Research Article

Cancer Epidemiology, Biomarkers & Prevention

Lipophilic Statin Use and Risk of Breast Cancer Subtypes

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Abstract

Background/Aims: Statins are widely used and of high interest as potential chemopreventive agents for cancer. Preclinical studies suggest that lipophilic statins have anticancer properties targeting hormone receptor (HR)-negative breast cancer. Few epidemiologic studies have investigated the relationship between lipophilic statin use and risk for breast cancer, stratified by HR status. We conducted a large case-control study within Kaiser Permanente of Northern California (KPNC) to determine whether chronic use of lipophilic statins is associated with decreased risk of HR-negative breast cancer or other breast cancer subtypes.

Methods: We identified 22,488 breast cancer cases diagnosed from 1997 to 2007, and 224,860 controls matched to cases based upon birth year and duration of KPNC pharmacy coverage. Use of lipophilic statins was ascertained using the comprehensive electronic pharmacy records of KPNC.

Results: We found no association between lipophilic statin use (≥2 y versus never) and overall breast cancer risk (odds ratio$_{adj}$, 1.02; 95% CI, 0.97-1.08) in conditional logistic regression models adjusted for oral contraceptive and hormone therapy use. Women who used lipophilic statins did not have a decreased risk of HR-negative breast cancer (odds ratio$_{adj}$, 0.98; 95% CI, 0.84-1.14) nor altered risk of HR-positive disease (odds ratio$_{adj}$, 1.03; 95% CI, 0.97-1.10). Furthermore, lipophilic statin use was not associated with risk of any of the intrinsic subtypes, luminal A, luminal B, human epidermal growth factor receptor 2 positive/estrogen receptor negative, or triple negative.

Conclusions: Our results do not support an association of lipophilic statin use with the risk for breast cancer in general or with risks of HR-negative or other breast cancer subtypes specifically.

Impact: These findings do not confirm previous reports of a possible preventive association. *Cancer Epidemiol Biomarkers Prev; 19(10); 2479–87. ©2010 AACR.*

Introduction

Breast cancer is the most frequently diagnosed cancer and second leading cause of cancer death among U.S. women (1). This heterogeneous disease is composed of distinct tumor subtypes with characteristic molecular profiles (2), patient prognoses (3, 4), and treatment options (5). Hormone receptor (HR) negative subtypes develop earlier, are more aggressive, and contribute disproportionately to breast cancer mortality (6-8). They also include the triple negative subtype, for which targeted treatment options are limited, because these tumors lack the therapeutic targets: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2/ERBB2; ref. 9). Statins or f3-hydroxy-f3-methylglutaryl CoA reductase inhibitors can affect a wide range of molecular processes such as inflammation, cell migration, proliferation, and apoptosis (10, 11), which are integral to the development of cancer, including breast cancer. Furthermore, they are widely prescribed for their cholesterol-lowering capabilities and are well tolerated. Therefore, even a small effect of statins on the risk of breast cancer, particularly the HR-negative subtypes, would have a significant public health impact in reducing breast cancer mortality.

Preclinical studies suggest that lipophilic statins may have anticancer effects specifically targeting HR-negative breast cancer. Lipophilic statins, such as atorvastatin, simvastatin, and lovastatin (12) constitute most statin medications prescribed today. This class of statins can freely diffuse across cell membranes, unlike hydrophilic statins, leading to greater bioavailability in peripheral tissues such as breast. *In vitro* studies have shown that lipophilic statins cause significant growth inhibition in HR-negative breast cancer cell lines but only limited effects in HR-positive cell lines (13-15). Interestingly, the human breast cancer cell line MD-231, which corresponds to the triple-negative breast cancer phenotype, is particularly susceptible to lipophilic statins (14). *In vivo* studies in mouse models of breast cancer have shown that the chemopreventive effects of lipophilic statins on decreasing tumor multiplicity seem to depend on the mode of

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doi: 10.1158/1055-9965.EPI-10-0524

administration, proving modestly effective when injected i.p. (16, 17) but ineffective when dosed orally (18).

Epidemiologic studies have also suggested that use of lipophilic statins may reduce breast cancer risk; however, several questions remain unanswered. A study including 4,383 incident cases of invasive breast cancer within the Women's Health Initiative cohort found that use of lipophilic statins specifically, but not statins generally, was associated with a modest reduction in overall breast cancer risk (19). Three subsequent meta-analyses found no differences in overall breast cancer risk associated with lipophilic statin use (20-22). Unfortunately, these previous studies did not stratify their findings for lipophilic statins by tumor hormone-receptor status, perhaps because of small numbers. Most recently, a case-only study of 2,830 breast cancer patients diagnosed in 2003 within Kaiser Permanente of Northern California (KPNC) by Kumar *et al.* (23) found that women with HR-negative breast tumors were less likely than those with HR-positive tumors to have used lipophilic statins. This finding has evoked debate on whether lipophilic statins, specifically, might reduce risk of HR-negative disease. However, as Byers (24) pointed out in an accompanying editorial, the absence of a control group limited the ability of this study to distinguish between a reduction in risk of HR-negative breast cancers, an increase in risk of HR-positive tumors, or both.

