Progesterone and Estrogen Receptors and Mammary Neoplasia in the Iowa Women’s Health Study: How Many Kinds of Breast Cancer Are There?1

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Abstract
Characterization of breast tumors on both estrogen receptor (ER) and progesterone receptor (PR) status suggests distinct biological and clinical profiles. We hypothesized that these tumor subtypes might also show specific differences in their relations with epidemiologic risk factors. Risk factors were assessed via a questionnaire mailed in January 1986 to 37,105 cancer-free women, ages 55–69 years: the Iowa Women’s Health Study. To the end of 1992 (241,627 person-years of follow-up), 939 incident breast cancers were ascertained by the Iowa population-based Surveillance, Epidemiology, and End Results Cancer Registry. Joint ER and PR status was determined on a total of 610 (65%) tumors. Three patterns of association were seen in relation to epidemiologic risk factors. Endogenous hormone exposure variables—parity, age at first birth, age at menarche, body mass index, and body fat distribution as defined by waist-to-hip ratio—showed their expected pattern of associations only with PR+ breast cancers. In age-adjusted and polychotomous logistic regression analyses, both ER−PR− and ER+PR− breast cancers showed evidence of an inverted pattern of associations with several risk factors compared with that seen for ER+PR+ cancers [including parity (ER−PR−), waist-to-hip ratio (ER−PR−), body mass index (both), body mass index at age 18 years (ER−PR−), history of bilateral oophorectomy (ER+PR−), and oral contraceptive use (ER+PR+)]. Family history was not associated with ER+PR− cancers; only 8 (8%) of 99 patients with this subtype had a family history of breast cancer compared with 16% of all other types combined. This pattern of prospective findings persists when analyses are confined to tumors less than 2 cm and are therefore probably not explained by association with tumor size. This suggests that receptor status may define fundamental types of breast tumors, each with different risk factors and, therefore, potentially different etiologies.

Introduction
The ER3 is a nuclear receptor protein that has an estrogen binding domain and a DNA binding domain (1). The estrogen-ER complex binds directly to the DNA and regulates the expression of other genes including the PR (1). The PR is a heterodimer encoded by a single gene (1). ER and PR are the specific receptors for estrogen and progesterone, are found in hormone-dependent organs, and can be expressed or overexpressed in a variety of malignant tissue, both from hormone-dependent organs and others.

The ER and PR status of breast cancer has been used to predict a patient’s response to hormonal manipulation, predict a patient’s clinical course, and select patients for adjuvant systemic therapy with tamoxifen. However, there is controversy on the value of ER or PR status, separately, as prognostic indices and for their use in the selection of patients for adjuvant therapy (1–6). Predictive power is enhanced when ER and PR status are considered jointly. For example, tumors that are both ER+ and PR+ (approximately 70% of breast tumors) show the best response to either additive or ablative endocrine therapy, whereas tumors which are ER−PR− show the poorest response (approximately 10% of tumors) and tumors that are discordant for ER and PR show an intermediate response, irrespective of which receptor is positive (30–40%; Refs. 5 and 6). Women with tumors that are ER+PR+ have longer disease-free and overall survival compared to those identified with ER−PR− cancers, while women with ER+PR− tumors show intermediate survival (2, 3). Although it has been concluded that ER+PR+ (“hormonally responsive”) tumors and ER−PR− tumors (“hormonally unresponsive”) represent two distinct tumor types from a biological and clinical perspective (6, 7), tumors discordant for ER and PR status may represent additional profiles (7–10). Clinical data have shown that ER−PR− and ER+PR+ tumors differ from each other in their natural history, their response to therapy, and their biological characteristics (1, 3, 6, 7, 11). In contrast, limited current evidence suggests that women with ER−PR+ tumors appear to fit a profile which strongly resembles ER+PR+ tumors, and that these tumors may represent a subset of ER+PR+ tumors or be false negative for ER status (7). Some may be characterized by a mutant ER with constitutive transcriptional activity (9, 10).

