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12. Abbreviations for the amino acid residues are: A, Ala; C, Cys D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
13. To mutagenize residues 52 to 58 simultaneously, the corresponding codons of one DNA strand of a cassette were synthesized by using a mixture of 62.5% of the wt base and 12.5% of each of the other three bases. This level of mutagenesis resulted in an average of two to three amino acid sequence changes per mutant protein. Synthesis of the second, complementary strand was performed enzymatically with a self-priming strategy [A. K. Oliphant, A. L. Nussbaum, K. Struhl, *Gene* **44**, 177 (1986)]. Double-stranded cassettes were generated by restriction cleavage and ligated into pUCroRS. This plasmid consists of the Eco RI to Sma I fragment of pAP119 (*3*) cloned between the Eco RI and Hinc II sites of pUC18 [C. Yanisch-Perron, J. Vieira, J. Messing, *Gene* **33**, 103 (1985)]. Plasmids were transformed into a strain in which Cro-mediated repression of a ribosomal protein s12 gene was required for resistance to streptomycin [M. C. Mossing and R. T. Sauer, in preparation; for an analogous selection, see (*4*)]. Active genes (identified as streptomycin resistant colonies) were recovered in <1% of transformants. The level of activity required for resistance varied depending on the level of Cro expression. When Cro expression was fully induced, <5% of wt activity was required.
14. A pUC19 [C. Yanisch-Perron, J. Vieira, J. Messing, *Gene* **33**, 103 (1985)] derivative plasmid containing a synthetic consensus Cro operator between the Xba I and Pst I sites was opened at the Bam H1 site and filled in with α-^{32}P–labeled dNTPs (deoxyribonucleotide triphosphates) by using the Klenow fragment of DNA polymerase. Subsequent digestion with Hind III and Kpn I yielded 12- and 45-bp (operator-containing)–labeled fragments as well as an unlabeled backbone fragment of 2.6 kb. This crude mixture was diluted into low salt buffer (final operator concentration 1 nM) and mixed with Cro or Cro.mDG, incubated for 20 min on ice, and subjected to DNAse digestion as described (*9*).
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Inheritance of Proliferative Breast Disease in Breast Cancer Kindreds

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Previous studies have emphasized that genetic susceptibility to breast cancer is rare and is expressed primarily as premenopausal breast cancer, bilateral breast cancer, or both. Proliferative breast disease (PBD) is a significant risk factor for the development of breast cancer and appears to be a precursor lesion. PBD and breast cancer were studied in 103 women from 20 kindreds that were selected for the presence of two first degree relatives with breast cancer and in 31 control women. Physical examination, screening mammography, and four-quadrant fine-needle breast aspirates were performed. Cytologic analysis of breast aspirates revealed PBD in 35% of clinically normal female first degree relatives of breast cancer cases and in 13% of controls. Genetic analysis suggests that genetic susceptibility causes both PBD and breast cancer in these kindreds. This study supports the hypothesis that this susceptibility is responsible for a considerable portion of breast cancer, including unilateral and postmenopausal breast cancer.

BREAST CANCER HAS LONG BEEN RECognized to be a familial disease (*1*). Population-based studies have shown that a woman's risk of developing breast cancer is increased 1.5- to 3-fold if one first degree relative (mother, daughter, or sister) had breast cancer and 5- to 10-fold if the relative had bilateral cancer or if more than one first degree relative had breast cancer (*2*). The hypothesis of a rare breast cancer–susceptibility allele that would be dominantly inherited has been supported by several studies (*3–6*) that have produced estimates of the gene frequency of a dominant susceptibility allele of 0.0006 to 0.008 in the population and a lifetime probability of breast cancer of 0.57 to 0.92 in genetically susceptible individuals. The following analysis suggests that genetic predisposition to breast cancer may be more common in postmenopausal breast cancer than previously thought and that this predisposition is expressed as PBD.

Proliferative breast disease has been used to describe several benign breast lesions characterized by excessive but nonmalignant proliferation of breast epithelial cells located in the terminal portions of the ducts of the breast (*7–8*). It has been suggested that PBD represents a premalignant state, because (i) breast cancer most often originates in the terminal ductal-lobular unit, (ii) proliferative lesions exhibit a spectrum of morphologic changes, the most severe of which resemble non-invasive cancer, and (iii) foci of PBD are often found in breast cancer mastectomy specimens (*9*). Several cohort studies have demonstrated that women with PBD have a two- to fivefold increased risk of developing breast cancer when compared to women with nonproliferative breast lesions (*10–12*). A family history of breast cancer in addition to the presence of PBD further increases the risk of breast cancer (*11*). In these studies, PBD was detected when women underwent surgical biopsy of clinically suspicious lesions.

