Twinship and Risk of Postmenopausal Breast Cancer


Background: Intrauterine exposure to high levels of endogenous estrogens has been hypothesized to increase the risk of breast cancer. Because estrogens and other pregnancy hormones are substantially elevated in twin pregnancies, and possibly more so in dizygotic twin pregnancies, we evaluated the association between aspects of twin membership (i.e., belonging to a twin pair) and the risk of breast cancer.

Methods: In a cohort of 29,197 postmenopausal Iowa women with no prior diagnosis of cancer (except for nonmelanoma skin cancer), breast cancer risk factors were determined by use of a mailed questionnaire in 1986 (baseline); twin membership, sex of the twin, and zygosity were determined by use of a follow-up questionnaire in 1992. Results: Within the cohort, 1.8% (n = 538) of the women reported being a twin; of these, 24% (n = 130) were monozygotic twins, 63% (n = 337) were dizygotic twins, and 13% (n = 71) did not know their zygosity. From 1986 through 1996, 1230 breast cancers in the cohort were ascertained by linkage to the Iowa Cancer Registry. Compared with singletons, women who belonged to a twin pair were at elevated risk of breast cancer (multivariate-adjusted risk ratio [RR] = 1.72; 95% confidence interval [CI] = 1.22–2.42), with adjustment for educational level, family history of breast cancer, height, body mass index, body fat distribution, age at menarche, age at first live birth, use of hormone replacement therapy, and alcohol use. Multivariate-adjusted risk was elevated (in comparison with singletons) if the sex of the other twin was female (RR = 1.82; 95% CI = 1.20–2.75); however, this risk was limited to female dizygotic twins (RR = 2.14; 95% CI = 1.21–3.79), since no excess risk was evident for monozygotic twins (RR = 1.04; 95% CI = 0.43–2.50). The risk to women with a male twin was also elevated (RR = 1.49; 95% CI = 0.80–2.78) in comparison with singletons, but this estimate was not statistically significant. Conclusions: This cohort study lends further support to the theory that there are important intrauterine influences on carcinogenesis of the breast. [J Natl Cancer Inst 2000;92:261–5]

Trichopolous (1) has hypothesized that breast cancer might originate from in utero exposure to elevated concentrations of estrogens. Twin pregnancy is associated with an approximate doubling of maternal estrogen levels (2–5) compared with a singleton pregnancy. Most (6–8), but not all (9), studies have suggested that being a twin, compared with being a singleton, is associated with an increased risk of breast cancer, although only one reported association (7) achieved statistical significance. More limited data (7,8) suggest that risk may be limited to dizygotic twins, and this could be related to the presence of a single placenta (monochorionicity) in the majority of monozygotic pregnancies, in contrast to the presence of two placentas (dichorionicity) in virtually all dizygotic pregnancies (10).

Data on the relationship between zygosity status and the levels of estrogen and other pregnancy hormones are available from only a single small study (4), which found that dizygotic (compared with monozygotic) twin pregnancies have elevated levels of human placental lactogen but not of urinary estrogen. Levels of serum estrogen (11) and gonadotropin (11,12) are higher in mothers who have had dizygotic twins than in mothers who have had monozygotic twins or singletons, which is consistent with the relationship between hormone levels and dizygotic twinning (13). Finally, estrogen levels are higher in singleton pregnancies...
with a female fetus (14,15), but no data exist on whether maternal or fetal estrogen levels vary by zygosity and sexes of the twins in a pregnancy, although levels of human chorionic gonadotropin in both maternal and cord blood are higher in female–female and female–male twins than in male–male twins at delivery (16). Given this background, we evaluated whether membership in a twin pair, zygosity, and sex of the co-twin were associated with postmenopausal breast cancer risk in a cohort of older women from the state of Iowa.