Given the clinical relevance of hormone receptors, there has been great interest in determining whether epidemiologic

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3 The abbreviations used are: ER, estrogen receptor; PR, progesterone receptor; BMI, body mass index; WHR, waist-to-hip ratio; MPLR, multivariate polychotomous logistic regression.
risk factors for breast cancer differ by ER or PR status. Variation in risk factor profiles by ER or PR status would support the hypothesis that classification by hormone receptors identifies different forms of breast cancer with contrasting etiologies (7) rather than different stages of the same disease pathway. However, results of epidemiologic studies that have stratified cases on either ER status (12–27) or PR status (14, 23, 25–27) have shown generally weak, overlapping, and nonsignificant associations that are inconsistent across studies. Reasons suggested for this lack of clarity and consistency include small sample sizes, the frequent lack of an undiseased comparison group, differing study populations (especially differences in age and menopausal status), and the use of variable cutoff points to define receptor positivity (20). However, an alternative reason for these findings may be that stratifying cases on ER or PR status alone obscures associations. Thus, a better approach may be to consider ER and PR status jointly, particularly given the known clinical and biological differences in tumor profiles discussed above. We therefore investigated differences in epidemiologic risk factors for breast cancer jointly stratified by ER and PR status, hypothesizing that the risk factors thought to operate via an endogenous hormonal mechanism would be most strongly associated with ER+PR+ tumors. The hormonal variables included obesity (because of the capacity of adipose tissue to convert adrenal androstenedione to estrone), age at menarche, age at menopause, type of menopause, oophorectomy, parity, and age at first birth. Furthermore, noting that ER−PR− cancers are more common among premenopausal women and that premenopausal cancers are often inversely associated with some of the hormonal and reproductive risk factors that are largely predictive of breast cancer risk as a whole, we postulated that we might see a pattern of associations between ER−PR− cancers and these risk factors that were inverted from the pattern for the ER+PR+ type.

Materials and Methods
The Iowa Women’s Health Study Cohort. A questionnaire, mailed in January 1986, was returned by 41,837 women 55–69 years of age who had a valid Iowa driver’s license in 1985 (28, 29). Self-reported items included education, income, and menopausal status, family history of breast cancer (among first- and second-degree female relatives), use of exogenous estrogens, and average alcohol intake (calculated as g/week) over the past year (assessed by the Willett food frequency questionnaire; Ref. 30). Prevalent cancers were identified by asking participants if they had ever been diagnosed by a physician with any form of cancer other than skin cancer. The validity of responses to this question compared to the physician report was found to be good (31). BMI (kg/m²) was calculated from self-reported current height and weight; BMI at age 18 years was calculated from self-reported weight at that age and current height. To assess body fat distribution, a paper tape was enclosed along with instructions in order to have a friend measure the circumference of the waist (1 inch above the umbilicus) and hips (maximum protrusion); measurements by this method have been shown to be both valid and reliable (32). WHR was calculated as the ratio of these two circumferences.

Follow-up of the Iowa Women’s Health Study Cohort. Vital status and breast cancer incidence were ascertained through 7 years of follow-up (1986–1992). Follow-up questionnaires were mailed in October 1987 and August 1989 to establish vital status and change of address. Nonrespondents to the follow-up questionnaires were traced through the National Change of Address Service. Deaths were ascertained through the Iowa Department of Health and the National Death Index. There were 2267 deaths up to the end of October 1992. Vital status was unknown for less than 1% of the cohort.

Incident breast cancers (code 174 of the International Classification of Diseases for Oncology) were ascertained by the State Health Registry of Iowa, part of the National Cancer Institute’s Surveillance, Epidemiology, and End Results Program (28, 29). Field representatives routinely visited hospitals and clinics in and around Iowa. For cancer patients who were Iowa residents at the time of diagnosis, information including personal identifiers, demographic data, date of diagnosis, primary site, tumor size and grade, and extent of disease were recorded. ER and PR status (positive/negative) were also recorded by Surveillance, Epidemiology, and End Results when available in the medical record; cases which were coded as borderline were considered to be receptor-positive for these analyses. The borderline category for ER accounts for less than 1% of all cancers and for PR, less than 2%. The Iowa Women’s Health Study cohort was matched to the registry with combinations of first, last, and maiden names, zip code, birthdate, and Social Security number.

Data Analysis. Participants with the following baseline characteristics were excluded: premenopausal (n = 569), history of mastectomy (n = 1764) or partial removal of a breast (n = 106), or history of any cancer other than skin cancer (n = 2293). After exclusion of these women, there were 37,105 who comprised the at-risk cohort for breast cancer.