We examined the frequency of PBD in families that were ascertained by two first degree relatives with breast cancer and tested the hypothesis that PBD and breast cancer are inherited lesions in these families (Fig. 1). All kindreds meeting this criteria were ascertained from the hematology/oncology clinic at the University of Utah, from the private clinic of one of the authors (H.H.), and the Utah Family Health Tree Project (*13*). One kindred, K1900, was selected with a third diagnosed individual (proband). All probands were classified as premenopausal or postmenopausal. If menopausal status was unknown, women diagnosed with breast cancer at age 50 or less were classified as premenopausal. The probands had premenopausal breast cancer in five kindreds and postmenopausal breast cancer in seven kindreds; seven kindreds had both one premenopausal and one postmenopausal proband. K1900 had one premenopausal and two postmenopausal probands. Nineteen kindreds were of

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Northern European descent; one kindred was Hispanic. The Utah population is representative of a Northern European gene pool because of its large founding Northern European population and low inbreeding (*14*).

After obtaining permission from a proband's attending physician, all available female first degree relatives of the probands were studied, as well as maternal and paternal aunts. If a woman was found to have PBD or breast cancer, or had a previous diagnosis of breast cancer, her first degree relatives were also studied. Sixteen additional relatives were studied even though the closest studied family member was unaffected with PBD or breast cancer. In all cases, the decision to sample was made conditional only on observations already made and all observed individuals were included in the analysis so that sampling procedures did not introduce bias (*15*). Wives of males in the kindreds, sisters of husbands marrying into the kindred, and their relatives were asked to participate as controls. All diagnoses of cancer of any site were recorded for all women who were within the sampling scheme.

After receiving approval from the Institutional Review Board of the University of Utah School of Medicine and the advisory committee of the Clinical Research Center, informed consent was obtained from all study participants. Subjects were evaluated in the Clinical Research Center where they underwent physical examination, screening mammography, and four-quandrant fine-needle aspiration. We modified the standard technique of fine-needle aspiration of a lesion (*16*) to screen for the presence of PBD in clinically normal women (*17*); all four quadrants of each breast were aspirated. A 1-inch, 22-gauge needle and a 20-ml syringe attached to a Cameco syringe pistol was inserted into each quadrant of the breast, 1 centimeter peripheral to the areola, under aseptic conditions. The needle was directed within each quadrant eight times while a

