Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and Breast Cancer Risk: Differences by Molecular Subtype

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Abstract

Use of non-steroidal anti-inflammatory drugs (NSAIDs) has been associated with reduced risk of breast cancer, though findings have been inconsistent. This inconsistency may result from differences in etiology for breast tumors of different subtypes. We examined the association between NSAID use and breast cancer characterized by molecular subtypes in a population-based case-control study in Western New York. Cases (n=1,170) were women with incident, primary, histologically confirmed breast cancer. Controls (n=2,115) were randomly selected from NY Department of Motor Vehicles records (<65yrs) or Medicare rolls (≥65yrs). Participants answered questions regarding their use of aspirin and ibuprofen in the year prior to interview and their use of aspirin throughout their adult life. Logistic regression models estimated odds ratios (OR) and 95% confidence intervals (95% CI). Recent and lifetime aspirin use was associated with reduced risk, with no differences by subtype. Recent use of ibuprofen was significantly associated with increased risk of ER+/PR+ (OR 1.33, 95% CI: 1.09-1.62), HER2- (OR 1.27, 95% CI: 1.05-1.53), and p53- breast cancers (OR 1.28, 95% CI: 1.04-1.57), as well as luminal A or B breast cancers. These findings support the hypothesis of heterogeneous etiologies of breast cancer subtypes and that aspirin and ibuprofen vary in their effects.

Introduction

Chronic inflammation is suspected to be associated with the initiation and promotion of carcinogenesis at several anatomic sites, including the breast (1, 2). Epidemiologic studies of non-steroidal anti-inflammatory drugs (NSAIDs) and breast cancer risk report inverse associations (3, 4), and NSAIDs have demonstrated anti-cancer properties *in vitro* and *in vivo* (5-8), adding further support to this hypothesis.

There is evidence that biologically distinct subtypes of breast cancer, distinguished on the basis of gene expression (9-11) or tumor marker expression (12), may also be etiologically distinct (13-16). Epidemiologic studies of the association of NSAIDs with breast cancer characterized by ER or PR status have been inconsistent (17-28). However, no previous studies have reported on the association of NSAID use with breast cancer risk according to HER2 protein expression, p53 mutation status, or joint ER, PR, and HER2 status.

We reported previously that recent and lifetime use of aspirin but not recent use of ibuprofen was inversely associated with breast cancer risk (29). In light of potential differences in breast cancer etiology for breast cancer classified by these subtypes, here we build upon our previous report and examine the associations of aspirin and ibuprofen use with risk of breast cancer subtypes defined by molecular characteristics in the Western New York Exposures and Breast Cancer (WEB) study.

Materials & Methods

The WEB study is a population-based case-control study of women living in Erie and Niagara counties of western New York State. Study methods have been described elsewhere (29). Briefly, cases and controls were identified between 1996 and 2001. Women were eligible to participate in the study if they were between the ages of 35 and 79 years, spoke English, had no

prior history of cancer other than non-melanoma skin cancer, and were current residents of Erie or Niagara counties. Cases were women with incident, first primary, histologically-confirmed breast cancer. Nurse case-finders who visited pathology departments of area hospitals identified potential cases. Patients' physicians were contacted to verify the diagnosis and to obtain permission to contact them. Once permission was granted, cases were interviewed within one year of diagnosis. Most cases were interviewed within 6 months (median 5.4 months). Controls were randomly selected from Department of Motor Vehicles records (<65yrs) or Medicare rolls (≥65yrs) and frequency matched to cases on age and race. Seventy-two percent of eligible cases (n=1,170) and 63% of eligible controls (n=2,115) were interviewed. Among those that provided a reason for non-participation, the primary reasons among eligible cases were physician refusal for permission to contact or patient disinterest; among eligible controls, the primary reasons were scheduling conflicts or illness. All participants provided informed consent and study protocols were approved by the Institutional Review Boards of the University at Buffalo and participating hospitals.

Data Collection

Trained interviewers conducted computer-assisted, in-person interviews and participants completed an extensive self-administered questionnaire. Participants were queried regarding breast cancer risk factors including medical and reproductive history. In particular, participants were asked to report their average monthly frequency of aspirin, ibuprofen, and acetaminophen use in the 12-24 months prior to interview and the average number of pills taken per day during that time period ("intensity"). Additionally, participants were asked about their average monthly frequency of aspirin use for each decade of adult life beginning at age 21.

Women were classified according to their reported frequency of aspirin and ibuprofen use as non-users (0 days/month), infrequent users (≤14 days/month), or regular users (>14 days/month). Intensity was categorized as non-use (0 pills/day), low intensity (<2 pills/day) and high intensity (≥2 pills/day). The high category cut-point of NSAID frequency and intensity was determined using the highest tertile of aspirin use among controls. From data on adult lifetime aspirin use, participants were classified based upon their average monthly aspirin use throughout their adult lifetime [non-users (0 days/month), irregular users (≤10 days/month), regular users (>10 days/month)].

Biological Specimens

Archived tumor blocks were obtained for 922 (79%) of cases. ER and PR status was determined by a single pathologist using immunohistochemistry (IHC) as described by Allred et al (30). For patients for whom tumor blocks were unavailable or for whom hormone receptor status was unable to be determined (e.g., insufficient tumor tissue) (n=222), hormone receptor status was obtained from hospital chart review. We compared results of our assessment with medical chart assignment of ER and PR status among participants for whom we had data from both sources (ER: n=682, PR: n=668); values were in good agreement (ER: κ=0.66, PR: κ=0.73). Using both methods, ER status was determined for 91% (n =752 ER+ and 315 ER-) and PR status for 90% (n=656 PR+ and 392 PR-) of cases.