Because breast cancers were detectable only for Iowa residents, each woman was allocated person-years of follow-up from the date of the 1986 baseline questionnaire to one of the following events: (a) date of breast cancer diagnosis; (b) date of death, if the death occurred in Iowa; (c) date of a move out of Iowa; (d) the midpoint between the date of last contact and date located outside of Iowa (if date of move from Iowa not known); or (e) midpoint between date of last contact and date of death, if the death did not occur in Iowa to the end of 1989. If none of these events occurred, the person was assumed to be living in Iowa and contributing person-years of follow-up to the end of December 1992.

Each breast cancer was categorized by the joint classification of ER and PR status (i.e., ER+PR+, ER+PR−, ER−PR+, or ER−PR−). If either ER or PR status or both were unknown, women were categorized as unknown. Women were stratified into two or three categories for all independent variables. Uppermost categories for BMI, BMI at age 18 years, and WHR for these analyses were the top quintile of the distribution for the entire cohort.

Incidence rates were calculated by dividing the number of events by the number of person-years of follow-up. Relative risks and their 95% confidence intervals were computed within categories of risk factors for each subtype of breast cancer (i.e., ER+PR+, ER+PR−, ER−PR+, ER−PR−, or ER/PR−). If the person-years from each of the other receptor status-defined subtypes of breast cancer were excluded. To assess the effects of multiple variables and multiple outcomes, MPLR was used, which permitted modeling of the ER+PR+ case, ER+PR− case, ER−PR+ case, unknown receptor status case, or non-case as the dependent variable. The dependent variable was treated as a polychotomous nominal variable, and the logit estimator always compared non-case to receptor status-defined case (i.e., ER+PR+, etc.). We did not include ER−PR+ cases in this analysis because of the small numbers (n = 17). The
MPLR method allowed estimation of subgroup-specific risk parameters and direct statistical significance testing of differences between regression coefficients. The multivariate relative risk was estimated from the odds ratio, which was calculated using maximum likelihood methods. Since this method ignores person-years of follow-up, we also analyzed the data for each possible outcome separately in multivariate Cox regression models (i.e., separate Cox regressions were run for ER+PR+ versus non-case, ER+PR− versus non-case, etc.). These results agreed very closely with those found with the polychotomous logistic regression model (data not presented) showing that not taking person-years of follow-up into account in the MPLR model did not affect the results.

Analyses were conducted using the SAS programs PHREG and CATMOD (SAS Institute, Cary, NC).

### Results

During 241,627 person-years of follow-up, there were 939 incident breast cancers. Combined ER and PR status was available for 610 (65%) of these tumors. There was no difference by age, education, or urban versus rural residence between women whose ER and PR status was known and those with missing values. For women with known receptor status, 68% were ER+PR+, 16% were ER+PR−, 3% were ER−PR+, and 13% were ER−PR−. The mean age at diagnosis did not differ between tumor groups (P = 0.37): 66.1 years for women with ER+PR+ tumors, 66.3 years for ER+PR− tumors, 65.1 years for ER−PR+, 65.6 years for ER−PR−, and 65.6 years for unknown receptor status. Tumors that were ER−PR− tended to be larger in size at diagnosis and to involve more regional spread than the other tumor types (Table 1). Those with unknown receptor status were smaller and more local, in keeping with the observation that, at least in the first few years of follow-up, there was often too little tissue for receptor status to be determined. The tumor types differ by tumor grade (P = 0.047), although there was a substantial number of missing values for this variable.

The age-adjusted analysis for each specific tumor type is shown in Table 2. The direction of the relative risks for ER+PR+ tumors and ER−PR+ tumors was the same for BMI (increased), BMI at age 18 years (decreased), WHR (increased), high parity (decreased), and alcohol use (essentially null). Family history had the strongest association with the risk of ER−PR+ tumors but the confidence limits were wide. In addition, women who had a surgical menopause, and perhaps a pre-diagnosis history of bilateral oophorectomy, or had ever used contraceptive estrogens were at increased risk for ER−PR+ tumors but not for ER+PR+ tumors. It should be recalled that the estimates for the ER−PR+ tumors are based on only 17 cases; nonetheless, the associations between BMI and type of menopause and risk of the ER−PR+ subtype were both statistically significant. It is notable that whereas only 35% of those with ER+PR+ tumors had a history of surgical hysterectomy, 59% of those with ER−PR+ tumors had such a history.