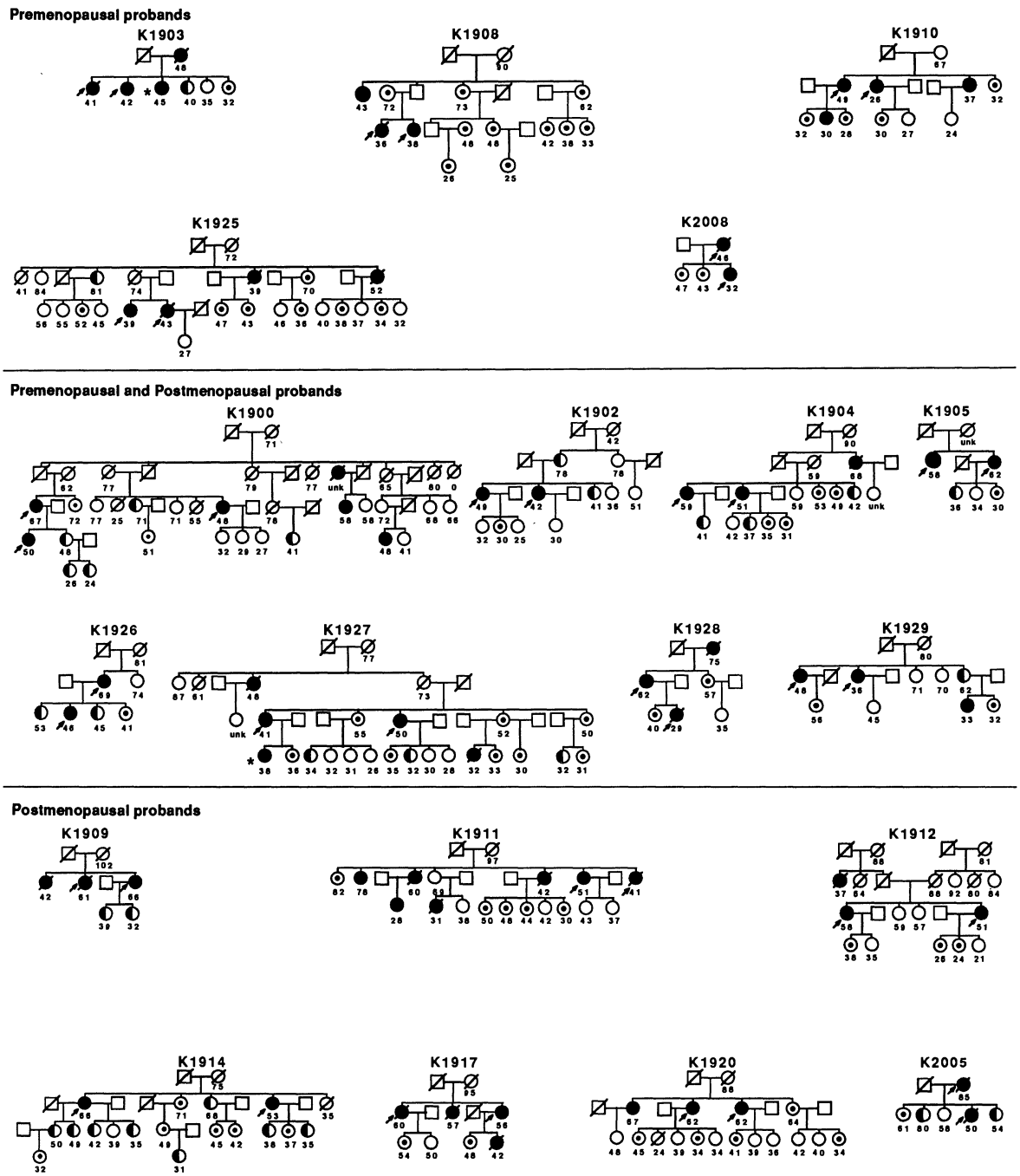

Fig. 1. Twenty pedigrees ascertained for two first degree relatives with breast cancer in which we were able to study at least two additional female first degree relatives not known to be affected with breast cancer. The pedigrees are separated into groups by the menopausal status of the probands, as shown. Probands are indicated by arrows. Women's status is indicated as breast cancer (●), PBD (◐), unaffected (⊙), or unknown (○); deceased individuals are indicated with diagonal lines through the symbol. For women with breast cancer, age at diagnosis is indicated. Unaffected women and women with PBD have age at examination indicated. For all other women, current age or age at death is indicated. Two individuals with PBD who were subsequently diagnosed with breast cancer are marked by asterisks. □, Men.

constant negative pressure of 15 ml was maintained to broadly sample the breast parenchyma. The aspirated material from each quadrant was expelled separately into 5 ml of 0.9% NaCl. The needle was washed with an additional 5 ml of 0.9% NaCl to expel any remaining material from each quadrant. The clinician and cytopathologist were blinded as to the subject's clinical status.

Fine-needle aspirates were obtained from 103 relatives and 31 controls. All four quadrants of both breasts were sampled in 126 subjects. The other eight women stopped the procedure after sampling two quadrants (four women) or four quadrants (four women) because of discomfort. In addition, one quadrant sample of one woman was contaminated with blood and one quadrant sample from one woman was lost in processing. Four women declined the examination and three women were ineligible as they had previously undergone prophylactic mastectomy. The only complication was two breast hematomas, which resolved without treatment. Women with one or more cytologic specimen showing moderate, marked, or atypical ductal hyperplasia were considered affected with PBD (*18*) (Fig. 2). Women with no such findings in whom at least five quadrants were tested were considered unaffected. Four relatives and one control with no observed PBD who had four or fewer quadrants sampled were considered unknown.

Cytologic analysis demonstrated moderate to marked ductal hyperplasia or atypical hyperplasia in 4 of the 30 control women (13%), with only one control demonstrating atypical ductal hyperplasia. This rate was compared to that found in the subset of 77 family members studied who were first degree relatives of breast cancer cases. We found moderate to severe ductal hyperplasia or atypical ductal hyperplasia in 27 of these women (35%), a significantly higher frequency than that observed in the controls (P = 0.02) (*19*). Atypical hyperplasia was found in four first degree relatives of breast cancer cases. Women with PBD were approximately the same age as those without PBD, average age 49 and 48, respectively. The mean age of the family members was 46, and the mean age of the controls was 52.

