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Possible Linkage of the Estrogen Receptor Gene to Breast Cancer in a Family with Late-Onset Disease

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Summary

The estrogen-receptor locus is a candidate gene for inherited susceptibility to human breast cancer, particularly among families with later onset, primarily estrogen-receptor-positive tumors. For one extended family with eight patients with late-onset disease, one estrogen-receptor haplotype was consistently coinherited with breast cancer, yielding a +1.85 lod score for linkage at zero recombination. Simulation of this pedigree assuming independent inheritance of breast cancer and estrogen-receptor genotypes led to a lod score ≥1.85 only once in 2,000 replicates. We suggest testing linkage of this gene to breast cancer in other families with late-onset disease.

Introduction

On the basis of its biological activity, the estrogen-receptor locus (ESR; McKusick 133430) is a candidate gene for human breast cancer. The cDNA and amino acid sequences of estrogen receptor are known (Walter et al. 1985; Green et al. 1986). The normal function of the cytoplasmic estrogen-receptor protein is to bind and transfer estrogens to nuclei, including nuclei of breast intraductal epithelial cells where breast cancer originates. Among breast cancer patients in the general population, the estrogen-receptor protein is detected in approximately 70% of malignant tumors from postmenopausal patients but in only approximately 30% of malignant tumors from premenopausal patients. The presence of estrogen receptor is associated with responsiveness to hormonal treatment and with favorable prognosis (McCarty et al. 1983).

Recent results suggest that chromosome 17q21 is the locale of a gene for susceptibility to early-onset, but not late-onset, familial breast cancer (Hall et al. 1990). This observation, coupled with the role of estrogen receptor in breast tumors of older patients, suggest that inherited alterations in ESR might be responsible for inherited susceptibility to late-onset human breast cancer. Consequently, we tested for linkage of ESR to human breast cancer in families with multiple relatives with late-onset disease.

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Material and Methods

Eleven families (families 13–23 in Hall et al. 1990) with multiple cases of breast cancer and whose average age at diagnosis age was ≥50 years were included in this analysis. The pattern of breast cancer in these families is consistent with inheritance of an autosomal dominant allele which is rare in the general population but highly penetrant among susceptible women, with lifetime risk of .86 (Newman et al. 1988). The maximum likelihood model also suggests that sporadic cases of breast cancer occur in these families, with a lifetime risk of .08 for nonsusceptible women. These parameters were incorporated into linkage analysis using the computer programs of LINKAGE with liability classes defined by age and sex (Lathrop et al. 1985). Homogeneity of lod score (Z) values among families was tested by using the computer program HOMOG (Ott 1985).

Genomic DNA was prepared from lymphoblastoid cell lines of informative relatives according to a method described elsewhere (Hall et al. 1989). South-

Table 1

Genomic Clones and Polymorphisms of ESR

Probe	Exon	Insert Size/Ends[a]	Contains Repeat Elements?	Polymorphism	Allele	Size (kb)	Frequency (*N*)
Q7	1	3.0 kd/*Eco*RI	No	. . .			
M72	2	1.6 kb/*Eco*RI	Yes	*Xba*I	A	10.5	.40 (146)
					B	7.5/3.0	.60
P18	3	2.8 kb/*Eco*RI-*Sac*I	Yes	. . .			
P1	4	2.7 kb/*Eco*RI	Yes	. . .			
H9	6	1.0 kb/*Eco*RI-*Hind*III	No	*Sac*I	A	22	.08 (136)
					B	11.5	.92
P5	7	7.5 kb/*Eco*RI	No	. . .			
R11	8	8.0 kb/*Eco*RI	Yes	*Hind*III	A	15	.65 (122)
					B	6.0	.35

[a] All inserts are in Bluescript plasmid (Stratagene).

ern blotting was carried out according to standard procedures (Maniatis et al. 1982; Feinberg and Vogelstein 1983, 1984). Clones with human repeated sequences were treated with placental DNA prior to hybridization (Litt and White 1985). Seven genomic clones containing seven exons of the large ESR gene (Ponglikitmongkol et al. 1988) were screened for polymorphism with restriction enzymes *Eco*RI, *Hin*dIII, *Bam*HI, *Pvu*II, *Pst*I, *Msp*I, *Taq*I, *Rsa*I, *Xba*I, *Stu*I, *Bgl*I, *Bgl*II, and *Sac*I. Polymorphisms were detected for three of the seven clones, each with one enzyme (Zuppan et al. 1989; table 1). For linkage analyses, recombination among the polymorphisms at the ESR locus was assumed to be zero.