In contrast to the ER+PR+ tumors, the relative risks for ER+PR− tumors associated with BMI, WHR, and family history of breast cancer were in the opposite direction (i.e., if a point estimate greater than 1.0 was found for one tumor type, an inverse association was seen for the other). The family history association is particularly notable; only 8 (8%) of 99 women in this subgroup had a positive family history compared with 16% for all other subgroups together. Parity showed no association with the risk of ER+PR− tumors compared with at least a suggestion of a reduced risk with higher parity in ER+PR+ tumors. Associations for ER+PR+ and ER+PR− tumors, however, were similar and essentially null for use of noncontraceptive estrogen and use of alcohol.

This inversion of direction of the associations between ER+PR+ and ER+PR− tumors was also seen with ER−PR− tumors for BMI, WHR, and parity. ER−PR− tumors, however, showed a positive association with family history. There were essentially null associations with age at menopause, history of oophorectomy, and BMI at age 18 years. There was a 55%
Table 2: Age-adjusted relative risk of postmenopausal breast cancer stratified by joint ER and PR status (n = 939)

<table>
<thead>
<tr>
<th>BMI</th>
<th>ER + PR + (n = 414)</th>
<th>ER + PR - (n = 99)</th>
<th>ER - PR + (n = 17)</th>
<th>ER - PR - (n = 80)</th>
<th>ER or PR unknown (n = 329)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Person-yr RR* (95% CI)</td>
<td>Person-yr RR (95% CI)</td>
<td>Person-yr RR (95% CI)</td>
<td>Person-yr RR (95% CI)</td>
<td>Person-yr RR (95% CI)</td>
</tr>
<tr>
<td>&lt;30</td>
<td>290</td>
<td>184,665 1.00</td>
<td>86</td>
<td>183,870 1.00</td>
<td>9</td>
</tr>
<tr>
<td>≥30</td>
<td>124</td>
<td>56,962 1.38</td>
<td>13</td>
<td>56,541 0.94</td>
<td>8</td>
</tr>
</tbody>
</table>

BMI at age 18

<23 | 325 | 178,312 1.00 | 76 | 177,375 1.00 | 14 | 177,128 1.00 | 56 | 177,292 1.00 | 263 | 177,936 1.00 |
≥23 | 86 | 62,218 0.76 | 21 | 61,950 0.80 | 2 | 61,893 0.41 | 23 | 61,969 1.18 | 64 | 62,905 0.70 |

WHR

<0.90 | 291 | 186,183 1.00 | 78 | 185,329 1.00 | 11 | 185,094 1.00 | 63 | 185,311 1.00 | 243 | 185,871 1.00 |
≥0.90 | 121 | 54,399 1.37 | 21 | 54,043 0.89 | 6 | 53,973 1.85 | 15 | 53,994 0.79 | 85 | 54,210 1.19 |

Age at menarche (yr)

≤12 | 204 | 101,557 1.00 | 43 | 100,945 1.00 | 7 | 100,792 1.00 | 32 | 100,910 1.00 | 146 | 101,210 1.00 |
≥13 | 206 | 137,239 0.74 | 54 | 136,648 0.92 | 10 | 136,501 1.05 | 48 | 136,624 1.10 | 180 | 137,091 0.91 |

Age at menopause (yr)

<50 | 197 | 115,630 1.01 | 56 | 114,881 0.55 | 20 | 114,494 0.26 | 101 | 114,912 0.26 | 227 | 115,433 1.15 |
≥50 | 199 | 115,630 1.01 | 56 | 114,881 0.55 | 20 | 114,494 0.26 | 101 | 114,912 0.26 | 227 | 115,433 1.15 |

Type of menopause

All other | 263 | 160,812 1.00 | 71 | 160,055 1.00 | 7 | 159,831 1.00 | 57 | 160,103 1.00 | 223 | 160,344 1.00 |
Surgical | 139 | 74,868 1.16 | 26 | 74,448 0.80 | 10 | 74,371 3.13 | 22 | 74,424 0.84 | 99 | 74,649 0.96 |