Pedigree analysis (*20*) was used to examine the pattern of disease inheritance in the kindreds. We examined the fit of postulated genetic models that define the basis of susceptibility to PBD. The major-gene models assumed a single autosomal locus with two alleles determining susceptibility to the trait; "A" is termed the susceptibility allele and "a" is the normal allele. Thus, individuals have genotypes AA (homozygous for the susceptibility allele), Aa (heterozygous for the susceptibility allele), or aa (homozygous for the normal allele). Mendelian segregation and Hardy-Weinberg equilibrium were assumed for this locus. The probability of the expression of PBD in an individual with a given genotype, called the penetrance of the genotype for PBD, was estimated. The relationship between the hypothesized susceptibility locus and breast cancer was assessed as described below. As described (*5*), the probability of being diagnosed with breast cancer in the nth 5-year age interval given genotype j at the postulated PBD and breast cancer susceptibility locus is equal to

$$[\prod_{i=1}^{n-1}(1-k_j a_i)]k_j a_n$$

where a_i is the average 5-year age-specific Utah incidence rate between 1973 and 1988. The k_j are parameters that assess the relationship between the PBD susceptibility locus genotypes and breast cancer risk, where $k_j = 1$ indicates breast cancer risk equal to the Utah age-specific incidence rates for genotype j. The probability of being unaffected at the nth age interval for genotype j

$$\prod_{i=1}^{n}(1-k_j a_i)$$

Men were always considered of unknown phenotype. All models were compared under the constraint that the parameters had to conform to the observed population frequency of PBD, 0.13, as there was insufficient pedigree data to obtain meaningful estimates of gene frequency. In this model, expression of PBD and breast cancer are independent, given that an individual has the susceptibility allele.

The results of pedigree analysis with the models described are shown in Table 1. Parameters are constrained to define specific models; unconstrained parameters are estimated to best fit the observed data. The