HER2 protein expression was determined in a manner similar to that for ER and PR status. A single pathologist determined HER2 expression for each sample using IHC. HER2 was scored using the guidelines of HerceptTest^{TM}. We classified tumors with scores 0-2+ (negative-equivocal) as HER2-negative (HER2-) and tumors with a score of 3+ (strongly positive) as HER2-positive (HER2+). A HER2 score of 2+ (equivocal) was determined by IHC for 31 cases

and classified as HER2-. For patients for whom HER2 expression could not be determined (n=111), this data was obtained from patients' hospital charts. Among participants for whom we had data from both sources (n=285), values were in good agreement (κ=0.65). HER2 expression data were available from one source or the other for 64% (n=74 HER2+ and 677 HER2-) of cases.

P53 mutation status was determined from tumor blocks using the Affymetrix p53 Genechip System (Santa Clara, CA), which consists of a multiplex PCR amplification of exons 2-11 of the p53 gene , followed by hybridization of the PCR product on the array. All mutation calls from the array data were confirmed by direct sequencing. This method has been previously described in detail (31). Data were available for 63% (n=205 p53+ and 528 p53-) cases.

Statistical Analysis

We classified breast cancer cases into subtypes according to ER/PR, HER2, and p53 status, and by joint combinations of ER, PR, and HER2 (10, 12). Luminal-like cases were ER+ or PR+ and HER2- (luminal A, n=540) or HER2+ (luminal B, n=39). HER2 expressing tumors were defined as ER-/PR- and HER2+ (n=34). There were 134 triple-negative (ER-/PR-/HER2-) cases.

We used unordered polytomous logistic regression models to estimate multivariable-adjusted odds ratios (OR) and 95% confidence intervals (95% CI) for the association of recent NSAID use and lifetime aspirin use with breast cancer stratified by molecular subtypes. *P*-values for heterogeneity (*P*-heterogeneity) of the OR between breast cancer subtypes were calculated using adjusted unconditional (2 case groups) or polytomous logistic regression (≥3 case groups) models. Exposure-response trends were calculated with logistic regression models from beta-coefficients of NSAID frequency and intensity. All reported *P*-values are two-tailed and α=0.05.

We considered as potential confounders in multivariable modeling known and suspected risk factors for breast cancer including age, education, age at menarche, age at first, birth, parity, body mass index (BMI) [measured, kg/m2], lifetime physical activity [sports or exercise; hours/week], menopausal status, postmenopausal hormone use, family history of breast cancer, and history of benign breast disease. We also assessed potential confounding by correlates of NSAID use, including histories of hypertension, arthritis, or cerebrovascular disease. We adjusted models for the frequency matching variables (age and race) and years of education and menopausal status. The remaining factors did not alter point estimates by ≥10% and were excluded from final models. Thus, final multivariable models were adjusted for age (years), years of education, race (white/non-white), and menopausal status (premenopausal/postmenopausal), and were simultaneously adjusted for use of other NSAIDs. Regression models were run among 1,111 (95%) cases and 2,052 (97%) controls with complete data on exposure and adjustment variables.

Results

Characteristics of the WEB study population have been described previously (29). The associations of NSAID use with breast cancer stratified by joint-ER and PR status are given in Table 1. Recent aspirin use was associated with reductions in breast cancer risk across cases groups, regardless of frequency or intensity of use; however with the exception of ER-/PR+ cancers, findings were not statistically significant and no exposure-response gradient was observed for frequency or intensity of use. In contrast, compared to non-users, recent ibuprofen users had a statistically significant 33% increased risk of ER+/PR+ breast cancer (OR 1.33, 95% CI: 1.09-1.62) but not ER+/PR-, ER-/PR+, or ER-/PR- breast cancers. For ibuprofen use versus non-use, differences across subtypes were not statistically significant, with the exception that the

P-heterogeneity of the OR for ER+/PR+ vs. ER-/PR- was 0.02. For ER+/PR+ breast cancer, point estimates were also elevated for increasing frequency (>14 days/month: OR 1.23, 95% CI: 0.85-1.77; *P*-trend=0.31), and intensity of ibuprofen use (≥2 pills/day: OR 1.32, 95% CI: 1.06-1.63; *P*-trend=0.38). These findings were also statistically different than those of ER-/PR- breast cancer (*P*-heterogeneity=0.04 and 0.02, respectively); however the *p*-trend for frequency or intensity of use of ibuprofen among ER+/PR+ breast cancers did not achieve statistical significance. In a sensitivity analysis, comparing users to non-users of aspirin and ibuprofen, respectively, results were unchanged when ER and PR were examined separately and there were no differences when stratified on menopausal status (data not shown).