History of bilateral oophorectomy

No | 338 | 198,733 1.00 | 78 | 197,699 1.00 | 11 | 197,465 1.00 | 65 | 197,670 1.00 | 273 | 198,316 1.00 |
Yes | 76 | 42,428 1.07 | 21 | 42,246 1.28 | 6 | 42,175 2.57 | 15 | 42,211 1.09 | 56 | 42,339 0.97 |

Family history of breast cancer

No | 332 | 205,826 1.00 | 87 | 204,855 1.00 | 13 | 204,584 1.00 | 64 | 204,775 1.00 | 259 | 205,257 1.00 |
Yes | 58 | 28,149 1.25 | 8 | 27,985 0.66 | 4 | 27,962 2.24 | 14 | 28,004 1.57 | 60 | 28,173 1.68 |

Parity

0 | 45 | 21,392 1.00 | 9 | 21,250 1.00 | 3 | 21,222 1.00 | 4 | 21,336 1.00 | 35 | 21,336 1.00 |
1–2 | 133 | 77,089 0.84 | 31 | 76,715 0.96 | 4 | 76,603 0.36 | 27 | 76,930 1.88 | 104 | 76,930 0.82 |
≥3 | 233 | 141,626 0.82 | 57 | 140,935 1.00 | 10 | 140,779 0.51 | 49 | 141,345 1.88 | 188 | 141,345 0.83 |

Age at first live birth (parous only)

<30 yr | 328 | 203,753 1.00 | 80 | 202,756 1.00 | 13 | 202,520 1.00 | 69 | 202,741 1.00 | 257 | 203,311 1.00 |
≥30 yr | 35 | 13,618 1.50 | 7 | 13,539 1.22 | 1 | 13,510 1.11 | 7 | 13,528 1.44 | 32 | 13,604 1.83 |

Contraceptive estrogen use

Never | 341 | 196,127 1.00 | 75 | 195,109 1.00 | 12 | 194,887 1.00 | 62 | 195,074 1.00 | 264 | 195,713 1.00 |
Ever | 73 | 45,084 1.08 | 24 | 44,884 1.63 | 5 | 44,884 1.91 | 18 | 44,857 1.40 | 65 | 44,990 1.12 |

Noncontraceptive estrogen use

Never | 245 | 148,751 1.00 | 62 | 148,040 1.00 | 11 | 147,858 1.00 | 47 | 147,984 1.00 | 197 | 148,438 1.00 |
Ever | 169 | 92,032 1.10 | 37 | 91,526 0.96 | 6 | 91,404 0.87 | 33 | 91,519 1.12 | 131 | 91,832 1.07 |

Alcohol use within the last yr

None | 230 | 136,994 1.00 | 53 | 136,303 1.00 | 10 | 136,139 1.00 | 37 | 136,242 1.00 | 184 | 136,733 1.00 |
Any | 184 | 104,633 1.08 | 46 | 104,108 1.17 | 7 | 103,967 0.92 | 43 | 104,105 1.55 | 145 | 104,388 1.04 |

* RR, relative risk; 95% CI, 95% confidence interval. Adjusted for age according to the method of Mantel and Haenszel.
increased risk of this tumor type in women who had ever drunk alcohol.

Parity was of particular interest because, although age at first birth was consistent in direction across all tumor types, parity showed an association in women with ER-PR- tumors opposite to that seen for those with the other three tumor types and opposite to that seen, somewhat inconsistently, in the literature. In these data, none of the point estimates for parity showed an association in women with ER-PR- tumors although age at menopause and use of noncontraceptive estrogen, which were inversely associated risk, whether increased or decreased, was statistically significant.