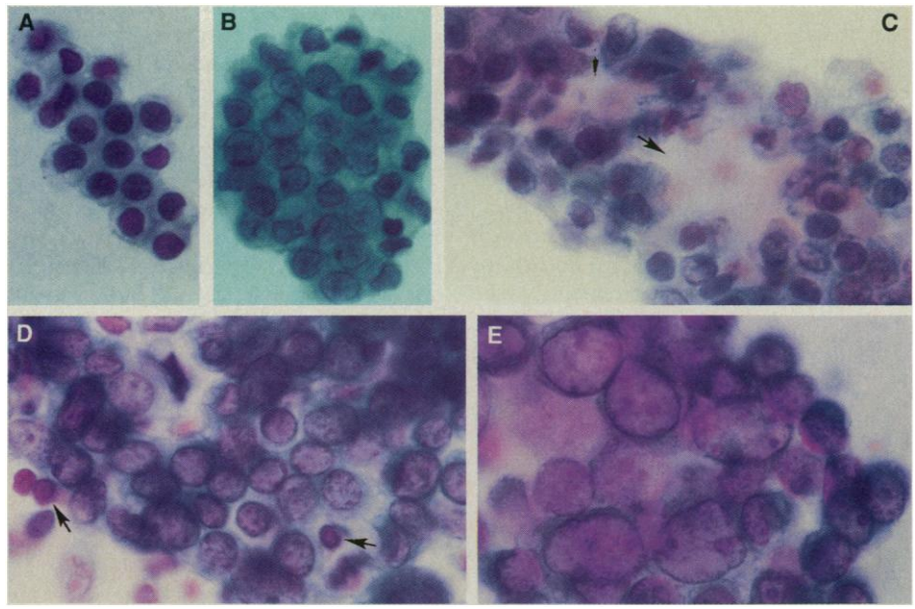

Fig. 2. Samples for cytological examination were refrigerated at 5°C until prepared (within 24 hours). For maximal cell recovery and preservation of cellular arrangements, a membrane-filtration technique was used. The entire 10 ml of saline containing each aspirate specimen and a 5-ml wash was filtered over a small-diameter (25 mm) 5-μm membrane filter (millipore) with a gravity filtration apparatus. After filtration, preparations were fixed in 95% ethanol for at least 15 min and stained with a modified Papanicolaou (Pap) stain on an automatic stainer. Stained filters were mounted on single-end frosted slides for subsequent evaluation. Filter preparations were evaluated cytomorphologically for the presence of fibroadipose tissue and ductal epithelium. Fragments of epithelial cells were divided into five classifications with published criteria (*18, 31, 32*). (**A**) Normal ductal epithelium. A small cohesive fragment exhibiting regular nuclear spacing and uniform nuclear shape, size, and staining characteristics. (**B**) Mild benign ductal hyperplasia. A cohesive fragment exhibiting mild nuclear enlargement and variation with evenly distributed chromatin and occasional small nucleoli. Although mild crowding is present, the cellular arrangement is only slightly disordered. (**C**) Moderate to marked benign ductal hyperplasia. A cohesive multilayered fragment exhibiting a disordered cellular arrangement and heterogeneity of nuclear features, including shape, size, and staining intensity. Nuclear size in some cells is increased beyond that seen in mild hyperplasia. Note the presence of irregular luminal outlines (arrows) suggestive of a cribriform arrangement. (**D**) Atypical ductal hyperplasia. Multilayered fragment exhibiting slight lack of cohesion, marked crowding and nuclear enlargement, and uniform nuclear features. The prominent nucleoli are not a constant feature of atypical hyperplasia. The presence of myoepithelial cells (arrows) excludes the diagnosis of malignancy. (**E**) Adenocarcinoma. Extreme nuclear enlargement and pleomorphism with nuclear membrane irregularities, irregular chromatin distribution, and macronucleoli. All photomicrographs are Pap stained cells at ×1260.

Table 1. Parameter estimates for genetic models. The proportionality constants for breast cancer are labeled k(AA), k(Aa), and k(aa), reflecting their dependence on genotype. Corrections for the original ascertainment shown in Fig. 1 were made to remove the effect of having sampled through probands. All analyses were done with the Pedigree Analysis Package (*29*). Degrees of freedom (df) represent the difference in the number of parameters estimated. Bold numbers indicate terms that are constrained to be equal by the model.

Model	Gene frequency (q)	Penetrance for PBD			Proportionality constant for breast cancer			χ^2	df
		P (AA)	P (Aa)	P (aa)	k(AA)	K(Aa)	k(aa)		
				Inherited excess breast cancer					
General	0.14	1.00	0.47	0.00	0.0	10.8	0.1		
Dominant I	0.12	**0.59**	**0.59**	0.07	**11.1**	**11.1**	0.6	1.6	2
Dominant II	0.13	**0.56**	**0.56**	0.00*	**8.4**	**8.4**	1.0*	2.6	4
Recessive I	0.47	0.60	**0.00**	**0.00**	9.6	**0.6**	**0.6**	2.1	2
Recessive II	0.46	0.63	**0.00**	**0.00**	9.7	**1.0***	**1.0***	2.3	4
				No excess breast cancer					
General	0.11	0.13	0.68	0.00	1.0*	1.0*	1.0*	47.9	3
Sporadic	1.00	0.13*	0.13*	0.13*	1.0*	1.0*	1.0*	64.3	6
				Non-inherited excess breast cancer					
General	0.11	0.13	0.68	0.00	**4.4**	**4.4**	**4.4**	12.0	2
Sporadic	1.00	0.13*	0.13*	0.13*	**4.4**	**4.4**	**4.4**	28.4	5

*Parameters were not estimated.

general model allows all parameters to vary without constraint. Dominant models constrain the penetrance for PBD and the proportionality constant for breast cancer risk of the two susceptible genotypes to be equal; the recessive models similarly constrain the two nonsusceptible genotypes. In both cases, model I allows nonsusceptible individuals to express PBD and allows their risk for breast cancer to vary from population rates, whereas model II assumes that all nonsusceptible individuals have no risk for PBD and a risk for breast cancer equal to the Utah population rates. In the sporadic models, all individuals have the population risk of PBD regardless of genotype. Models with no excess breast cancer risk assume Utah population rates for all individuals. Models with noninherited excess breast cancer risk assume all women have equal but elevated risk of breast cancer. Specific models are compared to the general model to determine whether the reduction in the number of parameters estimated significantly alters the fit of the model to the data. A model with no excess risk of breast cancer and no excess risk of PBD could be strongly rejected when compared to the general model ($\chi^2 = 64.3$, df = 6, $P < 0.0001$); a model with excess breast cancer but no excess PBD was rejected ($\chi^2 = 28.4$, df = 5, $P < 0.0001$), as was a model that allowed for genetic susceptibility to PBD but no excess risk of breast cancer ($\chi^2 = 47.9$, df = 3, $P < 0.0001$). Genetic susceptibility for PBD combined with an excess risk of breast cancer independent of the PBD susceptibility genotype was also rejected ($\chi^2 = 12$, df = 2, $P < 0.005$). Thus, the most parsimonious models involved a genetic susceptibility for PBD that was also expressed as increased breast cancer risk. The analysis could not distinguish between recessive and dominant modes of inheritance as an explanation of the data given the relatively low penetrance of the gene in women and nonpenetrance in males. For both dominant and recessive models, penetrance for PBD was 56 to 63% and the breast cancer risk in susceptible individuals was 8.4 to 11.1 times the Utah age-specific rates, equivalent to a lifetime probability of breast cancer in susceptible individuals of approximately 52 to 63%. There was no evidence for PBD in nonsusceptible individuals when either a recessive or dominant mode of inheritance was assumed. The penetrance of PBD did not differ significantly as a function of age, and a mixed model for PBD indicated that a major gene with a residual polygenic component was significantly preferred to either a major gene or polygenic inheritance alone (*21*).

