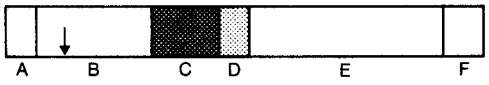

Fig 1—Schematic representation of the human estrogen receptor gene.

The exact function of region A is unknown. Region B plays a part in transcription enhancement of ER-regulated genes. Region C is the DNA-binding domain. Region D is a hinge region. Region E is the steroid-hormone-binding domain. The function of region F is unknown. The vertical arrow indicates the location of the ER B-polymorphism evaluated in this study.

A variant human oestrogen receptor (ER) gene has been found in ER-positive breast carcinomas.[4] Because the variant has also been detected in uterine tissue obtained at hysterectomy from women without breast cancer, it is a genetic polymorphism, rather than a somatic mutation in the breast tumour samples. The variant ER gene differs from the wild-type gene within the B region,[5] the exon coding for the aminoterminal portion of the protein (fig 1). Two nearby point mutations in the B region change codon 86 from Ala to Val and create a silent mutation in codon 87.[6] We describe here an association between the presence of the B-variant gene and spontaneous abortion.

Materials and methods

The identification of women who carry the variant ER gene has been described elsewhere.[4,5] Briefly, frozen biopsy specimens of primary breast tumours were analysed for the concentration and structure of ER messenger RNA (mRNA) by means of a solution hybridisation/RNase protection assay[6] that used, as hybridisation probes, ^{32}P-antisense RNAs produced from subcloned plasmids corresponding to portions of the protein-coding region of the human ER cDNA.[4] All samples thus identified as B-variants seemed to be heterozygous for the gene,[5] and 3 of the women have been confirmed to be heterozygotes by polymerase chain reaction (PCR) amplification and DNA sequence analysis.[7] Nonetheless, the RNase digestion at the point of RNA/RNA hybrid mismatch may not be complete (ref 6 and Sanchez M, Schachter B, unpublished), so samples may seem to be heterozygous when they are actually homozygous variants—ie, some women may be homozygous ER B-variants rather than heterozygotes.

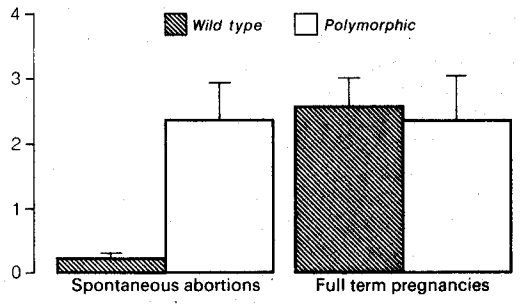

Fig 2—Mean (SE) number of spontaneous abortions per woman and mean (SE) number of full-term pregnancies per woman.

There was a significantly higher number of spontaneous abortions per woman in polymorphic women ($t' = 3{\cdot}14$, $df = 7{\cdot}32$, $p = 0{\cdot}016$ by separate variance estimate), but the number of full-term pregnancies per woman was not significantly different ($t = 0{\cdot}41$, $df = 29$, $p = 0{\cdot}682$).

Data on the occurrence of spontaneous abortion was obtained from patients as part of a larger set of clinical questions. The information was obtained by two of us (E. L., P. S.), who had no knowledge of the ER genotype of the patients. Pregnancy and sponaneous abortion were defined as clinically recognised pregnancy and clinically recognised spontaneous abortion. Early, unrecognised pregnancy loss was not considered in this study. The information on number of pregnancies and number of spontaneous abortions was obtained retrospectively from women undergoing

ADDRESSES: **Departments of Radiotherapy** (S. Lehrer, MD, H. K. Song, MD, E. Levine, RN, J. Dalton, MD, P. Savoretti, RN), **Obstetrics and Gynaecology, and Cell Biology and Anatomy** (B. Schachter, PhD, M. Sanchez, PhD), **and Stratton Popper Department of Pathology** (S. N. Thung, MD), **Mount Sinai School of Medicine, New York.** Correspondence to: Dr S. Lehrer, Radiation Therapy, Box 1236, Mount Sinai Medical Center, New York, NY 10029, USA.