The pattern of relative risks for the receptor status-unknown group closely followed those for the ER+PR+ group, as would be expected if this group contained the same distribution for receptor status as the women with known receptor status. Because there were differences in the distributions of tumor sizes by receptor status and because there is evidence to support the position that receptor status may change with tumor progression, we repeated the above analyses, confining attention to those tumors that were less than 2 cm at diagnosis. There were essentially no differences in the pattern of associations seen (data not shown). Indeed, the inverted associations seen for ER-PR- compared with ER+PR+ tumors with BMI < 23 cm were more marked. To determine whether these risk factors were independent of each other, and to take into account five outcomes simultaneously (non-case, ER+PR+ case, ER+PR- case, or ER-PR- case, and cases where ER or PR status was unknown; note that the 17 ER-PR+ cases were not included), an MPLR model was fitted to the data (Table 3). All 11 variables were entered simultaneously as well as age at menopause and use of noncontraceptive estrogen. In this model, all 11 variables were entered simultaneously as well as age at menopause and use of noncontraceptive estrogen.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Relative risk of postmenopausal breast cancer by receptor-defined subtype: MPLR model*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ER+PR+ cases</td>
</tr>
<tr>
<td></td>
<td>RR* (95% CI)</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
</tr>
<tr>
<td>&lt;30</td>
<td>1.00 (1.13-1.86)</td>
</tr>
<tr>
<td>≥30</td>
<td>1.00 (0.47-0.81)</td>
</tr>
<tr>
<td>WHR</td>
<td>1.00 (1.05-1.70)</td>
</tr>
<tr>
<td>Age at menarche (yr)</td>
<td></td>
</tr>
<tr>
<td>&lt;13</td>
<td>1.00 (0.56-0.85)</td>
</tr>
<tr>
<td>≥13</td>
<td>0.69 (0.36-1.35)</td>
</tr>
<tr>
<td>Type of menopause</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1.00 (0.91-1.63)</td>
</tr>
<tr>
<td>Surgical</td>
<td>1.22 (0.87-1.71)</td>
</tr>
<tr>
<td>History of bilateral oophorectomy</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00 (0.67-1.29)</td>
</tr>
<tr>
<td>Yes</td>
<td>0.93 (0.88-1.58)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
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<tr>
<td>0</td>
<td>1.00 (0.52-1.10)</td>
</tr>
<tr>
<td>1–2</td>
<td>0.76 (0.52-1.06)</td>
</tr>
<tr>
<td>≥3</td>
<td>0.75 (0.52-1.06)</td>
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<tr>
<td>Age at first live birth, parous only (yr)</td>
<td></td>
</tr>
<tr>
<td>&lt;30</td>
<td>1.00 (1.21-2.56)</td>
</tr>
<tr>
<td>≥30</td>
<td>1.00 (0.79-1.37)</td>
</tr>
<tr>
<td>Contraceptive estrogen use</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.00 (0.95-1.44)</td>
</tr>
<tr>
<td>Ever</td>
<td>1.00 (0.95-1.44)</td>
</tr>
<tr>
<td>Alcohol use within the last yr</td>
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</tr>
<tr>
<td>None</td>
<td>1.00 (0.95-1.44)</td>
</tr>
<tr>
<td>Any</td>
<td>1.17 (0.95-1.44)</td>
</tr>
</tbody>
</table>

In this model, all 11 variables were entered simultaneously as well as age at menopause and use of noncontraceptive estrogen.

* Iowa Women’s Health study, 7-year follow-up (n = 939).

a RR, relative risk, 95% CI, 95% confidence interval.

b P < 0.05 for β coefficient different from respective β coefficient for ER+PR+ cases.

c 0.15 > P > 0.05 for β coefficient different from respective β coefficient for ER+PR+ cases.

d 0.05 for β coefficient different from respective β coefficient for ER+PR+ cases.
showed almost identical results between the multivariate and age-adjusted estimates. The “inverted” pattern of relative risk estimates seen in Table 2 for ER−PR− tumors appeared also in the MPLR model for BMI, BMI at age 18 years, WHR, age at menarche, type of menopause, and parity. BMI, type of menopause, history of bilateral oophorectomy, and oral contraceptive use appear to be different for ER+PR− compared with ER+PR+. In this model, the association between alcohol and risk of ER−PR− tumors is attenuated but the lack of an association between ER+PR− and family history persists.