We conducted a case-control study of 22,488 invasive breast cancer cases diagnosed from 1997 to 2007 and 224,860 matched controls within the KPNC healthcare system to examine whether chronic use of lipophilic statins is associated with (*a*) decreased risk of HR-negative breast cancer without affecting risk of HR-positive disease; (*b*) increased risk of HR-positive breast cancer without affecting risk of HR-negative disease; or (*c*) decreased risk of HR-negative breast cancer and increased risk of HR-positive disease. Furthermore, we investigated whether lipophilic statins are associated with the risk of any of the intrinsic breast cancer subtypes, luminal A, luminal B, HER2+/ER–, and triple negative, as defined by ER, PR, and HER2 status. We also attempted to replicate the case-only study findings of Kumar *et al.* (23). KPNC is a nonprofit integrated healthcare-delivery system with electronic records capturing inpatient and outpatient services, pharmacy information, and central laboratory results for over 3 million members. To our knowledge, this study is the largest case-control study of lipophilic statin use and breast cancer risk to date and the first to examine the risk of intrinsic breast cancer subtypes in relation to statin use.

Materials and Methods

Study population

We conducted a case-control study within the member population of the KPNC healthcare delivery system. Statin use was ascertained through the KPNC Pharmacy Information System, which records prescription medications dispensed at all KPNC pharmacies since August 1994. We restricted our analysis to cases and controls with follow-back periods of ≥2 years from the diagnosis/index date to the beginning of their prescription drug coverage (coverage). Cases were identified through KPNC Cancer Registry (KPNCCR) records. Cases consisted of all women diagnosed with invasive breast cancer with known HR status between January 1, 1997, and December 31, 2007. Controls were matched to cases based upon exact year of birth and duration of coverage at a ratio of 10:1 if available (mean, 9.9; range, 6-10 controls per case).

This study was approved by the Institutional Review Board of the Kaiser Foundation Research Institute.

Statin use

Nearly 98% of all dispensed statins by KPNC pharmacies are composed of the lipophilic statins, lovastatin, simvastatin, and atorvastatin (25). Therefore, we restricted our analysis to these three agents and will henceforth refer to them simply as statins. Using the KPNC automated pharmacy database, we ascertained statin brands and refill dates for all study participants. We restricted our analysis to statin use that preceded the breast cancer diagnosis/index date. Women had to receive at least two prescriptions to be considered statin users because, without a refill, statin use may have been discontinued promptly due to side effects or other reasons. Women with zero or one statin prescription were considered never users.

In our primary analysis, statin use was defined as at least 2 years of use to reduce the possibility of detection bias, that is, the detection of a tumor as a consequence of a physician visit at which statins were prescribed. Therefore, cases and controls were restricted to those with at least 2 years of enrollment and pharmacy benefits before diagnosis/index date. To explore a possible duration effect, statin use was also categorized as never, as defined above, <1 year, 1 to <2 years, 2 to <3 years, 3 to <5 years, and ≥5 years of use. Duration of statin use before the diagnosis/index date was calculated by adding up the days of supply of all prescriptions, accounting for dispensing before the previous prescription was to be used up, and not differentiating between receipt of a single statin and sequential combination of any of the three statins studied.

We also obtained prescription information on oral contraceptive use and menopausal hormone therapy use from the KPNC pharmacy database. A minimum of two filled prescriptions of oral contraceptive or hormone therapy was required to be classified as a user. We considered women to be oral contraceptive users if they had documented oral contraceptive use within 10 years and hormone therapy users if they used hormone therapy within 5 years before diagnosis in accordance with the estimated periods when breast cancer risk is elevated (26, 27). Hormone therapy included estrogens and progestins because a previous study on lipophilic statin use and overall breast cancer risk in the KPNC health

care system showed no appreciable confounding by hormone therapy when adjusting separately for estrogens, progestins, and other female hormone preparations (25).

Tumor characteristics

Information on tumor characteristics was obtained from the KPNCCR. Histologic grade is scored based on Scarff Bloom Richardson criteria as I (low grade), II (moderate grade), or III (high grade). Tumor stage is recorded as localized malignancy, regional malignancy, or distant metastases in accordance with the Surveillance, Epidemiology, and End Results reporting guidelines (28).

Immunohistochemistry testing was conducted by the KPNC Immunohistochemistry Laboratory, a high volume reference laboratory with >11,000 patients yearly, of which approximately one third are breast cancer cases. Breast cancer cases are routinely tested for ER, PR, and HER2. Immunohistochemistry stains are manually interpreted by four full-time immunopathologists. The laboratory has been designated as a center of excellence for HercepTest interpretation by the DAKO Corporation, licensed by the Clinical Laboratory Improvement Amendments, and certified by the College of American Pathologists. Fluorescence *in situ* hybridization (FISH) assays were conducted by the KPNC Genetics Laboratory.