**Subjects and Methods**

The institutional review boards of the University of Minnesota and the University of Iowa both reviewed and approved this study. In 1986, a 16-page mailed questionnaire was returned by 42% of 98,030 randomly selected Iowa women, aged 55–69 years; full details are available elsewhere (17,18). The self-administered questionnaire ascertained major breast cancer risk factors, including reproductive history, family history of breast cancer, and anthropometric data. Respondents also measured their waist and hip circumferences with a paper tape measure, using a valid and reliable methodology (19). Follow-up questionnaires were mailed to cohort members in 1987, 1989, 1992, and 1997 to identify residence changes, to update vital status, and to collect additional risk factor data. On the 1992 questionnaire, participants were asked if they were a twin, the sex of their co-twin, and whether they were an identical or fraternal twin; this questionnaire was completed by 33,017 of 39,789 women who were still alive at that follow-up, for a response rate of 83%.

Total mortality and cancer incidence from 1986 through 1996 were determined by linkages to the Iowa death certificate database and the Iowa Cancer Registry, the latter of which is part of the National Cancer Institute’s Surveillance, Epidemiology, and End Results (SEER) Program (20). Linkage was based on a combination of Social Security number; first name, last name, and maiden name; birthdate; and ZIP code. The linkage data were supplemented by information from the four follow-up surveys and, for survey nonrespondents, by linkage to the National Death Index.

For these analyses, we excluded women who were premenopausal, had had a mastectomy or partial breast removal, or had reported any cancer other than skin cancer at baseline in 1986, which left 37,105 women in the at-risk cohort. We further excluded women who were deceased at the time of the 1992 questionnaire or who did not respond to it (n = 7618), as well as women who responded to it but who had missing data on twin status (n = 290), which left 29,197 women in the at-risk cohort; the latter two exclusions eliminated 290 cases of breast cancer that occurred among the nonrespondents through 1996.

Length of follow-up time for each woman was calculated from the date of return of the baseline questionnaire to 1) the date of a breast cancer diagnosis in Iowa, 2) the date of her emigration from Iowa, or 3) the date of her death. If none of these events occurred, the date of last follow-up was considered to be December 31, 1996. Cox proportional hazards regression (21) was used to estimate the age- and multivariate-adjusted risk ratios (RRs) and their 95% confidence intervals (CIs) for the association between aspects of twin membership—i.e., whether the woman belonged to a pair of twins and the nature of that pair—and breast cancer incidence. The assumption of proportional hazards for the main exposure of interest (twin membership) was tested and found not to be violated.

Multivariate RRs were adjusted for factors associated with breast cancer risk in this dataset, including educational level (less than high school, some high school or graduated from high school, or more than high school), family history of breast cancer (no or yes), age at menarche (<12 years, 12 years, 13 years, 14 years, or >14 years), age at first live birth (<20 years, 20–24 years, 25–29 years, >30 years, or nulliparous), height in meters (as a continuous variable), body mass index (i.e., weight in kg/height in m²) at baseline (as a continuous variable), body mass index at age 18 years (as a continuous variable), waist-to-hip ratio (as a continuous variable), use of hormone replacement therapy (never, former, or current), and alcohol use (none, <4 g/day, or ≥4 g/day).

**Results**

Participants who completed the 1992 follow-up questionnaire were slightly younger (mean age, 61.6 years) and had a somewhat higher level of education (41% greater than high school) compared with nonrespondents (mean age, 62.2 years; 31% greater than high school education) (statistical significance not evaluated). Beyond this, there were no—or only trivial—differences between respondents and nonrespondents in other breast cancer risk factors, including height, body mass index at baseline, body mass index at age 18 years, waist-to-hip ratio, age at menarche, age at menopause, age at first live birth, use of hormone replacement therapy, and use of alcohol. In addition, breast cancer incidence from 1986 through 1992 was similar for respondents and nonrespondents (22).