In order to determine whether the differences in patterns of association between receptor-defined subsets and specific risk factors were measurably different, we tested whether the regression coefficients for each risk factor differed between ER+PR+ tumors and ER+PR− tumors, and between ER+PR+ tumors and ER−PR− tumors and between ER+PR+ tumors and those where receptor-status was unknown. The only statistically significant differences ($P < 0.05$) were between ER+PR+ tumors and ER−PR− tumors for BMI at age 18 years and between ER+PR+ tumors and ER+PR− tumors in BMI and a history of bilateral oophorectomy. However, there were borderline differences in the patterns (0.15 < $P > 0.05$) as noted above.

**Discussion**

This study provides several new pieces of information on the role of receptor status in breast cancer. First, although previous epidemiologic studies have produced inconsistent data on the relationship between ER status and risk factors, the joint stratification on estrogen and progesterone receptors produces a somewhat more interpretable as well as intriguing pattern. Second, the appearance of differences in patterns of association between receptor-defined subtypes and specific known risk factors for breast cancer, plus the data suggesting tumor size differences across subtypes do not explain these patterns, provide some answer to the question of whether the receptor profile of a particular breast cancer is evidence of discrete tumor types or merely a marker (modifiable over time) of the degree of malignant behavior. Third, some of the specific associations suggest new directions for study.

Before expanding on these points, it is appropriate to consider the quality of the outcome data. Receptor status was considered at a variety of clinical laboratories rather than at a single reference laboratory. There are, nonetheless, two reasons to argue for the usefulness of the data: (a) the methodology for establishing receptor status has become increasingly routine and standardized (7) and (b) empirical observation that the distribution of ER/PR status seen in Table 1 essentially is identical to that seen in the reference laboratory studies. For example, Thorpe (7) reported a distribution in postmenopausal women for ER+PR+, ER+PR−, ER−PR+, and ER−PR− of 64%, 17%, 3%, and 16%, respectively. The proportions from Bland et al. (34) were 63%, 15%, 5%, and 17%, respectively. These compare with the distribution among those with known receptor status in the population described here of 69%, 15%, 3%, and 13%, respectively. Of course, similarity of distributions still is compatible with some random misclassification.

Three important patterns suggest themselves: the PR+, the ER+PR+, and ER−PR−. The PR+ pattern is associated with family history and with variables that may be characterized as those related to endogenous hormone exposure, body mass, body fat distribution, age at menarche, and age at first birth (and perhaps parity). It previously has been thought that the associations, particularly with the “classic” reproductive variables, were defined largely by the ER+ status. For example, Ballard-Barbash et al. (17) and Hildreth et al. (15) reported differences between ER+ and ER− tumors for nulliparity and age at first birth; McTiernan et al. (21) reported similar findings, although only that for age at first birth was statistically significant; the data of Hislop et al. (19) are less clear but may be consistent with the other reports. Stanford et al. (22), in a study of women ages 20–54 years, showed differences in the association between parity and ER+ versus ER− status. As the data herein show, the association is with PR-positivity, a variable not measured in any of the above studies; in the presence of PR− status, several associations with ER+, even that with family history, are reversed in sign. Conversely, the risk factors for tumors that were PR+ show a similar pattern, irrespective of ER status; the only differences appear to be related to “menopausal” variables which may be more strongly associated with ER−PR+ (there are only 17 of these) than with ER+PR+.

The only large-scale epidemiologic study to address the role of progesterone receptors in relation to reproductive and other variables is the case-control study of 607 cases and 1214 controls conducted by Krieger et al. (25). Yoo et al. (27) reported differences between individuals with PR+ versus PR− tumors in relation to specific risk factors but not between ER+ versus ER−. Krieger et al. (25) did not stratify jointly on ER and PR status but noted a stronger family history association with the ER− and PR− cases than their receptor positive counterparts, which is consistent with the data presented here. Their other findings were that ER and PR status, when considered separately, provide essentially similar data on the relationship between risk factors and outcome: they showed that the pattern seen in relation to age at first birth, parity, and Quetelet’s Index for ER status was identical to that seen for PR status.