ER and PR testing results have been routinely documented in the electronic pathology records of the KPNCCR since 1996. Tumors with >5% nuclear staining were considered positive for ER and PR expression. Tumor expression of HER2 was assessed by immunohistochemistry and, if 2+ or specifically requested by a clinician, by FISH. Tumors with immunohistochemistry staining of 0, 1+, or 2+ and negative FISH results were considered HER2 negative, whereas tumors with either immunohistochemistry staining of 3+ or positive FISH results (ratio, ≥2.0) were considered HER2 positive in accordance with the guidelines of the American Society of Clinical Oncology and College of American Pathologists (29). Although HER2 testing has been routinely done on invasive breast cancers since 2000, we restricted our analyses requiring HER2 data to the years 2002 to 2007, when the HER2 results were considerably more complete in the KPNCCR than in previous years.

HR-positive tumors were ER positive, PR positive, or both. HR-negative tumors were ER negative/PR negative or ER negative/PR unknown. We defined the intrinsic breast cancer subtypes as (2, 3) luminal A, luminal B, HER2+/ER−, and triple negative based on tumor ER, PR, and HER2 expression, similarly to what was done by Carey et al. (7). Intrinsic subtypes classified by immunohistochemistry-based tumor markers have been shown to be equally predictive of patient prognosis as their gene expression-based counterparts (7). Subtype luminal A was defined as ER positive and/or PR positive and HER2 negative; luminal B as ER positive and/or PR positive and HER2 positive; HER2+/ER- as ER negative, PR negative, and HER2 positive; and triple-negative tumors as ER negative, PR negative, and HER2 negative. The triple-negative tumor category contains the basal-like and the normal breast-like subtypes. Immunohistochemistry testing is not routinely done for the other two markers (Cytokeratin 5/6 and HER1) used by Carey *et al.* to identify the basal-like group.

Statistical analyses

We compared the distribution of individual characteristics among various patient groups using χ^2 tests for discrete variables and *t*=tests for continuous variables. In matched case-control analyses, odds ratios for specific breast cancer subtypes associated with statin use were estimated using conditional logistic regression models and adjusted for oral contraceptive and hormone therapy use. Adjustment for oral contraceptive and hormone therapy use resulted in a ~2% change in the odds ratio; these weak potential confounders were retained in all analyses to be conservative. We used unconditional logistic regression models (dichotomous outcomes) and polytomous logistic regression (multinomial outcomes) to estimate the odds ratios for the association of statin use and various tumor characteristics in case-only analyses. These unconditional regression models were adjusted for the matching variables age at diagnosis (age) modeled as age + age^2 and duration of prescription categorized into quartiles, in addition to oral contraceptive use, hormone therapy use, and race/ethnicity (race) modeled as a categorical variable (non-Hispanic White, Hispanic White, African American, Asian/Pacific Islander, other). In polytomous logistic regression models, we calculated odds ratios comparing each category to a common reference group and examined global changes in the distribution of categorical tumor characteristics between statin users and nonusers by the Maximum Likelihood ANOVA/χ^2 test. All analyses were done using SAS, version 9.1 (SAS Institute, Inc.).

Sensitivity analysis

To evaluate the sensitivity of our results to the effects of unmeasured confounders, we performed external adjustments (30, 31) for established risk factors for breast cancer that were also associated with statin use in the 2002 Member Health Survey of KPNC. We restricted the survey population to 6,985 women >40 years of age who had a minimum of 2 years of prescription to resemble the characteristics of our study cohort. In this random sample of KPNC members, statin use (≥2 y versus never) was significantly associated with body mass index (BMI; odds ratio, 1.67; ≥30 versus <30) and alcohol consumption (odds ratio, 0.52; ≥7 versus <7 drinks per week), and African American race (odds ratio, 1.50; African American versus non-Hispanic Whites) but not with any other racial/ethnic group or menopausal status (odds ratio, 1.01; postmenopausal versus premenopausal) after adjusting for age, coverage, oral contraceptive, and hormone therapy use. The prevalence of statin use (≥2 y), obesity (BMI, ≥30), alcohol consumption (≥7 drinks per week), and African Americans was 0.091, 0.238, 0.151, and 0.060, respectively,

in the 2002 Member Health Survey population. We used the following literature-based estimates of the relative risks of HR-negative and -positive breast cancer, respectively: 1.06 and 1.82 in association with BMI of ≥30 versus <30 (32); and 1.10 and 1.22 in association with ≥7 versus <7 alcoholic drinks per week (33).