There were 538 twins (1.8%) in the at-risk cohort of 29,197. Of these twins, 24% (n = 130) reported being monozygotic, 63% (n = 337) reported being dizygotic, and 13% (n = 71) were not sure of their zygosity status or their zygosity status information was missing; of the women who reported that they were not sure of their zygosity status, all reported a female co-twin. Of the 463 women with complete data on both zygosity and sex of the co-twin (four women had missing data on the sex of their twins), 40% had a male co-twin; 32% had a dizygotic female co-twin, and 28% had a monozygotic female co-twin. There were 25 pairs of twins who both participated in the Iowa Women’s Health Study: 12 pairs who were concordant for dizygotic status, seven pairs who were concordant for monozygotic status, and three pairs who were concordant for reporting “not sure of zygosity status.” Only three pairs of twins were discordant in their report of zygosity: In two pairs, one twin reported being dizygotic, while the other twin reported being unsure of her zygosity status; and in one pair, one twin reported being monozygotic, while the other twin reported not being sure of her zygosity status.

There were no striking differences in the major known breast cancer risk factors in this dataset between singletons and twins (Table 1). From 1986 through 1996 (301,777 person-years of follow-up), 1,230 breast cancers were identified in the at-risk cohort. Compared with singletons, women who were members of a twin pair showed a statistically significant increase in their risk of breast cancer after adjustment for other breast cancer risk factors (RR = 1.72; 95% CI = 1.22–2.42) (Table 2). Elevated risk was confined to dizygotic twins (RR = 1.77; 95% CI = 1.16–2.70), as there was no elevation in risk for women who were a monozygotic twin (RR = 1.04; 95% CI = 0.43–2.50). In addition, risk was higher if the sex of the other twin was female (RR = 1.82; 95% CI = 1.20–2.75) rather than male (RR = 1.49; 95% CI = 0.80–2.78), and the highest risk was seen for female dizygotic twins (RR = 2.14; 95% CI = 1.21–3.79).

Because a large percentage of the breast cancer cases (51%) were prevalent at the time of ascertainment of twin status, we next conducted an analysis based only on cases occurring after receipt of the third (1992) follow-up questionnaire. Compared with singletons, women who were members of a twin pair (19 case patients; age-adjusted RR = 1.67; 95% CI = 1.06–2.64) or were a dizygotic twin (13 case patients; age-adjusted RR = 1.84; 95% CI = 1.06–3.19) exhibited a statistically significant elevation in their risk of breast cancer. Women in the group who had a male co-twin (seven case patients; age-adjusted RR = 1.80; 95% CI = 0.86–3.80) or had a female dizygotic co-twin (six case patients; age-adjusted RR = 1.95; 95% CI = 0.87–4.35) were at elevated risk, but the risks were not statistically significant, perhaps because of the small numbers of cases;
Table 1. Comparison of baseline breast cancer risk factors by twin status, Iowa Women’s Health Study, 1986

<table>
<thead>
<tr>
<th>Factor</th>
<th>Singleton</th>
<th>Twin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± standard deviation</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>61.6 ± 4.2</td>
<td>61.5 ± 4.2</td>
</tr>
<tr>
<td>Age at menarche, y</td>
<td>12.8 ± 1.4</td>
<td>13.0 ± 1.4</td>
</tr>
<tr>
<td>Age at menopause, y</td>
<td>47.7 ± 6.3</td>
<td>47.2 ± 6.4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>69.1 ± 13.2</td>
<td>67.9 ± 13.3</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.63 ± 0.06</td>
<td>1.62 ± 0.06</td>
</tr>
<tr>
<td>Body mass index, kg/m², at baseline</td>
<td>26.9 ± 5.0</td>
<td>26.6 ± 5.0</td>
</tr>
<tr>
<td>Body mass index, kg/m², at age 18</td>
<td>20.9 ± 3.0</td>
<td>20.7 ± 2.8</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.835 ± 0.084</td>
<td>0.842 ± 0.109</td>
</tr>
</tbody>
</table>