Associations of recent NSAID use with breast cancer stratified by HER2 expression status are given in Table 2. Associations with aspirin use did not differ by HER2 status. Compared to non-use, aspirin use was associated with reduced risks of HER2+ (OR 0.84, 95% CI: 0.52-1.36) and HER2- breast cancers (OR 0.91, 95% CI: 0.76-1.09) (*P*-heterogeneity=0.76). Again, there was no clear exposure-response gradient with increasing frequency or intensity of aspirin use. Use of ibuprofen in the previous year was associated with a statistically significant 27% increased risk of HER2- breast cancers (OR 1.27, 95% CI: 1.05-1.53) and was not associated with HER2+ breast cancers (OR 0.83, 95% CI: 0.51-1.35). Compared to non-use, categories of ibuprofen frequency and intensity were associated with elevated risks of HER2- tumors, however the association was strongest amongst infrequent users (≤14 days/ month: OR 1.28, 95% CI: 1.05-1.56) rather than frequent users (>14 days/month: OR 1.11, 95% CI: 0.78-1.58) and low intensity of use (<2 pills/day: OR 1.36, 95% CI: 1.04-1.80) rather than high (≥2 pills/day: OR 1.21, 95% CI: 0.99-1.48) giving no evidence of an exposure-response gradient.

Similar to our findings of aspirin use with breast cancer stratified by ER/PR or HER2, separately, we observed no clear differences in the associations of aspirin use with breast cancer characterized by combinations of ER, PR, and HER2 (Table 3). There was a suggestion of an increased risk of luminal B tumors and a large inverse association for HER2 expressing tumors, although confidence intervals were very wide and the findings were not statistically different. However, use of ibuprofen was associated with 34-40% increased risks of luminal A (OR 1.34, 95% CI: 1.09-1.65) and luminal B (OR 1.41, 95% CI: 0.69-2.87) breast cancer. Risks of these subtypes were similarly elevated for the highest categories of frequency and intensity of ibuprofen use. Use of ibuprofen was suggestive of a 50% reduction in risk of HER2 expressing tumors (OR 0.50, 95% CI: 0.25-1.02), although not statistically significant. The *P*-heterogeneity compared to the luminal A subtype was <0.01. The highest categories of frequency (>14 days/month: OR 0.43, 95% CI: 0.10-1.89) and intensity of use (≥2 pills/day: OR 0.42, 95% CI: 0.19-0.94) were associated with similar reductions in risk of HER2 expressing breast cancer. This latter association was significantly different than for the luminal A subtype (*P*-heterogeneity<0.01). Ibuprofen use was not associated with risk of triple-negative breast cancer (OR 0.99, 95% CI; 0.68-1.45).

We observed no significant differences in risk by p53 status associated with aspirin use (Table 4). Recent ibuprofen use was not associated with p53+ tumors (OR 0.96, 95% CI: 0.71-1.31), while it was positively associated with risk of p53- tumors (OR 1.28, 95% CI: 1.04-1.57). However, point estimates were not statistically different (*P*-heterogeneity=0.11) and we observed no exposure-response gradients for frequency or intensity of ibuprofen use for p53- breast cancers.

Analyses of the associations of adult lifetime aspirin use with breast cancer stratified by breast cancer subtypes were limited by small numbers for several case groups (Supplemental Table). We did find that women who used aspirin regularly throughout their adult life had similar reduction of risk of breast cancers defined by either ER or PR. The strongest finding was for ER-/PR- tumors. Regular use was associated with a 48% reduction in risk of this subtype (OR 0.52, 95% CI: 0.24-1.14), albeit based upon 8 exposed cases. Although the finding was not statistically different than that of ER+/PR+ (*P*-heterogeneity=0.36), there was evidence of a significant trend (*P*-trend=0.02). Lifetime aspirin use was also similarly associated with significant reductions in risks of HER2- (OR 0.60, 95% CI: 0.38-0.95; *P*-trend=0.04) and p53- (OR 0.56, 95% CI: 0.33-0.95; *P*-trend=0.02) breast cancers; however the point-estimates were not statistically different than those for HER2+ (*P*-heterogeneity=0.20) or p53+ tumors (*P*-heterogeneity=0.48). Regular lifetime aspirin use was associated with a 41% reduction in risk of luminal A breast cancer (OR 0.59, 95% CI: 0.36-0.97; *P*-trend=0.05) and a statistically non-significant 48% reduction in risk of triple-negative breast cancer (OR 0.52, 95% CI: 0.18-1.54; *P*-trend=0.26). There was no clear association of lifetime aspirin with either the luminal B or HER2 expressing phenotypes.

Discussion

In this study population of women living in Western New York, we previously found that recent use of aspirin but not ibuprofen was inversely associated with breast cancer risk (29). We found here that, for aspirin, this association did not differ by molecular subtype. For ibuprofen, recent use was associated with an increased risk of breast cancer defined as ER+/PR+, HER2-, p53- or luminal A/B, and a decreased risk of HER2 expressing tumors. With few exceptions, we observed no statistically significant exposure-response gradients for increasing frequency or intensity of aspirin or ibuprofen use in association with any molecular subtype. Similar to our

findings with recent aspirin use, aspirin use during a woman's adult lifetime was associated with similar reductions in risks for most tumor subtypes.