We carried out external adjustment using the method of Schneeweiss (30) for BMI and alcohol consumption and the method of Suissa (31) for African-American race to use the available race information in cases only. Briefly, in the Schneeweiss (30) external adjustment procedure, the prevalence of the dichotomized confounders and the multivariate-adjusted odds ratios for their relationship with statin use were estimated from the 2002 Member Health Survey data. Estimates of the relative risks of HR-specific breast cancer associated with the potential confounders were obtained by using the most extreme estimate reported in the most recent meta-analyses (32, 33) found by conducting a PubMed literature search. Finally, the net bias of all confounders was estimated by the weighted average of the percent bias attributed to each confounder, in which the weights correspond to the prevalence of each confounder. The Suissa (31) external adjustment procedure is similar except that it does not rely upon literature-based estimates of the confounder-disease relationship, using the confounder information available among cases and inferred for controls using data from an external population instead.

Results

Study population

Our case-control study population consisted of 224,860 controls and 22,488 invasive breast cancer cases. Cases and controls were well matched with respect to age at time of diagnosis/index date and duration of follow-up in the KPNC pharmacy database (Table 1). As expected, we observed higher frequencies of oral contraceptives and menopausal hormone therapy in cases compared with controls. The prevalence and duration of statin use was similar between cases and controls (Table 1).

Of the 22,488 invasive breast cancer cases in our study population, 82.2% were diagnosed with HR-positive and 17.8% with HR-negative tumors. Women diagnosed with HR-negative breast cancer were more likely to have used oral contraceptives within 10 years before diagnosis ($P < 0.0001$) and less likely to have used hormone therapy within 5 years before diagnosis ($P < 0.0001$) compared with HR-positive cases. HR-negative cases were less likely than HR-positive cases to have used statins (20.9% versus 24.7%; $P < 0.0001$) and tended to have used statins for shorter durations (0.44 versus 0.56 years on average; $P < 0.0001$), in part because of their younger age. HR-positive cases were more likely to be non-Hispanic White and less likely to be Hispanic or African American than were HR-negative cases. The distribution of Asians and women within the other

Table 1. Characteristics of breast cancer cases diagnosed from 1997 to 2007 at KPNC and matched controls by disease and HR status

Characteristic	All controls, *n* = 224,860	All cases, *n* = 22,488	HR-negative cases, *n* = 3,996	HR-positive cases, *n* = 18,492
Age at diagnosis/index date, mean year ± SD	61.11 ± 13.32	61.11 ± 13.32	57.68 ± 13.46	61.85 ± 13.17
Years of coverage, mean ± SD	7.28 ± 3.19	7.28 ± 3.19	7.20 ± 3.15	7.30 ± 3.19
Oral contraceptive use, *n* (%)*	19,080 (8.5)	2,220 (9.9)	468 (11.7)	1,752 (9.5)
Menopausal hormone therapy use, *n* (%)†	89,664 (39.9)	9,593 (42.7)	1,553 (38.9)	8,040 (43.5)
Lipophilic statin use, *n* (%)				
Never use	169,816 (75.5)	17,079 (76.0)	3,160 (79.1)	13,919 (75.3)
Ever use	55,044 (24.5)	5,409 (24.0)	836 (20.9)	4,573 (24.7)
≥2-y use	18,614 (8.3)	1,888 (8.4)	260 (6.5)	1,628 (8.8)
Years of lipophilic statin use, mean ± SD				
Among ever users	0.53 ± 1.38	0.54 ± 1.41	0.44 ± 1.22	0.56 ± 1.45
Among ≥2-y users	4.47 ± 2.20	4.55 ± 2.28	4.34 ± 2.27	4.58 ± 2.28
Race, *n* (%)‡				
Non-Hispanic White	n/a	16,256 (72.3)	2,485 (62.2)	13,771 (74.5)
Hispanic White	n/a	1,574 (7.0)	360 (9.0)	1,214 (6.6)
African American	n/a	1,803 (8.0)	590 (14.8)	1,213 (6.6)
Asian/Pacific Islander	n/a	2,410 (10.7)	463 (11.6)	1,947 (10.5)
Other	n/a	455 (2.0)	98 (2.5)	347 (1.9)

*Oral contraceptive ever use within 10 years before diagnosis/index date.
†Hormone therapy ever use within 5 years before diagnosis/index date.
‡Race information was captured by the KPNCCR and was available for cases only.