NUMBER OF SPONTANEOUS ABORTIONS AND FULL-TERM PREGNANCIES IN THE TWO GROUPS

—	Number with wild-type gene (n=23)	Number with variant gene (n=8)
Number of spontaneous abortions		
0	16	2
1	7	1
2	0	1
4	0	4
Number of full-term pregnancies		
0	0	2
1	4	0
2	10	3
3	5	1
5	3	2
7	1	0

For spontaneous abortions: $\chi^2 = 17{\cdot}145$, $p = 0{\cdot}0007$.
For full-term pregnancies: $\chi^2 = 8{\cdot}3$, $p = 0{\cdot}14$.

treatment for breast cancer. To eliminate any effect that ER level might have on the incidence of spontaneous abortion, we chose as controls homozygous women with wild-type ER at concentrations in the same range as those of the B-polymorphic women—less than 200 fmol/mg.

The two groups, wild-type homozygous and polymorphic, were selected according to whether or not the polymorphism was present in the ER mRNA of the breast tumour biopsy specimen. Biopsies and interviews were done between 1985 and 1989. Only women on whom reproductive data were available were included in this study.

Results

The eight B-variant women had had 19 spontaneous abortions in 38 pregnancies (50%), compared with 7 spontaneous abortions in 68 pregnancies (10%) for the controls ($\chi^2 = 20{\cdot}76$, $p < 0{\cdot}001$). Cross-tabulation of the data showed a significantly higher proportion of abortions among the B-variant women; and the abortion rate per woman was also significantly higher in the B-variant women (table and fig 2).

All the tumours in B-variant women and controls had an oestrogen receptor. Because the method used to detect the polymorphism required ER m-RNA, we were unable to study tumours without an oestrogen receptor; therefore, the exact frequency of the ER B-variant allele in women with ER-negative breast cancer is uncertain.

Discussion

Spontaneous abortion is common and occurs in 12–16% of normal pregnancies.[8,9] The 50% of pregnancies that ended in spontaneous abortion in the eight B-variant women was thus a high proportion, whereas the 10% in the controls was similar to that reported for normal pregnancies.

Several studies have shown a need for oestrogen in early pregnancy. Pregnancy in bonnet monkeys has been inhibited by giving oral tamoxifen, which blocks the oestrogen receptor, during the post-ovulatory period.[10] Fetal resorption occurred when tamoxifen was given orally to rabbits on day 10 after implantation,[11] and when it was given later in the course of a pregnancy it reduced significantly the length of gestation and number of livebirths. Since oestrogen most probably exerts its effect through its interaction with cellular ER, naturally occurring genetic variation in the structure of the ER could result in a corresponding phenotypic difference in oestrogen-dependent events, including those involved in pregnancy maintenance.

Recent studies, employing mutated ER expression vectors in in-vitro transfection studies, have begun to delineate the function(s) of the B region of the ER protein. For example, an ER mutant lacking aminoacids 67–131 retained full oestrogen-dependent transcriptional enhancement on the vitellogenin oestrogen response element (ERE) but had only 60% of the wild-type ER enhancement activity on the pS2 ERE.[12] Also, a deletion mutant lacking aminoacids 1–178 (the entire A/B region) completely retained its ability to transactivate vitellogenin ERE but could not transactivate ovalbumin ERE.[13] Thus the amino terminal portion of the ER probably influences the magnitude of transactivation of some, but not all, ER-regulated genes, perhaps through interaction with other chromatin-associated proteins.

Further indication that the region containing the B-variant sequence has an important function is inferred from the sequence conservation among species. The stretch of aminoacids 80 to 89 in the wild-type human ER has an identical counterpart in the B-region of the rat[14] and mouse[15] ER. Thus, it is conceivable that the association between the B-variant genotype and high incidence of miscarriage reflects altered bioactivity of the ER B-variant protein in some (maternal or fetal) cell types needed for pregnancy maintenance. Alternatively, or in addition, individuals carrying a B-variant gene may, under some circumstances, produce insufficient amounts of ER protein.

It is of interest that clinically recognised pregnancy occurred more commonly among the B-variant women (38 pregnancies in eight women) than in the controls (68 pregnancies in 23 women), whereas the two groups did not differ in full-term pregnancy rate (2·7 versus 2·4, fig 2).