Because COX-2 expression has been associated *in vitro* with estrogen synthesis (7, 8), we hypothesized that NSAID use would be inversely associated with risk of hormone receptor positive tumors. Authors of several case-control (18, 21, 22) and cohort studies (17, 19, 20, 23, 24, 27), and one randomized controlled trial (26, 28), have examined the association of NSAID use and breast cancer stratified by hormone receptor status; however findings have been inconsistent (17-28). Because previous studies have reported associations using different combinations of ER/PR expression status (e.g., ER+/PR+ and ER-/PR-), or ER and PR status separately, comparisons between our findings and others are challenging. Comparisons are further limited in that not all studies report associations for individual NSAIDs, assuming equal effects of this broad class of medications. In this study, aspirin use was associated with reduced risks of breast characterized by joint ER/PR status. Several others have reported similar findings (18, 24, 27). In a recent analysis of the Iowa Women's Health Study, Bardia et al. (27), reported that ever aspirin use was associated with similar reductions in risk for ER+ (RR 0.77, 95% CI: 0.67-0.89), ER- (RR 0.78, 95% CI: 0.56-1.08), PR+ (RR 0.79, 95% CI: 0.68-0.92), and PR- (RR 0.73, 95% CI: 0.56-0.95) tumors; similar reductions in risk were observed when ER and PR were combined. In contrast, others have observed reduced risks of hormone receptor positive (i.e., ER+, ≥1 positive hormone receptor, or ER+/PR+) breast cancer only (21-23), increased risks of hormone receptor negative breast cancers (19), or no association (17, 20). In a randomized controlled trial of low-dose (100mg) aspirin taken every other day for 10 years compared to a placebo, there was no association with breast cancer risk overall (26) or with breast cancer

defined by ER or PR (28). It is widely speculated, however, that this dose may be too low for chemoprevention (28).

In contrast to our hypothesis, we observed increased risks of ER+/PR+ breast cancer in association with recent ibuprofen use. Several others have observed similar results. In the Multiethnic Cohort Study, there was an increase in hormone receptor positive breast cancer among women who were recent, short-term (≤1 year) users of non-aspirin NSAIDs compared to non-users (HR 1.29, 95% CI: 1.02-1.63), and no association among women with hormone receptor negative tumors (HR 1.06, 95% CI: 0.73-1.53), although greater duration of use at baseline was inversely associated with risk of hormone receptor positive tumors (17). In the California Teachers Study, daily ibuprofen use was associated with increased breast cancer risk that did not differ by ER and PR status (19), and in the Nurse's Health Study II cohort of premenopausal women, non-aspirin NSAID use 2-3 times per week was associated with increased breast cancer risk (RR 1.37, 95% CI: 1.09-1.67), with no difference by hormone receptor status (25). Our observation of inverse associations of breast cancers defined by ER and PR with aspirin use and an increased risk of hormone receptor positive tumors with ibuprofen use are inconsistent with our hypothesis that inhibition of COX-2 through the use of NSAIDs would result in the strongest reduction in risk of hormone receptor positive tumors.

This study is the first, to our knowledge, to report on the association of NSAID use with breast cancer characterized by HER2 expression, p53 mutation status, or combinations of ER, PR, and HER2. We found no difference in the association of aspirin use with tumors characterized by HER2 or p53, and use of ibuprofen was associated with increased risks of HER2- and p53- breast cancers. We know of only two other reports of the association of NSAIDs with any cancer characterized by p53 status and no others of breast cancer (32, 33).

Freedman et al. (33), reported no differences in the association of aspirin use with colon cancer stratified by p53 expression. Figueroa et al. (32), reported no differences in the association of aspirin or any NSAID with esophageal or gastric cancers by p53 status in a population-based case-control study. Nevertheless, our findings suggest further investigations are needed.

The primary strength of this report is that it is the first to comprehensively examine the association of NSAIDs, and use of aspirin during a woman's adult lifetime, with breast cancer subtypes defined by ER, PR, HER2, and p53. An additional strength is our ability to measure of the frequency and intensity of use of aspirin and ibuprofen.

This study also has several important limitations. Foremost, we were limited in power to detect differences between rare breast cancer subgroups. Our findings nevertheless merit further investigation. In addition, our classification of subtypes based on ER, PR, and HER2 is a surrogate for a more comprehensive nomenclature determined by tumor marker expression (12); therefore the subtypes defined in this study, particularly the differences between luminal A and B tumors, may be misclassified. Because fluorescence in situ hybridization was not performed to validate tumors with an equivocal (i.e., 2+) HER2 score, and the agreement between IHC and medical records was good but not excellent, misclassification of HER2 status is possible. In addition, data collected on recent NSAID use were limited to frequency and intensity of use and information on NSAID dose, duration, or indication for use was not collected. Our classification of infrequent and regular users may misclassify participants who were regular users at an earlier time. However it is unlikely that such misclassification would differ by disease status or case group and would likely result in a bias of point estimates towards the null and widening of confidence intervals (34). Doses of aspirin and ibuprofen made available over the counter and by prescription vary substantially; the absence of exposure-response gradients may be because of

measurement error related to the lack of dose data. It is also possible that our results may be confounded by other unmeasured factors, in particular by the indication for NSAID use or use of other NSAIDs such as naproxen or selective COX-2 inhibitors such as celecoxib. However, adjustment for history of arthritis, hypertension, diabetes, or cerebrovascular disease had no effect on point estimates. Additionally, for analyses of adult lifetime aspirin, we were unable to assess confounding by an overall healthy lifestyle. As with any case-control study, possible effects of recall bias need to be considered, although it is unlikely that participants would differ in their ability to recall their NSAID exposure depending upon the molecular classification of their cancer. It is possible that our findings may be due, in part, to self-selection into the study. Though unlikely, if selection into the study was based upon use of aspirin or ibuprofen or some correlate of their use, our findings could be biased. Again, it is unlikely that that this bias would differ by tumor characteristics. Comparing cases with tumors blocks vs. those without, use of aspirin and ibuprofen was similar.