Table 2. Risk of HR-negative or -positive breast cancer associated with statin use

Statin use	HR-negative cases, *n* = 3,996 (col%)	Matched controls, *n* = 39,960 (col%)*	OR (95% CI)†	HR-positive cases, *n* = 18,492 (col%)	Matched controls, *n* = 184,900 (col%)‡	OR (95% CI)§
Never	3,160 (79.1)	31,694 (79.3)	Reference	13,919 (75.3)	138,122 (74.7)	Reference
≥2 y	260 (6.5)	2,612 (6.5)	0.98 (0.84-1.13)	1,628 (8.8)	16,002 (8.7)	1.03 (0.97-1.10)
Never	3,160 (79.1)	31,694 (79.3)	Reference	13,919 (75.3)	138,122 (74.7)	Reference
<1 y	440 (11.0)	4,477 (11.2)	1.02 (0.91-1.16)	2,299 (12.4)	24,107 (13.0)	1.00 (0.95-1.05)
≥1-2 y	136 (3.4)	1,177 (2.9)	1.17 (0.97-1.41)	646 (3.5)	6,669 (3.6)	0.97 (0.89-1.05)
≥2-3 y	93 (2.3)	831 (2.1)	1.13 (0.91-1.42)	475 (2.6)	4,827 (2.6)	0.98 (0.89-1.08)
≥3-5 y	96 (2.4)	1,040 (2.6)	0.93 (0.75-1.16)	604 (3.3)	6,099 (3.3)	0.99 (0.90-1.08)
≥5 y	71 (1.8)	741 (1.9)	0.97 (0.75-1.25)	549 (3.0)	5,076 (2.7)	1.08 (0.98-1.19)

Abbreviation: OR, odds ratio

*Controls matched to HR-negative cases bases on age at diagnosis/index date and years of coverage.

†Odds ratios based on conditional logistic regression among HR-negative cases and their matched controls, adjusted for oral contraceptive and hormone therapy use.

‡Controls matched to HR-positive cases bases on age at diagnosis/index date and years of coverage.

§Odds ratios based on conditional logistic regression among HR-positive cases and their matched controls, adjusted for oral contraceptive and hormone therapy use.

category was similar between HR-negative and HR-positive cases.

Risk of breast cancer in relation to statin use

In case-control analyses, we found no statistically significant association between statin use (≥2 years versus never) and overall breast cancer risk (odds ratio, 1.02; 95% CI, 0.97-1.08; $P = 0.42$). Women who used statins for ≥2 years did not have a decreased risk of HR-negative breast cancer (odds ratio, 0.98; 95% CI, 0.84 -1.14; $P = 0.74$) or an increased risk of HR-positive breast cancer (odds ratio, 1.03; 95% CI, 0.97-1.10; $P = 0.31$; Table 2). These null findings did not vary significantly among women with the age of 55 years or older versus younger than 55 years. Longer duration of statin use was also not consistently associated with decreasing risk of HR-negative breast cancer ($P_{trend} = 0.86$) or increasing risk of HR-positive breast cancer ($P_{trend} = 0.58$).

Subtype-specific case-control analyses showed little evidence that statin use was associated with the risk of developing a particular intrinsic breast cancer subtype (Table 3). Statin use of ≥2 years was associated with a slight, nonstatistically significant increase in risk for luminal A-type breast cancer (odds ratio,1.09; 95% CI, 1.00-1.18; $P = 0.057$). We found no evidence that statin use altered the risk of the luminal B, HER2+/ER−, or triple-negative breast cancer subtypes. Therefore, it is unlikely that statins affect the risk of the four intrinsic breast cancer subtypes differentially.

Tumor characteristics in relation to statin use

In analyses restricted to breast cancer cases, we examined whether HR status was associated with statin use to replicate the findings of one previous smaller study (23). Although statin use was slightly less likely among HR-negative breast cancer cases than among HR-positive cases (odds ratio,0.91; 95% CI, 0.78-1.05; $P = 0.18$), this difference was not statistically significant. To further understand the difference between our study findings based upon 11 years of data and a previous study, which only included data from 2003 (23), we repeated our case-only (Fig. 1A) and case-control (Fig. 1B) analyses stratified by year. We observed considerable fluctuations in the estimated odds ratio on a year-by-year basis. The year 2003

Table 3. Risk for intrinsic breast cancer subtypes associated with statin use

Study population	Statin use, ≥2 y	Statin use, never	OR* (95% CI)
Luminal A			
Controls	8,990 (17.5)	42,355 (82.5)	Reference
Cases	948 (18.2)	4,265 (81.8)	1.09 (1.00 - 1.18)
Luminal B			
Controls	1,841 (14.4)	10,910 (85.6)	Reference
Cases	178 (13.8)	1,110 (86.2)	0.99 (0.82 -1.19)
HER2+/ER−			
Controls	676 (12.9)	4,574 (87.1)	Reference
Cases	71 (13.0)	474 (87.0)	1.05 (0.78-1.42)
Triple negative			
Controls	1,118 (12.3)	7,984 (87.7)	Reference
Cases	111 (12.1)	810 (87.9)	0.93 (0.74 - 1.18)

*Odds ratios based on conditional logistic regression among cases classified by intrinsic subtype and respective matched controls, adjusted for oral contraceptive and hormone therapy use.