No doubt the B-variant women became pregnant more often to overcome their 50% likelihood of spontaneous abortion. However, once they had had two or three children, like the wild-type-ER women, they stopped trying to have more.

A history of spontaneous abortion has been associated with breast cancer,[16–18] although there is controversy over this association.[19–22] Also, as mentioned above, habitual spontaneous abortion is familial.[3] Finally, spontaneous abortion and breast cancer occurred in the sisters of one of our polymorphic breast cancer patients, who herself had had multiple spontaneous abortions. Therefore, one wonders whether there is an association between the B-polymorphism and breast cancer.

We thank Dr James Schmeidler for statistical advice; Dr Mark Bergman for helpful discussions; and Dr Teresa Garcia, for her contributions to the early part of the study.

The study was funded in part by NIH grants CA 45754 and CA49506-01A1.

REFERENCES

1. Maier DB. Genetic and infectious causes of habitual abortion. In: Gondos B, Riddick DH, eds. Pathology of infertility. New York: Thieme Medical Publishers, 1987: 201–18.
2. Simpson JL. Genes, chromosomes, and reproductive failure. *Fertil Steril* 1980; **33:** 107–16.
3. FitzSimmons J, Tunis S, Jackson D, Wapner RJ, Jackson L. Factors related to subsequent reproductive outcome in couples with repeated pregnancy loss. *Am J Med Genet* 1984; **18:** 407–11.

4. Ponglikitmongkol M, Green S, Chambon P. Genomic organization of the human estrogen receptor gene. *EMBO J* 1988; **7:** 3385–88.
5. Garcia T, Lehrer S, Bloomer WD, Schachter B. A variant estrogen receptor messenger ribonucleic acid is associated with reduced levels of estrogen binding in human mammary tumors. *Molec Endocrinol* 1988; **2:** 785–91.
6. Winter E, Yamamoto F, Almoguera C, Perucho M. A method to detect and characterize point mutations in transcribed genes: amplification and overexpression of the mutant c-Ki-ras allele in human tumor cells. *Proc Nat Acad Sci USA* 1986; **82:** 7575–79.
7. Garcia T, Sanchez M, Cox JL, et al. Identification of a variant form of human estrogen receptor with an aminoacid replacement. *Nucleic Acids Res* 1989; **17:** 8364.
8. Mills JL, Simpson JL, Driscoll S, et al. Incidence of spontaneous abortion among normal women and insulin-dependent diabetic women whose pregnancies were identified within 21 days of conception. *N Engl J Med* 1988; **319:** 1617–23.
9. Wilcox AJ, Weinberg CR, O'Connor JF, et al. Incidence of early loss of pregnancy. *N Engl J Med* 1988; **319:** 189–94.
10. Ravindranath N, Moudgal NR. Use of tamoxifen, an anti-estrogen, in establishing a need for estrogen in early pregnancy in the bonnet monkey *(Macaca radiata)*. *J Reprod Fertil* 1987; **81:** 327–36.
11. Furr BJA, Valcaccia B, Challis JRG. The effects of Nolvadex (tamoxifen citrate; ICI 46,474) on pregnancy in rabbits. *J Reprod Fertil* 1976; **48:** 367–69.
12. Kumar V, Green S, Stack G, Berry M, Jin J, Chambon P. Functional domains of the human estrogen receptor. *Cell* 1987; **51:** 941–51.
13. Tora L, Gaub MP, Mader S, Dierich A, Bellard M, Chambon P. Cell specific activity of a GGTCA half palindromic estrogen responsive element in the chicken ovalbumin gene promoter. *EMBO J* 1988; **7:** 3771–78.
14. Koike S, Sakai M, Muramatsu M. Molecular cloning and characterization of rat estrogen receptor cDNA. *Nucl Acids Res* 1989; **15:** 2499–513.
15. White R, Lees JA, Needham M, Ham J, Parker M. Structural organization and expression of the mouse estrogen receptor. *Molec Endocrinol* 1989; **1:** 735–44.
16. Pike MC, Henderson BE, Casagrande JT, Rosario I, Gray GE. Oral contraceptive use and early abortion as risk factors for breast cancer in young women. *Br J Cancer* 1981; **43:** 72–76.
17. Hadjimichael OC, Boyle CA, Meigs JW. Abortion before first livebirth and risk of breast cancer. *Br J Cancer* 1986; **53:** 281–84.
18. Brinton LA, Hoover R, Fraumeni JF. Reproductive factors in the aetiology of breast cancer. *Br J Cancer* 1983; **47:** 757–62.
19. Berkowitz GS. Risk Factors. In: Marchant DJ, Kase NG, Berkowitz RL, eds. Breast disease. New York: Churchill Livingston, 1986: 13–41.
20. Salber EJ, Trichopoulos D, MacMahon B. Lactation and reproductive histories of breast cancer patients in Boston 1965–66. *JNCI* 1969; **43:** 1013–24.
21. Paffenbarger RS, Kampert JB, Chang HG. Characteristics that predict risk of breast cancer before and after the menopause. *Am J Epidemiol* 1980; **112:** 258–68.
22. Helmrich SP, Shapiro S, Rosenberg L, et al. Risk factors for breast cancer. *Am J Epidemiol* 1983; **117:** 35–45.