Previous studies have suggested that genetic susceptibility to breast cancer is expressed primarily as premenopausal breast cancer, bilateral breast cancer, or both (*3–6*). In this study of the kindreds of pairs of affected relatives, a slight majority of the breast cancer cases were postmenopausal, and only 8% of the cases were bilateral. It has also been suggested that excesses of ovarian, uterine corpus, gastrointestinal, or brain cancer or leukemia may be associated with inherited breast cancer. In the women we studied, 8 cancers at these sites were observed when 9.9 were expected (*22*), whereas 26-nonproband breast cancer cases were observed when 8.6 were expected. We also found that the incidence of breast cancer was similar whether the probands were premenopausal, postmenopausal, or both (Table 2). Thus, the genetic susceptibility

Table 2. Frequency of detection of PBD in first degree relatives of breast cancer cases by menopausal status of the probands. Non–first degree relatives of breast cancer cases in the pedigree were excluded from this analysis as they are at varying degrees of risk.

Diagnostic criteria	Menopausal status of probands		
	Both probands premenopausal	Pre- and postmenopausal probands	Both probands postmenopausal
Number of first degree relatives	18	31	28
Frequency of PBD (%)	17	42	39
Number of breast cancer cases per kindred ($\bar{X}$)	1.4	1.0	1.4
Range	0–2	0–3	0–5
Nonproband age for breast cancer diagnosis ($\bar{X}$)	42.0	50.0	48.4
Range	30–52	32–75	28–78
Proband age for breast cancer diagnosis ($\bar{X}$)	39.2	51.0	58.7
Range	26–49	29–69	41–85
Proband age of relatives examined ($\bar{X}$)	47.2	45.4	46.4
Range	25–81	30–78	24–82

for PBD we describe offers an explanation for the fraction of breast cancer that has previously been described as familial but not due to a major gene and a fraction of what is considered to be nongenetic breast cancer (*23*). This interpretation is consistent with the suggestion from our study that the frequency of a breast cancer susceptibility allele is considerably higher than previously thought.

PBD was detected less frequently in first degree relatives in kindreds with two premenopausal probands than in the first degree relatives in the other kindreds, although the difference was not significant (Table 2, $P = 0.09$). PBD was not detected in 8 of the 20 kindreds. In five kindreds (1908, 1912, 1917, 1928, and 2008) this can be explained by the small number of at-risk individuals sampled. In the six kindreds with only two breast cancer cases, 64% of the first degree relatives had PBD compared to 24% in kindreds with breast cancer cases in addition to the probands ($P < 0.001$). Thus, in kindreds in which breast cancer was the primary expression, either PBD had progressed to breast cancer or PBD was not an expression of the susceptibility.