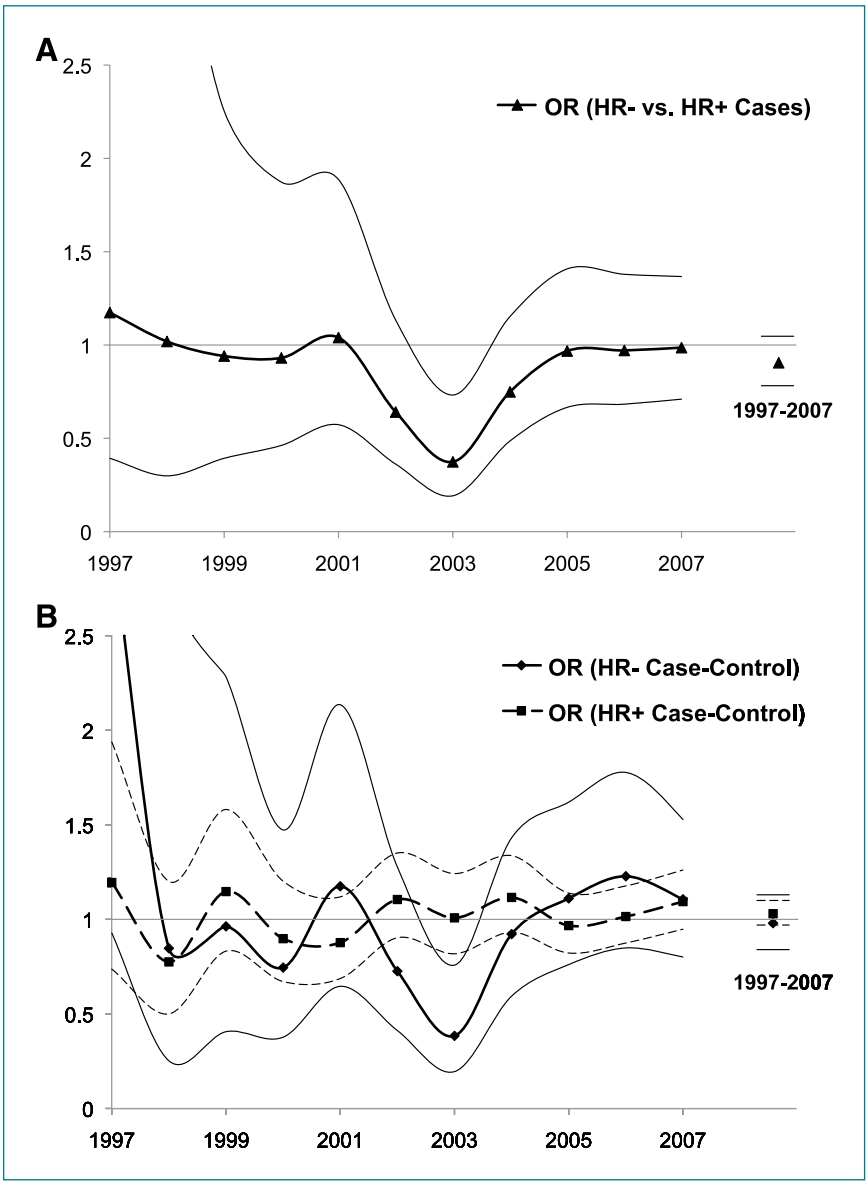

Figure 1. Trends in annual estimates of the association between statin use and breast cancer by HR status. A, annual estimates of the odds ratios (triangles) with 95% CIs for the association of statin use (≥2 y versus never) with HR status (HR- versus HR+) in case-only analyses during the years 1997 to 2007. B, annual estimates of the odds ratios with 95% CIs for the association of statin use (≥2 y versus never) with risk for HR-negative (diamonds, solid lines) and HR-positive (squares, dashed lines) breast cancer in case-control analyses during the years 1997 to 2007. Summary estimates for the entire observation period are in each graph. OR, odds ratio.

seemed to be an outlier because it was the only year during the 11-year study period with a statistically significantly lower proportion of HR-negative cases in statin users (Fig. 1A). This result was likely due to an unusually low risk of HR-negative breast cancer in statin users, particular to the year 2003, because the risk of HR-positive disease in statin users seemed to be stable across the 11-year study period (Fig. 1B).

Among cases only, we also examined whether other tumor characteristics were associated with statin use (Table 4). After adjusting for race in addition to age, coverage, oral contraceptive, and hormone therapy use, cases who were statin users were slightly less likely than nonusers to have tumors that were negative for ER expression (odds ratio,0.88; 95% CI, 0.76-1.02; $P = 0.086$), PR expression (odds ratio,0.89; 95% CI, 0.80-0.99; $P = 0.036$), and aberrant HER2 overexpression/amplification (odds ratio,0.90; 95% CI, 0.77-1.06, $P = 0.22$). Only the result for PR expression reached borderline statistical significance and may be explained by chance in light of the multiple comparisons that were done. There were no statistically significant differences in the global distributions of the intrinsic breast cancer subtypes between cases that were statin users versus nonusers ($\chi^2_{3df} = 5.16$; $P = 0.16$). Finally, statin use did not correlate with downshifts in either stage of diagnosis ($\chi^2_{2df} = 0.53$; $P = 0.77$) or histologic tumor grade ($\chi^2_{2df} = 2.22$; $P = 0.33$).