Table 2. Relative risk of breast cancer according to characteristics of twin membership, Iowa Women’s Health Study, 1986–1996*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
<th>Case patients</th>
<th>Person-years</th>
<th>Age-adjusted risk ratio (95% CI)</th>
<th>Multivariate risk ratio† (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singleton</td>
<td>28659</td>
<td>1195</td>
<td>296257</td>
<td>1 (referred)</td>
<td>1 (referred)</td>
</tr>
<tr>
<td>Twin</td>
<td>538</td>
<td>35</td>
<td>5520</td>
<td>1.58 (1.13–2.21)</td>
<td>1.72 (1.22–2.42)</td>
</tr>
<tr>
<td>Zygosity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monozygotic</td>
<td>130</td>
<td>5</td>
<td>1347</td>
<td>0.92 (0.38–2.21)</td>
<td>1.04 (0.43–2.50)</td>
</tr>
<tr>
<td>Dizygotic</td>
<td>337</td>
<td>23</td>
<td>3443</td>
<td>1.67 (1.10–2.52)</td>
<td>1.77 (1.16–2.70)</td>
</tr>
<tr>
<td>Not sure/missing</td>
<td>71</td>
<td>7</td>
<td>738</td>
<td>2.36 (1.12–4.95)</td>
<td>2.78 (1.32–5.85)</td>
</tr>
<tr>
<td>Sex of other twin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>184</td>
<td>10</td>
<td>1891</td>
<td>1.32 (0.71–2.46)</td>
<td>1.49 (0.80–2.78)</td>
</tr>
<tr>
<td>Female</td>
<td>340</td>
<td>24</td>
<td>3486</td>
<td>1.71 (1.14–2.56)</td>
<td>1.82 (1.20–2.75)</td>
</tr>
<tr>
<td>Female/dizygotic</td>
<td>149</td>
<td>13</td>
<td>1509</td>
<td>2.15 (1.25–3.71)</td>
<td>2.14 (1.21–3.79)</td>
</tr>
</tbody>
</table>

*CI = confidence interval.
†Adjusted for age, educational level, family history of breast cancer, age at menarche, age at first live birth, height, body mass index at baseline, body mass index at age 18 years, waist-to-hip ratio, alcohol use, and hormone replacement therapy.

There were relatively few studies of twinship and breast cancer risk. In a medical records-based case–control study nested in a cohort of births at five Swedish hospitals from 1874 through 1961, Ekbom et al. (8) found that dizygotic twins (crude odds ratio [OR] = 1.52) but not monozygotic twins (OR = 0.59) were at increased breast cancer risk (mainly after menopause) compared with singletons (no CIs reported). Adjustment for maternal age, socioeconomic status, parity, pregnancy toxemia, neonatal birthweight, prematurity, and jaundice did not attenuate the results obtained by Ekbom et al. for dizygotic twins (OR = 1.72; 95% CI = 0.92–3.20). Hsieh et al. (6), using data from an international case–control study, found an elevated OR for breast cancer risk among the monozygotic twins in this group.