Codominant segregation of the alleles for each of the three ESR polymorphisms was confirmed by screening the CEPH families. Caucasian population frequencies were estimated from CEPH parents and from unrelated individuals from Caucasian families in our series (table 1). Linkage disequilibrium among M72, H9, and R11 was tested by using the frequency distribution of 100 haplotypes of the three markers obtained from CEPH and breast cancer families. These haplotypes indicated no linkage disequilibrium among the three markers in this large gene. Testing random association of pairs of markers produced the following values $\chi_1^2 = .00$ for H9 and M72, $\chi_1^2 = .62$ for H9 and R11, and $\chi_1^2 = .62$ for M72 and R11. For the three markers as a group, the $2 \times 2 \times 2$ homogeneity $\chi_1^2 = .19$. Haplotype frequencies were estimated from the frequencies of the component alleles.

Results

Coinheritance of breast cancer with ESR haplotypes in family 22 is shown in figure 1. In this family, breast cancer was consistently inherited with the *AAA* haplotype of ESR, which has a frequency of .02 in the Caucasian population. For close linkage of ESR to breast cancer in family 22, $Z = 1.85$. In order to estimate how frequently a value of $Z \geq 1.85$ could be expected by chance in a family of this structure, we simulated 2,000 replicates of family 22 with genotypes for the three ESR markers under the null hypothesis of no linkage of ESR to breast cancer and estimated Z values by using LINKAGE as before. $Z \geq 1.85$ at a recombination fraction of .001 occurred in one of the 2,000 replicates, corresponding to an approximate P value of .0005, with a 95% confidence interval of 0 to .0024. However, $Z = 1.85$ corresponds to odds in favor of linkage that are only 70:1, well below the odds of 1,000:1 required for significance when multiple linkages are tested.

For the late-onset families as a group, ESR did not appear linked to breast cancer: $Z = -5.66$ at close linkage and $Z < -2$ for genes within approximately 5% recombination of ESR on chromosome 6q. However, the P value for homogeneity of recombination fractions between ESR and a breast cancer gene in these families was .04, indicating that the sample included both families with linkage of breast cancer to ESR and families in which breast cancer was not linked to this locus. The maximum likelihood estimate of the number of families in the sample that have linkage of