The second risk factor pattern that emerges is that seen for the ER−PR− (the “receptor-negative”) tumors. These cancers show only a somewhat elevated association with alcohol. There is no evidence of the usual pattern of associations with any of the endogenous hormone exposure variables, except age at first live birth which shows a relative risk estimate comparable to that seen for ER+PR+. Conversely, and intriguingly, the associations with parity, age at menarche, WHR, and BMI appear to be the reverse of that usually seen. These differences are not readily explained by the differences in tumor size noted in Table 1 as the pattern of associations persists when comparisons are made just between the receptor-defined subsets of tumors that are less than 2 cm at diagnosis. The association between alcohol and hormonal status is a complex one. There are data to show that chronic high intake is associated with early menopause, lower postmenopausal gonadotrophin levels, but elevated concentrations of estrogen and progesterone. Short-term feeding trials produced elevations in estrogens and dehydroepiandrosterone but not progesterone (36). Feeding of low doses (0.34 g/kg) of ethanol results in very rapid elevations of plasma testosterone in women but not men (37). Furthermore, we have shown recently (38) as have the Nurses Health Study group (39) that alcohol appears to interact with estrogen replacement therapy in elevating the risk of breast cancer. We have also shown that this interaction appears to be particularly marked for ER−PR− cancers (40). This provides some additional evidence for receptor-defined subtype differences in etiology. Nonetheless, the mechanisms remain elusive.

The third pattern is seen for the ER+PR− tumors. Although these show a pattern of risk factors with some similarity to that for ER−PR−, there are important differences in the direction and strength of association with age at menopause, age at first birth, parity, and, most intriguingly, family history. They might reason-
ably be called true sporadic breast cancers. The ER+PR− group includes a subset that has been shown recently by Fuqua et al. (8, 10) to show a splice-site mutation. Because PRs are induced by estrogen (2, 41, 42), the ER+PR− configuration should not occur in the presence of estrogens. Fuqua et al. (8, 10) postulated that the ER in this group of cancers would be capable of binding estrogen but would be nonfunctional as a transcriptional inducer. They reported finding, in breast tumor cells, a truncated ER that lacks exon 7 and is unable to induce an estrogen-responsive gene construct (8, 10). Whether the group of tumors defined by the splice-site mutation contributes materially to the overall lack of association between the ER+PR− tumors and a family history of breast cancer (only 8% of these women had a family history of breast cancer compared with 16% for all other types combined) has not been established. At least one study has suggested linkage of the ER gene with familial postmenopausal cancer (43).

Overall, these data are consistent with the following hypotheses which are here proposed for further testing in other studies. First, there are distinct patterns of risk factors associated with breast cancers with different joint progesterone and estrogen receptor status; if this is true, it argues that the receptor status of a breast cancer is a characteristic of the tumor, not a marker of the degree of differentiation of the tumor cells. Thus, the hypothesis would be that types of breast cancer, defined by receptor status, are the consequence of different disease processes and receptor status can provide information on the etiology of breast cancer generally, not just on its prognosis in a specific patient.

Second, we would hypothesize that there is variability in the strength of association of family history with the receptor-defined subgroups. This observation may provide important clues regarding candidate genes involved in the heritability of some subtypes of breast cancer and the progression of true sporadic cancers.

Third, we hypothesize that the ER−PR− subset of breast cancers is not associated with the endogenous hormonal risk factors in the usual pattern but shows perhaps the strongest association with alcohol consumption. Considerable lack of clarity has surrounded the question of how alcohol increases the risk of breast cancer (38, 44); the focus on ER−PR− tumors may prove fruitful in that a clearer idea of mechanisms may emerge when this disease subtype is better specified. For instance, it is the ER−PR− subtype that is most frequently epidermal growth factor receptor positive (45, 46).

The fourth hypothesis is that the variability of the direction or strength of the association of specific risk factors (e.g., parity and alcohol) with receptor-defined breast cancers could account for the inconsistent and weak associations seen across different studies when breast cancer is treated as a single entity. In such studies, the proportions of subtypes will vary by age, menopausal status, etc. (7).

In summary, data are presented to suggest a specific pattern of associations between epidemiologic risk factors and subtypes of breast cancer defined by joint estrogen and progesterone receptor status. The pattern suggests that breast cancer is more than one disease. If these observations are confirmed, a new typology may be needed that expands upon (perhaps replaces) the existing premenopausal and postmenopausal designations.

References
Progesterone and estrogen receptors and mammary neoplasia in the Iowa Women’s Health Study: how many kinds of breast cancer are there?

J D Potter, J R Cerhan, T A Sellers, et al.