**Discussion**

We found that women who were twins had an approximately 1.7-fold greater risk of developing breast cancer than did singletons after adjustment for major breast cancer risk factors. The increase in multivariate-adjusted risk was confined to dizygotic twins (RR = 1.77) and, in particular, to women whose dizygotic co-twin was female (RR = 2.14). There was no evidence for an increase in risk in monozygotic twins, but the small number of exposed cases did not allow a precise estimation of risk. The strengths of this study include the use of a large, community-based cohort of women, case ascertainment using a SEER cancer registry, and control for major breast cancer risk factors. The prevalence of monozygotic and dizygotic twins, as well as the sex ratio of dizygotic twins, is in agreement with other survey data from populations of Western European origin (23).
for women who were twins (crude OR = 1.58; no CI reported) compared with singletons, and this association was only slightly attenuated (OR = 1.40; 95% CI = 0.77–2.55) after adjustment for age, parity, age at first birth, age at menarche, height, body mass index, maternal age at birth, birth order, and menopausal status. However, in contrast to our study, risk was slightly higher if the co-twin was male (OR = 1.54; 95% CI = 0.64–3.71) rather than female (OR = 1.30; 95% CI = 0.58–2.92), and the association was weaker among postmenopausal women (OR = 1.14; 95% CI = 0.53–3.45) than among premenopausal women (OR = 2.03; 95% CI = 0.74–5.55); however, none of these estimates were statistically significant. In contrast to our findings, Sanderson et al. (9), using two population-based, case-control studies conducted in the Seattle, WA, area, found no association with being a twin (yes/no) for women aged 50–64 years (OR = 0.9; 95% CI = 0.4–2.2) and a suggestive inverse association for women aged 21–45 years (OR = 0.6; 95% CI = 0.3–1.3). This study did not include data on zyosity.

Studies of the breast cancer experience occurring in registries of twins compared with the general population have found both increased risk (7) and null results (24,25), although the design of the twin registry of one of the null studies (25) has been criticized on methodological grounds (24), and reanalysis of the other null study (24) suggests that dizygotic twins—but not monozygotic twins—are at elevated breast cancer risk compared with singletons (6). The positive twin study (7) found that the increased risk of breast cancer was confined to dizygotic twins aged 20–29 years (standardized incidence ratio = 6.7; 95% CI = 2.9–13.1).

At an ecologic level, there is a strong correlation (r = .74; P<.005) between the twinning rate, which is due almost exclusively to the dizygotic twinning rate (23), and breast cancer mortality (26).

Thus, in aggregate, our study is consistent with several other studies, using different study designs, which suggest that being a twin and being a dizygotic twin, in particular, are associated with an elevated breast cancer risk. Our findings that the relative risk was greatest for female dizygotic twins has, to our knowledge, not been reported previously. As noted above, there is little evidence that the association between twin membership and breast cancer risk is confounded by other perinatal or traditional reproductive and adult breast cancer risk factors. Other childhood and adolescent breast cancer risk factors (e.g., diet and anthropometric measurements) could be potential confounders, but few such factors are currently known and we had no data to address this issue.

The use of surrogate exposures such as twin membership to evaluate the hypothesis that intrauterine estrogen levels are associated with breast cancer risk has several important limitations. First, twin membership is an inexact surrogate for elevated estrogen levels, although any misclassification would be expected to make it more difficult to detect an association. Second, the levels of many other hormones, including human chorionic gonadotropin, human placental lactogen, follicle-stimulating hormone, luteinizing hormone, and progesterone, are elevated during pregnancy, and these hormones, or other hormonal changes during pregnancy, may be of greater relevance. Third, most hormone levels have been measured in the maternal circulation, while it is the fetal circulation that is of most interest, and the interactions between maternal, placental, and fetal steroid production and exchange are complex and incompletely understood. Finally, dizygotic twinning not only is a surrogate for elevated in utero estrogen or other hormonal levels but also has many other correlates—including race, older maternal age, higher maternal parity, greater maternal height, and a maternal family history of dizygotic twinning (27)—thus, alternate explanations of the twinning association must be considered.

In summary, we found a statistically significant, positive association between twin membership and breast cancer risk that was strongest for dizygotic female twins. These data provide support for the role of intrauterine influences on breast cancer risk.

REFERENCES

(19) Kushi LH, Kaye SA, Folsom AR, Soler JT, Prineas RJ. Accuracy and reliability of self-


NOTES

1Editor’s note: SEER is a set of geographically defined, population-based, central cancer registries in the United States, operated by local nonprofit organizations under contract to the National Cancer Institute (NCI). Registry data are submitted electronically without personal identifiers to the NCI on a biannual basis, and the NCI makes the data available to the public for scientific research.

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