Sensitivity analysis

External adjustment for obesity, alcohol consumption, and race showed that the magnitude of confounding due to these factors was small in our study. External adjustment

Table 4. Tumor characteristics associated with statin use

Tumor characteristic	Statin use, ≥2 y	Statin use, never	OR* (95% CI)
ER status			
ER+	1,623 (10.6)	13,730 (89.4)	Reference
ER–	265 (7.33)	3,349 (92.7)	0.88 (0.76-1.02)
PR status			
PR+	1,307 (10.5)	11,181 (89.5)	Reference
PR–	571 (8.9)	5,840 (91.1)	0.89† (0.80-0.99)
HER2 status‡			
HER2–	1,059 (17.3)	5,075 (82.7)	Reference
HER2+	250 (13.6)	1,585 (86.4)	0.90 (0.77-1.06)
Intrinsic subtype‡			
Luminal A	948 (18.2)	4,265 (81.8)	Reference
Luminal B	178 (13.8)	1,110 (86.2)	0.94 (0.85-1.04)
HER2+/ER–	71 (13.0)	474 (87.0)	0.93 (0.79-1.07)
Triple negative	111 (12.1)	810 (87.9)	0.90 (0.78-1.01)
Tumor stage			
Localized	1,325 (10.7)	11,011 (89.3)	Reference
Regional	493 (8.3)	5,422 (91.7)	0.99 (0.93-1.05)
Metastasis	60 (10.3)	520 (89.7)	1.05 (0.90-1.20)
Tumor grade			
Grade I	460 (11.6)	3,492 (88.4)	Reference
Grade II	783 (10.7)	6,544 (89.3)	1.02 (0.96-1.09)
Grade III	397 (7.8)	4,714 (92.2)	0.97 (0.90-1.05)

*Adjusted for age, coverage, oral contraceptive use, hormone therapy use, and race.
†$P < 0.05$.
‡HER2 status was only evaluated in tumors from 2002 to 2007.

for obesity and alcohol consumption using the Schneeweiss method (30) minimally changed the odds ratio for the association of statin use with the risk of breast cancer from 0.98 to 0.97 for HR-negative cancer and from 1.03 to 0.99 for HR-positive breast cancer. Similarly, external adjustment for race using the method of Suissa (31) minimally changed the crude odds ratio from 1.00 to 0.98 for HR-negative cancer while having no effect on the crude odds ratio of 1.01 for HR-positive breast cancer. Furthermore, adjustment for race using the available data in case-only analyses barely changed the odds ratio for the association of statin use with HR-negative versus HR-positive tumors from 0.91 to 0.92. These results indicated that the main results of our study were not sensitive to the effects of obesity, alcohol, or race and that other weaker unmeasured potential confounders were unlikely to influence our interpretation.

Discussion

We report the results of the largest case-control study on statin use and breast cancer risk to date, to our knowledge, with 22,488 cases, which allowed us to investigate associations with risk of specific breast cancer subtypes. The use of lipophilic statins was not associated with risk of breast cancer overall. Statin use was not associated with a reduced risk of HR-negative breast cancer or an increased risk of HR-positive disease. We also found no statistically significant association with risk of luminal A, luminal B, HER2+/ER–, or triple-negative subtypes. Furthermore, our study showed no evidence that statin use affects the tumor expression of ER, PR, or HER2, or the histologic grade or stage at diagnosis. Collectively, our results indicate that use of lipophilic statins neither increases nor decreases the overall risk of breast cancer and argue against tumor subtype-specific risk modulations.

Our finding that the overall risk for breast cancer is not associated with statin use contributes to existing evidence that statin medications are neutral with regard to breast cancer risk, a reassuring notion considering the widespread use of statins. Our relative risk estimate of odds ratio,1.02 is consistent with that of a recent meta-analysis, which reported an overall relative risk of 1.01 (95% CI, 0.79-1.30) based on seven randomized controlled trials (21). Such close agreement is important because it suggests minimal effects of unmeasured confounders, which can be problematic in observational studies but are accounted for by randomization in randomized controlled trials. Furthermore, our results agree with a large prospective cohort study in the KPNC membership, focusing on lipophilic statins specifically, which reported a relative risk of 1.02 (95% CI, 0.86-1.21) for any breast cancer with at least 5 years of use (25). We could not confirm the Women's Health Initiative findings of an overall reduction in breast cancer risk associated with lipophilic statin use (hazard ratio, 0.82; 95% CI, 0.70-0.97; ref. 19).

In our study, lipophilic statin use was not associated with either a reduction in risk of HR-negative or an increase in HR-positive breast cancer. These observations are consistent with the null findings of two earlier studies looking at HR-specific breast cancer risk (34, 35). However, these studies included hydrophilic and lipophilic statins and did not stratify by lipophilicity. Furthermore, lipophilic statin use was not significantly associated with the risk of developing any of the four intrinsic breast cancer subtypes. To our knowledge, this is the first study to examine the risk of intrinsic breast cancer subtypes in relation to statin use.