NSAIDs are thought to exhibit their anti-inflammatory and chemopreventive effects by non-selectively binding to the cyclooxygenase enzymes (COX-1 & 2; EC 1.14.99.1), which catalyze the synthesis of pro-inflammatory prostaglandins from arachidonic acid (35). Among the prostaglandins, prostaglandin E_2 is considered a powerful mitogen and potential chemopreventive target (35, 36). PGE_2 has been shown to induce aromatase expression and *de novo* estrogen synthesis in breast epithelia and stromal cells *in vitro*; introduction of NSAIDs has been shown to reduce estrogen levels in a dose-dependent manner (8). In humans, the association of NSAIDs with circulating estrogen levels has been inconsistent (37-39). We know of no mechanism, however, by which ibuprofen would increase risk of breast cancer subtypes.

There is limited evidence that COX-2 expression is correlated with ER, PR, HER2, and p53 expression in breast tumors (40-44). Findings of *in vitro* studies among human invasive breast cancer cells suggest that HER2 oncogene activation regulates COX-2 expression in breast cancer (43, 45, 46), inducing a positive feedback loop in which PGE_2 in turn further induces HER2 expression (47). Introduction of an NSAID has been shown to reduce HER2 expression (47). P53 may also be associated with COX-2 expression *in vitro* (48, 49) and animal models of breast cancer give limited evidence that p53 expression is associated with COX-2 expression (43, 50).

The findings of this large, population-based case-control study support existing evidence that aspirin is inversely associated with breast cancer risk. Our findings do not support, however, the hypothesis that aspirin's effects are differential by tumor subtype. Use of ibuprofen may be associated with increased risk of certain breast cancer subtypes associated with less aggressive phenotype and inversely associated with the risk of the aggressive HER2 expressing phenotype. Our findings provide preliminary evidence to suggest that aspirin and ibuprofen are heterogeneous in their effects, and lend further support to the hypothesis that the etiology of breast tumors differs by subtype. Epidemiologic studies of the association of NSAIDs with breast tumors characterized by molecular subtype should include detailed information of the timing and dose of a wide range of individual prescription and non-prescription NSAIDs. A better understanding of inflammation in relation to risk of breast cancer could significantly advance strategies for chemoprevention.

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Table 1. Association of recent NSAID use with breast cancer characterized by estrogen and progesterone receptor expression status among WEB study participants.

NSAID Use	ER+/PR+ *n* = 590, *n* (%)	ER+/PR- *n* = 150, *n* (%)	ER-/PR+ *n* = 66, *n* (%)	ER-/PR- *n* = 241, *n* (%)	Controls *n* = 2,115, *n* (%)	ER+/PR+ v. Controls OR (95% CI)[a]	ER+/PR- v. Controls OR (95% CI)[a]	ER-/PR+ v. Controls OR (95% CI)[a]	ER-/PR- v. Controls OR (95% CI)[a]
Aspirin									
Non-Users	331 (56.68)	80 (54.42)	46 (70.77)	146 (60.83)	1,137 (54.30)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Users	253 (43.32)	67 (45.58)	19 (29.23)	94 (39.17)	957 (45.70)	0.86 (0.71-1.04)	0.88 (0.61-1.25)	0.57 (0.33-0.98)	0.80 (0.60-1.05)
Frequency									
Non-Users	331 (56.68)	80 (54.42)	46 (70.77)	146 (60.83)	1,137 (54.30)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Infrequent Users (≤14 days/month)	171 (29.28)	40 (27.21)	13 (20.00)	71 (29.58)	665 (31.76)	0.86 (0.69-1.06)	0.77 (0.51-1.17)	0.51 (0.27-0.96)	0.83 (0.61-1.12)
Regular Users (>14 days/month)	82 (14.04)	27 (18.37)	6 (9.23)	23 (9.58)	292 (13.94)	0.84 (0.63-1.13)	1.10 (0.66-1.82)	0.73 (0.30-1.80)	0.67 (0.41-1.08)
					P-trend	0.33	0.67	0.47	0.12
Intensity									
Non-Users	331 (56.68)	80 (54.05)	46 (69.70)	146 (60.83)	1,137 (54.30)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Low (<2 pills/day)	121 (20.58)	42 (28.38)	9 (13.64)	43 (17.92)	414 (19.75)	0.94 (0.73-1.21)	1.26 (0.82-1.92)	0.65 (0.30-1.40)	0.92 (0.63-1.35)
High (≥2 pills/day)	136 (23.13)	26 (17.57)	11 (16.67)	51 (21.25)	545 (26.00)	0.83 (0.66-1.05)	0.60 (0.37-0.97)	0.54 (0.28-1.07)	0.71 (0.51-1.00)
					P-trend	0.39	0.02	0.05	0.11
Ibuprofen									
Non-Users	237 (40.44)	67 (46.53)	27 (41.54)	108 (45.38)	953 (45.62)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Users	349 (59.56)	77 (53.47)	38 (58.46)	130 (54.62)	1,136 (54.38)	1.33 (1.09-1.62)	1.10 (0.76-1.58)	0.97 (0.57-1.65)	0.93 (0.70-1.24)*
Frequency									
Non-Users	237 (40.44)	67 (46.53)	27 (41.54)	108 (45.38)	953 (45.62)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Infrequent Users (≤14 days/month)	298 (50.85)	65 (45.14)	34 (52.31)	116 (48.74)	963 (46.10)	1.35 (1.09-1.66)	1.10 (0.75-1.60)	1.00 (0.58-1.73)	0.99 (0.74-1.33)*
Regular Users (>14 days/month)	51 (8.70)	12 (8.33)	4 (6.15)	14 (5.88)	173 (8.28)	1.23 (0.85-1.77)	1.04 (0.53-2.04)	0.77 (0.26-2.27)	0.62 (0.34-1.13)*
					P-trend	0.31	0.73	0.66	0.27
Intensity									
Non-Users	237 (40.44)	67 (45.58)	27 (41.54)	108 (45.38)	953 (45.62)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Low (<2 pills/day)	84 (14.43)	24 (16.33)	14 (21.54)	32 (13.39)	284 (13.54)	1.28 (0.95-1.72)	1.21 (0.73-2.03)	1.41 (0.70-2.82)	1.01 (0.65-1.56)
High (≥2 pills/day)	261 (44.85)	56 (38.10)	24 (36.92)	99 (41.42)	860 (41.01)	1.32 (1.06-1.63)	1.02 (0.69-1.52)	0.77 (0.43-1.38)	0.91 (0.67-1.24)*
					P-trend	0.38	0.62	0.51	0.89