It has been hypothesized that in hereditary cancer, the initial alteration, or susceptibility to it, is inherited as a dominant gene, and that further mutational events cause lesion appearance and progression to malignancy (*24*). The analysis of the adenomatous polyp as the precursor for colon cancer is the most refined demonstration of a cancer precursor. Adenomatous polyps are known to be inherited (*25*), and deletions of tumor suppressors and activation of oncogenes in adenomatous polyps have been demonstrated in their conversion to malignancy (*26*). We postulate that this process is analogous to the development of PBD and its conversion to breast cancer, except that PBD appears to be a more diffuse lesion. Evidence to support this model of breast cancer etiology is provided by the segregation of both breast cancer and PBD in these families. This can best be explained as two developmental stages of a single genetic susceptibility, with PBD occurring at an earlier age, and breast cancer developing from the PBD precursor lesions. This model is supported by the two cases of breast cancer initially detected by our screening of the 77 first degree relatives. In one case, fine-needle aspirates showed atypical ductal hyperplasia in three quadrants of the right breast 1 year before microcalcifications in that breast were detected by mammography. A second set of fine-needle aspirate samples was obtained at the time of the abnormal mammography; these showed malignant cells in the same quadrant in which the abnormality was identified, as well as atypical hyperplasia in both the right and left breast. Subsequent biopsy revealed intraductal carcinoma, confirmed by histology. In the second case, the initial fine-needle aspirates showed atypical ductal hyperplasia in one breast concurrent with a suggestion of breast cancer in that breast on mammography. Breast cancer was subsequently confirmed by histology.

Table 3. Linkage analysis with D17S74. Model I assumed a rare, dominant susceptibility locus ($q = 0.003$); unaffected individuals were considered unknown, and all breast cancer cases were considered to be due to genetic susceptibility. Model II adds age-specific penetrance and sporadics (*6*) to model I, and model III extends the phenotype of model I to include PBD. D17S74 was typed with pCMM86 (obtained from American Type Culture Collection) with Taq I and Hinf I restriction enzymes by methods previously described (*30*). D17S74 was analyzed as six observed alleles, each with a 10% gene frequency, and a seventh unobserved allele. ND, not done.

Kindred	Age at onset	Lod score $\theta = 0.02$ for models		
		I	II	III
		Early-age onset		
1910	35.5	0.27	−0.15	0.27
1908	39.0	−1.00	−0.15	−1.00
1929	39.0	−1.00	−0.51	−1.00
2008	39.0	0.00	−0.18	0.00
1927	41.8	−1.05	−0.18	−1.95
1001	42.4	−1.96	−1.53	−1.96
1925	43.3	0.29	0.79	−0.95
1903	44.0	−1.02	−0.54	−0.96
1901	44.7	0.98	0.92	0.98
1902	45.5	0.01	0.24	−1.00
		Late-age onset		
1911	47.3	−0.73	−0.82	−0.72
2010	49.0	0.24	0.19	0.24
2012	51.0	−0.04	−0.05	−0.04
1912	48.6	−0.07	0.00	−0.07
1917	53.8	−0.26	−0.13	−0.26
1900	54.2	−0.54	0.09	−2.78
1928	55.3	0.00	0.00	0.00
2006	55.7	0.00	−0.01	0.00
1909	56.3	ND	ND	ND
1924	57.0	0.00	0.00	0.00
1926	57.5	0.00	−0.02	0.57
1921	58.7	−1.05	−0.05	−1.05
1904	59.3	0.00	−0.01	−1.53
1914	59.5	0.28	0.00	−0.43
1905	60.0	−1.05	−0.05	−1.05
1920	63.7	−0.26	−0.04	−0.26
2013	64.0	0.00	−0.03	0.00
2014	65.6	0.00	−0.03	0.00
2005	67.5	0.00	0.00	0.21
2009	69.0	0.05	0.00	0.05
Total early-onset breast cancer		−4.48	−1.29	−7.57
Total late-onset breast cancer		−3.43	−0.96	−7.12
Total		−7.91	−2.25	−14.69

We have attempted to confirm the report of linkage of an early-age onset breast cancer susceptibility locus to D17S74 (*27*), a highly polymorphic marker locus on the long arm of chromosome 17 by studying it in our kindreds (Table 3). We have included the 20 PBD kindreds in this study, 9 other kindreds in our current study that did not qualify for this report at the time of data closure, and K1001, a kindred ascertained for a cluster of breast cancer cases (*28*). Many of the kindreds were uninformative, as they were not specifically selected to be informative for linkage analysis of breast cancer alone. For each model, we calculated a lod score for linkage between a marker and a susceptibility locus, calculated as the logorithm of the ratio of the likelihood at a given recombination fraction (θ) to the likelihood of free recombination ($\theta = 0.5$). For brevity, the lod scores are reported for $\theta = 0.02$, the most likely hypothesized genetic distance between the susceptibility locus and D17S74 (*27*). Three models of susceptibility were tested (Table 3); model II corresponds to the model for which linkage was reported (*27*). For all three models tested, linkage of a susceptibility locus to D17S74 was not supported for the set of early-age onset breast cancer kindreds. Some kindreds showed negative lod scores suggestive of nonlinkage and several kindreds showed slightly positive lod scores. Further studies are needed to determine whether these reports taken together represent lack of link-

age to 17q or genetic heterogeneity.