Detection of anti-listeriolysin O for serodiagnosis of human listeriosis

PATRICK BERCHE KARL A. REICH MARIE BONNICHON
JEAN-LUC BERETTI CHRISTIANE GEOFFROY JACQUES RAVENEAU
PASCALE COSSART JEAN-LOUIS GAILLARD PIERRE GESLIN
HENRI KREIS MICHEL VERON

To see whether detection of antibodies against listeriolysin O (LLO) could be used to diagnose human listeriosis, sera from 28 patients infected with *Listeria monocytogenes* and 101 controls were tested by dot-blot titration with purified LLO. 27 patients (96·4%) with listeriosis produced specific anti-LLO. Anti-LLO was detected in 8 (15·6%) of 51 healthy controls and in 6 (12·0%) of 50 controls who had various bacterial, fungal, and viral infections. Anti-LLO titres did not exceed 100 in these two control groups. Anti-LLO could be detected soon after clinical onset of listeriosis, and antibodies persisted for at least several months. This test might be useful for epidemiological surveys and for serodiagnosis of listeriosis, especially when bacteria cannot be isolated.

Lancet 1990; **335:** 624–27.

Introduction

Listeria monocytogenes is believed to be transmitted to man mainly via contaminated food.[1] Although immunocompromised patients and pregnant women are especially susceptible to listeriosis, the disease can also develop in seemingly healthy individuals. A vital stage of the infection process is the multiplication of the organism within the cytoplasm of host cells;[2] genetic studies have shown that an extracellular haemolysin, listeriolysin O (LLO), is essential for this intracellular multiplication.[3-7] LLO is a 58 kDa protein belonging to the group of SH-activated haemolysins; it is antigenically related to streptolysin O (SLO), pneumolysin, and perfringolysin,[8,9] and is produced by all pathogenic strains of *L monocytogenes*. We here investigate whether detection of specific anti-LLO could be used for serodiagnosis of human listeriosis.

Patients and methods

Patients

28 patients with severe listeriosis (septicaemia and/or meningoencephalitis) were studied. Three groups were assessed: (1) 13 newborn babies with listeriosis (12 at birth, 1 at age 11 days), and 2 pregnant women, aged 23 and 25 years, with bacteraemia

ADDRESSES: **Laboratoire de Microbiologie** (Prof P. Berche, MD, M. Bonnichon, J. L. Beretti, J. Raveneau, J. L. Gaillard, MD, M. Veron, MD) **and Unité de Transplantation, Département de Néphrologie** (H. Kreis, MD), **Hôpital Necker-Enfants Malades, Paris; Unité de Génie Microbiologique, Département des Biotechnologies** (K. A. Reich, PhD, P. Cossart, PhD), **and Unité des Antigènes Bactériens** (C. Geoffroy, PhD), **Institut Pasteur, Paris; and Centre National de Référence des Pneumocoques** (P. Geslin, MD), **Creteil, France.** Correspondence to Prof P. Berche, Laboratoire de Microbiologie, Hôpital Necker-Enfants Malades, 75730 Paris Cedex 15, France.