Abbreviations: NSAID, non-steroidal anti-inflammatory drug; ER, estrogen receptor; PR, progesterone receptor; OR, odds ratio; CI, confidence interval

* *P*-heterogeneity < 0.05 for comparisons of ORs in each breast cancer subtype versus ER-/PR-

[a] Adjusted for age, education, race, menopausal status and other NSAID use

Table 2. Association of recent NSAID use with breast cancer characterized by HER2 expression status among WEB study participants.

NSAID Use	HER2+ Cases *n* = 74, *n* (%)	HER2- Cases *n* = 677, *n* (%)	Controls *n* = 2,115, *n* (%)	HER2+ v. Controls OR (95% CI)[a]	HER2- v. Controls OR (95% CI)[a]
Aspirin					
Non-Users	45 (60.81)	373 (55.75)	1,137 (54.30)	1.00 (reference)	1.00 (reference)
Users	29 (39.19)	296 (44.25)	957 (45.70)	0.84 (0.52-1.36)	0.91 (0.76-1.09)
Frequency					
Non-Users	45 (60.81)	373 (55.75)	1,137 (54.30)	1.00 (reference)	1.00 (reference)
Infrequent Users (≤14 days/month)	19 (25.68)	206 (30.79)	665 (31.76)	0.75 (0.43-1.31)	0.91 (0.74-1.11)
Regular Users (>14 days/month)	10 (13.51)	90 (13.45)	292 (13.94)	1.19 (0.58-2.48)	0.87 (0.58-2.48)
			P-trend	0.27	0.21
Intensity					
Non-Users	45 (60.81)	373 (55.42)	1,137 (54.25)	1.00 (reference)	1.00 (reference)
Low (<2 pills/day)	18 (24.32)	140 (20.80)	414 (19.75)	1.55 (0.84-2.84)	0.97 (0.76-1.24)
High (≥2 pills/day)	11 (14.86)	160 (23.77)	545 (26.00)	0.50 (0.25-0.98)	0.88 (0.70-1.09)
			P-trend	0.18	0.08
Ibuprofen					
Non-Users	32 (32.24)	275 (41.11)	953 (45.62)	1.00 (reference)	1.00 (reference)
Users	52 (56.76)	394 (58.89)	1,136 (54.38)	0.83 (0.51-1.35)	1.27 (1.05-1.53)
Frequency					
Non-Users	32 (32.24)	275 (41.11)	953 (45.62)	1.00 (reference)	1.00 (reference)
Infrequent Users (≤14 days/month)	34 (45.95)	339 (50.67)	963 (46.10)	0.81 (0.48-1.36)	1.28 (1.05-1.56)
Regular Users (>14 days/month)	8 (10.81)	55 (8.22)	173 (8.28)	1.03 (0.46-2.32)	1.11 (0.78-1.58)
			P-trend	0.21	0.79
Intensity					
Non-Users	32 (43.24)	275 (41.11)	953 (45.45)	1.00 (reference)	1.00 (reference)
Low (<2 pills/day)	15 (20.27)	106 (15.84)	284 (13.54)	1.27 (0.66-2.44)	1.36 (1.04-1.80)
High (≥2 pills/day)	27 (36.49)	288 (43.05)	860 (41.01)	0.68 (0.39-1.17)	1.21 (0.99-1.48)*
			P-trend	0.12	0.31

Abbreviations: NSAID, non-steroidal anti-inflammatory drug; OR, odds ratio; CI, confidence interval

* *P*-heterogeneity < 0.05 for comparisons of HER2- versus HER2+ ORs

[a] Adjusted for age, education, race, menopausal status and other NSAID use

Table 3. Association of recent NSAID use with breast cancer characterized by molecular subtype among WEB study participants.