The results of our 1997 to 2007 case-only analysis did not support the finding of a previous case-only study by Kumar *et al.* (23) conducted among KPNC breast cancer patients diagnosed in 2003. Although our study found that HR-negative breast cancer patients were slightly less likely than HR-positive patients to have used statins (odds ratio, 0.91; 95% CI, 0.78-1.05), this difference was not statistically significant. Interestingly, the year 2003 represented an outlier in the context of our 11-year study period. It was the only year for which HR-negative cases were statistically significantly less likely than

HR-positive cases to have used lipophilic statin. To our knowledge there were no changes in reporting practices at KPNC, statin use, or breast cancer incidence that might explain the exceptional pattern in the year 2003. Therefore, although we were able to duplicate the findings of Kumar *et al* (23), based on 34 HR-negative and 269 HR-positive cases who had used statins for ≥1 year, these observations for the year 2003 likely reflect random variation and small numbers rather than a true association between lipophilic statin use and breast cancer risk. In addition, we found no evidence of significant alteration in ER, PR, or HER2 expression with statin use that would be suggestive of a phenotypic switch to a more favorable breast cancer subtype as postulated based on the findings of Kumar *et al.* (23, 24).

Our study design has several important strengths. First, the large size of our study allowed us to examine subtype-specific risks and provided high power to detect even modest associations between lipophilic statin use and HR-specific breast cancer risk. The size of our study further enabled us to stratify our analysis by calendar year, uncovering considerable variation, especially during the year 2003, upon which previous findings have been based (23). Second, KPNC electronic pharmacy records enabled accurate ascertainment of the timing and duration of lipophilic statin use. Electronic pharmacy records are highly accurate compared with interview or questionnaire-based methods, which rely on patient recall. Third, because nearly 98% of statins prescribed at KPNC are lipophilic and all incident invasive breast cancer cases who met the eligibility criteria were included in our study, we minimized the potential for bias due to either sampling of cases or exclusion of hydrophilic statin users. Finally, the KPNC membership of >3 million residents resembles the underlying general population in the greater Bay area (36) and more closely represents the racial/ethnic composition of the U.S. population than patients in randomized controlled trials (37), adding to the external validity of our results.

The main limitation of our study was that information on potential confounding factors, such as obesity, alcohol consumption, race, or socioeconomic status, were not available in the electronic records for all cases and controls. However, our sensitivity analysis showed minimal changes in the HR-specific relative risk estimates after externally adjusting for obesity, race, and alcohol consumption, suggesting that these unmeasured confounders did not have a major impact on our results. This is consistent with the observations of other population-based studies on statins and breast cancer, which ascertained many potential confounders but reported little difference between age-adjusted and fully adjusted risk estimates (34, 35). A second limitation of our study is that ductal carcinomas *in situ* (DCIS), which today make up nearly 25% of newly diagnosed breast lesions, were not evaluated. We decided to focus our analysis on invasive breast cancer cases only because routine testing of DCIS tumors for ER and PR was not done until 2000 in KPNC and HER2 testing is rarely done on DCIS tumors. In contrast to our study, Kumar *et al.* (23) included 11.2% cases diagnosed at *in situ* stage; however, we obtained similar results for the year 2003, indicating that the exclusion of DCIS was unlikely to affect our conclusions.

In conclusion, our results do not support an association between lipophilic statin use and breast cancer risk in general or the risk for ER−, PR−, and HER2-defined subtypes specifically. Any effect, if it exists at all, is likely to be much smaller than previous studies have suggested. These findings are reassuring with respect to the safety of chronic statin use and should be considered in the interpretation of several ongoing breast cancer chemoprevention trials of lipophilic statins (38), as well as in the planning of future interventional studies using statin medications.

Disclosure of Potential Conflicts of Interest

L.A. Habel is about to receive a research grant through the Kaiser Foundation Research Institute and the University of North Carolina from Sanofi-Aventis to examine risk for breast and other cancers associated with medications used to treat diabetes and has research grants from Genomic Health, Inc., and Bio Theranostics through the Kaiser Foundation Research Institute to examine gene expression profiles and breast cancer outcomes, from Genentech through the Kaiser Foundation Research Institute to examine patient and tumor factors associated with HER2 status, and from Merck through the Kaiser Foundation Research Institute to examine risk for herpes zoster among cancer patients.

Grant Support

Grant R01 CA 098838 from the National Cancer Institute, (L.A. Habel, G.D. Friedman), and support from the Cancer Prevention Fellowship Program, Office of the Director, National Cancer Institute (S. Woditschka).

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Received 05/15/2010; revised 07/23/2010; accepted 08/03/2010; published online 10/07/2010.

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