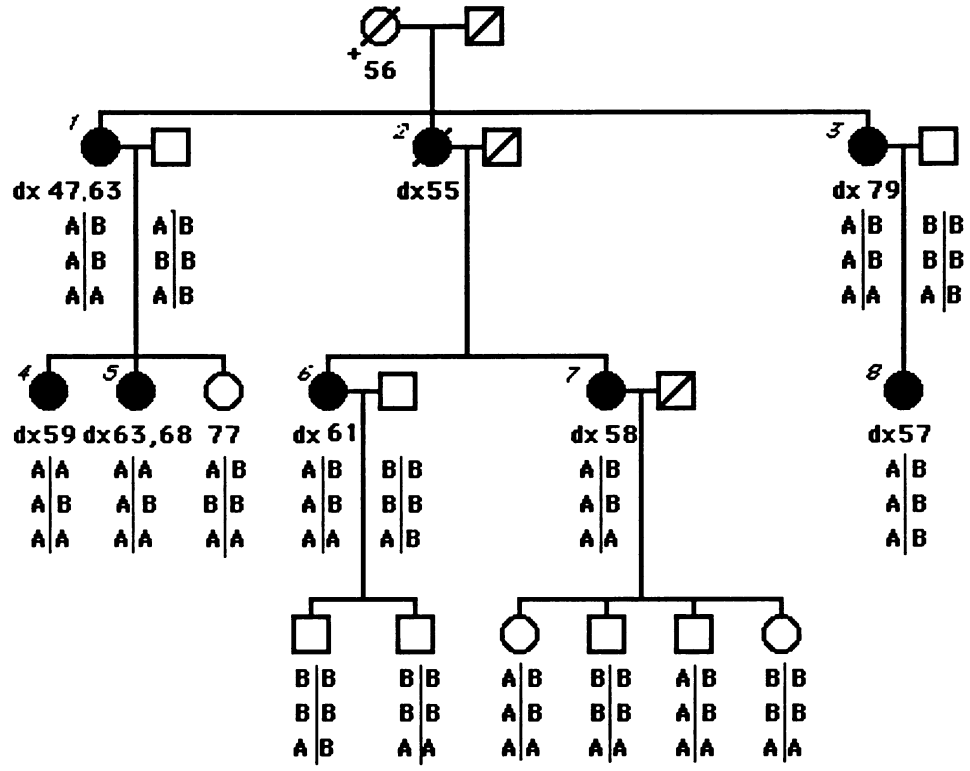

Figure 1 Linkage of breast cancer to ESR in family 22, for which $Z = +1.85$. Polymorphic markers of estrogen receptor are, from top to bottom, M72*Xba*I, H9*Sac*I, and R11*Hin*dIII. Alleles and their frequencies are listed in table 1.

breast cancer to ESR was one family, specifically family 22.

Discussion

Among breast cancer families with late-onset disease, in which susceptibility is not linked to chromosome 17q21, the locales of other disease genes remain open questions. The most conservative, and probably most likely, interpretation of the present results is that ESR is not linked to breast cancer and that the positive Z value in family 22 occurred by chance. However, because there exist other series of late-onset breast cancer families in which linkage of the disease to ESR could be tested, and because family 22 is unusual among breast cancer families, some comments about this family might be useful for future comparative purposes.

The breast cancers in family 22 were diagnosed at significantly older ages than were breast cancers among women in the other families in our sample. Only one of the 10 malignant breast tumors in family 22 was diagnosed in a premenopausal woman. A second primary in the same patient was diagnosed 17 years later, after menopause. This is in marked contrast to the majority of patients in our other families. Breast cancer is very common in family 22, even by the standards of families ascertained for multiple cases. Of the nine women >45 years of age in two generations of the family, eight have developed a total of 10 malignant breast tumors. (The founding female of the family died of cancer of an unspecified site at age 56 years.) Histologic types of the breast cancers were not unusual: nine tumors were intraductal adenocarcinomas, and one was a lobular carcinoma.

Breast tumors of patients 3–8, all of whom were postmenopausal at diagnosis, were tested for estrogen-receptor protein; patients 1 and 2 were diagnosed before this was routine clinical practice. Tumors from patients 4–8 were positive; only patient 3 was negative for estrogen receptor. Inherited alterations in the estrogen-receptor protein might be of particular importance to the postmenopausal development of tumors in which the protein will be expressed.

An ESR polymorphism has been described which causes an amino acid change in the protein. At amino acid 86 in exon 2, alanine may be substituted by valine. In a small series of 31 breast cancer patients with estrogen-receptor-positive tumors who were not selected for family history, the presence of valine at amino acid 86 was associated with a spontaneous abortion risk of 50%, compared with 10% among breast cancer patients with the more common sequence (Lehrer et al. 1990). We have not determined amino acid 86 for the breast cancer patients in family 22, but their pregnancies did not appear to be at high risk of miscarriage. Patients 3–8, for whom complete pregnancy histories were available, had a total of two spontaneous abortions (one each for patients 4 and 6) and 14 live births, an overall risk of 12%. Our next step will be further characterization of the ESR sequences of the breast cancer patients, compared with that of the one unaffected sister in family 22.

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