NSAID Use	Luminal A[a] *n* = 540, *n* (%)	Luminal B[a] *n* = 39, *n* (%)	HER2 Expressing[a] *n* = 34, *n* (%)	Triple-negative[a] *n* = 134, *n* (%)	Controls *n* = 2115, *n* (%)	Luminal A v. Controls OR (95% CI)[b]	Luminal B v. Controls OR (95% CI)[b]	HER2 Expressing v. Controls OR (95% CI)[b]	Triple-negative v. Controls OR (95% CI)[b]
Aspirin Use									
Non-Users	291 (54.60)	20 (51.28)	24 (70.59)	80 (60.15)	1,137 (54.30)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Users	242 (45.40)	19 (48.72)	10 (29.41)	53 (39.85)	957 (45.70)	0.93 (0.77-1.14)	1.29 (0.68-2.47)	0.52 (0.24-1.13)	0.82 (0.56-1.18)
Frequency									
Non-Users	291 (54.60)	20 (51.28)	24 (70.59)	80 (60.15)	1,137 (54.30)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Infrequent Users (≤14 days/month)	166 (31.14)	14 (35.90)	5 (14.71)	40 (30.08)	665 (31.76)	0.93 (0.75-1.17)	1.28 (0.63-2.58)	0.36 (0.14-0.97)	0.85 (0.57-1.27)
Regular Users (>14 days/month)	76 (14.26)	5 (12.82)	5 (14.71)	13 (9.77)	292 (13.94)	0.92 (0.68-1.25)	1.51 (0.54-4.24)	1.02 (0.37-2.83)	0.66 (0.35-1.26)
P-trend						0.44	0.20	0.71	0.17
Intensity									
Non-Users	291 (54.19)	20 (51.28)	24 (70.59)	80 (60.15)	1,137 (54.25)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Low (<2 pills/day)	116 (21.60)	12 (30.77)	6 (17.65)	23 (17.29)	414 (19.75)	1.00 (0.77-1.30)	2.63 (1.18-5.86)*	0.87 (0.33-2.27)	0.87 (0.52-1.44)
High (≥2 pills/day)	130 (24.21)	7 (17.95)	4 (11.76)	30 (22.56)	545 (26.00)	0.91 (0.72-1.16)	0.72 (0.30-1.73)	0.34 (0.12-0.98)	0.78 (0.50-1.20)
P-trend						0.15	0.61	0.18	0.27
Ibuprofen Use									
Non-Users	215 (40.26)	12 (30.77)	19 (55.88)	59 (44.70)	953 (45.62)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Users	319 (59.74)	27 (69.23)	15 (44.12)	73 (55.30)	1,136 (54.38)	1.34 (1.09-1.65)	1.41 (0.69-2.87)	0.50 (0.25-1.02)*	0.99 (0.68-1.45)
Frequency									
Non-Users	215 (40.26)	12 (30.77)	19 (55.88)	59 (44.70)	953 (45.62)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Infrequent Users (≤14 days/month)	270 (50.56)	21 (53.85)	13 (38.24)	67 (50.76)	963 (46.10)	1.34 (1.09-1.66)	1.27 (0.60-2.68)	0.55 (0.26-1.17)*	1.08 (0.73-1.60)
Regular Users (>14 days/month)	49 (9.18)	6 (15.38)	2 (5.88)	6 (4.55)	173 (8.28)	1.30 (0.89-1.89)	2.17 (0.78-6.04)	0.43 (0.10-1.89)	0.54 (0.23-1.29)
P-trend						0.29	0.05	0.88	0.13
Intensity									
Non-Users	215 (40.34)	12 (30.77)	19 (55.88)	59 (44.36)	953 (45.45)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Low (<2 pills/day)	88 (16.51)	10 (25.64)	5 (14.71)	18 (13.53)	284 (13.54)	1.45 (1.07-1.95)	2.10 (0.87-5.11)	0.76 (0.27-2.14)	1.10 (0.62-1.94)
High (≥2 pills/day)	230 (43.15)	17 (43.59)	10 (29.41)	56 (42.11)	860 (41.01)	1.28 (1.02-1.60)	1.16 (0.53-2.53)	0.42 (0.19-0.94)*	0.97 (0.65-1.45)
P-trend						0.36	0.75	0.07	0.60

Abbreviations: NSAID, non-steroidal anti-inflammatory drug; ER, estrogen receptor; PR, progesterone receptor; OR, odds ratio; CI, confidence interval

* *P*-heterogeneity < 0.05 for comparisons of ORs in each breast cancer subtype versus Luminal A

[a] Luminal A: ER+ or PR+, HER2-; Luminal B: ER+ or PR+, HER2+; HER2 Expressing: ER-/PR-, HER2+; Triple-negative: ER-/PR-/HER2-

[b] Adjusted for age, education, race, menopausal status & other NSAID use

Table 4. Association of recent NSAID use with breast cancer characterized by P53 mutation status among WEB study participants.