We anticipate that accurate phenotypic characterization of PBD in our kindreds will leadto linkage to candidate markers that can then be confirmed by analysis of breast cancer alone with an extended set of markers. Genetic mapping may in turn lead to molecular isolation of the locus or loci responsible for breast cancer susceptibility, which will permit further refinement of the underlying genetic model and analysis of interaction of genetic susceptibility with other risk factors. Four-quadrant fine-needle aspiration is a sensitive, rapid, minimally invasive test that could identify these high-risk lesions in the absence of a clinically identifiable mass and allow identification and close monitoring of genetically susceptible women. Women thus identified might also benefit from specific intervention therapy before the development of cancer, should this type of treatment become available.

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Induction by Antigen of Intrathymic Apoptosis of $CD4^+CD8^+$ TCR^{lo} Thymocytes in Vivo

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In order to examine the mechanisms by which clonal deletion of autoreactive T cells occurs, a peptide antigen was used to induce deletion of antigen-reactive thymocytes in vivo. Mice transgenic for a T cell receptor (TCR) that reacts to this peptide contain thymocytes that progress from the immature to the mature phenotype. Intraperitoneal administration of the peptide antigen to transgenic mice results in a rapid deletion of the immature $CD4^+CD8^+$ TCR^{lo} thymocytes. Apoptosis of cortical thymocytes can be seen within 20 hours of treatment. These results provide direct evidence for the in vivo role of apoptosis in the development of antigen-induced tolerance.

SELF TOLERANCE WITHIN THE T CELL repertoire is established in part by clonal deletion (clonal elimination) of self-reactive T cell clones (*1, 2*). Clonal deletion involves the presentation of self antigens and self–major histocompatibility complex (MHC) molecules to T cells that are developing within the thymus (*3–12*). Clonal deletion of I-E (MHC class II)–reactive T cells in I-E–expressing mice was first inferred by the absence of $V_\beta 17$-bearing T cells from the mature, but not the immature, thymocyte population (*3*). The deletion of $V_\beta 17$ from peripheral $CD8^+$- and $CD4^+$-bearing T cells suggested that deletion could occur at the $CD4^+CD8^+$ stage of thymocyte development (*4*). Tolerance induction to the Mls antigens involves deletion of reactive T cells from the mature thymocyte and peripheral T cell populations, but not from the immature pools (*5–9*).

The deletion of anti–H-Y and allospecific T cells was inferred from the absence of immature $CD4^+CD8^+$ thymocytes in mice expressing the H-Y or allospecific antigen (*10, 11*). The stage at which deletion is evident within thymocyte populations can depend on the antigen system examined. Pircher and co-workers (*12*) demonstrated this by examining deletion in a transgenic system having T cells of dual specificity—to lymphocytic choriomeningitis virus (LCMV) and to Mls^a. Tolerance to Mls^a deleted the mature but not the immature $CD4^+CD8^+$

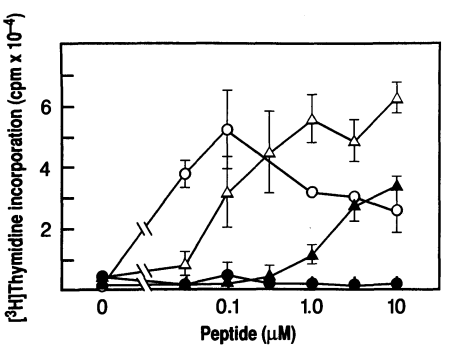

Fig. 1. Reactivity of peripheral transgenic T cells to OVA323-339 peptide analogs. Peptides used at the indicated concentration were OVA323-339 (○), OVA323-338 (△), OVA323-336 (▲), and OVA324-334 (●). Splenocytes from H-2^d haplotype mice expressing the DO11.10 αβ TCR were isolated as described (*15*) and cultured at 2.5×10^6 cells per milliliter with the indicated concentration of peptide. Triplicate 200-μl cultures were pulsed with 0.4 μCi of [^{3}H]thymidine on the second day of a 3-day culture. Data represent the mean and standard deviation of incorporated cpm of [^{3}H]thymidine. The experiment was repeated three times with similar results. Peptides were synthesized on an Applied Biosystems model 430 peptide synthesizer as described (*26*).

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