NSAID Use	P53+ Cases *n* = 205, *n* (%)	P53- Cases *n* = 528, *n* (%)	Controls *n* = 2,115, *n* (%)	P53+ v. Controls OR (95% CI)[a]	P53- v. Controls OR (95% CI)[a]
Aspirin					
Non-Users	117 (58.21)	305 (58.54)	1,137 (54.30)	1.00 (reference)	1.00 (reference)
Users	84 (41.79)	216 (41.46)	957 (45.70)	0.82 (0.61-1.12)	0.82 (0.67-1.01)
Frequency					
Non-Users	117 (58.21)	305 (58.54)	1,137 (54.30)	1.00 (reference)	1.00 (reference)
Infrequent Users (≤14 days/month)	58 (28.86)	150 (28.79)	665 (31.76)	0.81 (0.58-1.14)	0.82 (0.65-1.02)
Regular Users (>14 days/month)	26 (12.94)	66 (12.67)	292 (13.94)	0.81 (0.49-1.33)	0.82 (0.60-1.13)
			P-trend	0.46	0.26
Intensity					
Non-Users	117 (58.21)	305 (58.54)	1,137 (54.30)	1.00 (reference)	1.00 (reference)
Low (<2 pills/day)	38 (18.81)	104 (19.81)	414 (19.75)	0.88 (0.58-1.34)	0.94 (0.72-1.23)
High (≥2 pills/day)	47 (23.27)	116 (22.10)	545 (26.00)	0.83 (0.58-1.19)	0.75 (0.59-0.96)
			P-trend	0.42	0.02
Ibuprofen					
Non-Users	91 (44.83)	208 (40.00)	953 (45.62)	1.00 (reference)	1.00 (reference)
Users	112 (55.17)	312 (60.00)	1,136 (54.38)	0.96 (0.71-1.31)	1.28 (1.04-1.57)
Frequency					
Non-Users	91 (44.83)	208 (40.00)	953 (45.62)	1.00 (reference)	1.00 (reference)
Infrequent Users (≤14 days/month)	99 (48.77)	265 (50.96)	963 (46.10)	1.01 (0.73-1.40)	1.28 (1.03-1.59)
Regular Users (>14 days/month)	13 (6.40)	47 (9.04)	173 (8.28)	0.70 (0.36-1.34)	1.22 (0.84-1.77)
			P-trend	0.93	0.40
Intensity					
Non-Users	91 (44.83)	208 (40.00)	953 (45.62)	1.00 (reference)	1.00 (reference)
Low (<2 pills/day)	23 (11.33)	80 (15.38)	284 (13.54)	0.78 (0.48-1.28)	1.37 (1.01-1.86)
High (≥2 pills/day)	89 (43.84)	232 (44.62)	860 (41.01)	1.00 (0.72-1.40)	1.23 (0.98-1.53)
			P-trend	0.61	0.45

Abbreviations: NSAID, non-steroidal anti-inflammatory drug; OR, odds ratio; CI, confidence interval

* *P*-heterogeneity < 0.05 for comparisons of p53- versus p53+ ORs

[a] Adjusted for age, education, race, menopausal status and other NSAID use

Supplemental Table. Associations of lifetime aspirin use with subsets of breast cancer, among WEB study participants.

	Lifetime Aspirin						
	Non-users		Irregular Users (≤10 days/month)		Regular Users (>10 days/month)		
Molecular Subtype[a]	**Cases/Controls**	**OR (95% CI)**	**Cases/Controls**	**OR (95% CI)[b]**	**Cases/Controls**	**OR (95% CI)[b]**	***P*-trend**
ER+/PR+ vs. Controls	112/369	1.00 (reference)	424/1,554	0.85 (0.66-1.08)	34/138	0.74 (0.48-1.16)	0.23
ER+/PR- vs. Controls	25/369	1.00 (reference)	109/1,554	0.88 (0.55-1.39)	9/138	0.63 (0.27-1.50)	0.29
ER-/PR+ vs. Controls	12/369	1.00 (reference)	50/1,554	1.20 (0.63-2.32)	2/138	0.55 (0.12-2.52)	0.18
ER-/PR- vs. Controls	45/369	1.00 (reference)	178/1,554	1.03 (0.72-1.47)	8/138	0.52 (0.24-1.14)	0.02
HER2+ vs. Controls	11/369	1.00 (reference)	55/1,554	1.50 (0.77-2.94)	5/138	1.44 (0.48-4.28)	0.55
HER2- vs. Controls	124/369	1.00 (reference)	499/1,554	0.93 (0.73-1.17)	32/138	0.60 (0.38-0.95)	0.04
Luminal A	101/369	1.00 (reference)	398/1,554	0.88 (0.68-1.14)	27/1,554	0.59 (0.36-0.97)	0.05
Luminal B	6/369	1.00 (reference)	28/1,554	1.47 (0.59-3.66)	3/1,554	1.62 (0.39-6.72)	0.92
HER2 Expressing	5/369	1.00 (reference)	26/1,554	1.48 (0.55-3.94)	2/1,554	1.25 (0.24-6.60)	0.38
Triple-negative	23/369	1.00 (reference)	99/1,554	1.11 (0.69-1.79)	4/1,554	0.52 (0.18-1.54)	0.26
P53+ vs. Controls	36/369	1.00 (reference)	151/1,554	1.04 (0.71-1.55)	13/138	0.79 (0.38-1.65)	0.35
P53- vs. Controls	96/369	1.00 (reference)	390/1,554	0.96 (0.74-1.24)	21/138	0.56 (0.33-0.95)	0.02

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; OR, odds ratio; CI, confidence interval

* *P*-heterogeneity < 0.05 for comparisons of ORs in each breast cancer subtype versus ER-/PR-, HER2+, Luminal A, or p53+, respectively

[a] Luminal A: ER+ or PR+, HER2-; Luminal B: ER+ or PR+, HER2+; HER2 Expressing: ER-/PR-, HER2+; Triple-negative: ER-/PR-/HER2-

[b] Adjusted for age, education, race, and menopausal status