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Does mammographic density mediate risk factor associations with breast cancer: an analysis by tumor characteristics

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Abstract

Background—Though mammographic density (MD) has been proposed as an intermediate marker of breast cancer risk, few studies have examined whether the associations between breast cancer risk factors and risk are mediated by MD, particularly by tumor characteristics.

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Conflicts of Interest: None

Methods—Our study population included 3,392 cases (1,105 premenopausal) and 8,882 (3,192 premenopausal) controls from four case-control studies. For established risk factors, we estimated the percent of the total risk factor association with breast cancer that was mediated by percent MD (secondarily, by dense area and non-dense area) for invasive breast cancer as well as for subtypes defined by the estrogen receptor (ER+/ER−), progesterone receptor (PR+/PR−), and HER2 (HER2+/HER2−). Analyses were conducted separately in pre- and postmenopausal women.

Results—Positive associations between prior breast biopsy and risk of invasive breast cancer as well as all subtypes were partially mediated by percent MD in pre- and postmenopausal women (percent mediated=11–27%, p≤0.02). In postmenopausal women, nulliparity and hormone therapy use were positively associated with invasive, ER+, PR+, and HER2− breast cancer; percent MD partially mediated these associations (percent mediated≥31%, p≤0.02). Further, among postmenopausal women, percent MD partially mediated the positive association between later age at first birth and invasive as well as ER+ breast cancer (percent mediated=16%, p≤0.05).

Conclusion—Percent MD partially mediated the associations between breast biopsy, nulliparity, age at first birth, and hormone therapy with risk of breast cancer, particularly among postmenopausal women, suggesting that these risk factors at least partially influence breast cancer risk through changes in breast tissue composition.

Introduction

High percent mammographic density (MD), or the proportion of dense breast tissue on a mammogram, is one of the strongest risk factors for breast cancer.[1] Further, MD varies by anthropometric, reproductive, and lifestyle breast cancer risk factors, suggesting that MD may be a potential surrogate marker for breast cancer risk.[2] However, the extent to which MD acts as a mediator for established breast cancer risk factors is not well understood as few studies have examined whether MD mediates the associations between these factors and breast cancer risk.[3–6] In a recent analysis in the Nurses' Health Study (NHS) and NHSII, we observed that MD mediated the associations for some risk factors, including benign breast disease, but not others, such as family history of breast cancer.[5] One of the primary limitations of this prior analysis was limited power to assess mediation by breast cancer tumor characteristics. As the associations between some breast cancer risk factors, including MD, vary by tumor characteristics, the extent to which MD acts as a mediator of the associations with breast cancer risk factors may also vary by tumor characteristics. Therefore, the purpose of this analysis was to confirm our prior observations in the NHS/NHSII for all invasive breast cancer with the following exposures: current body mass index (BMI), height, age at menarche, parity, age at first birth, alcohol use, family history of breast cancer and hormone therapy (HT) use. Further, as the associations between breast cancer risk factors, including MD, may vary by breast tumor characteristics, we sought to quantify the extent to which the associations between these risk factors as well as prior breast biopsy and risk of breast cancer subtypes defined by tumor markers [specifically defined by the estrogen receptor (ER+, ER−), progesterone receptor (PR+, PR−), and human epidermal growth factor receptor 2 (HER2+, HER2−)] were mediated by percent MD in a larger study.[7] Lastly, in secondary analyses, we extended our prior work and investigated the extent to

which both absolute dense area as well as absolute non-dense area on a mammogram mediated these associations.

Materials and Methods

Study populations

We included data from four nested case-control studies of breast cancer: the Mayo Mammography Health Study (MMHS), the Nurses' Health Study (NHS), the Nurses' Health Study II (NHSII), and the San Francisco Bay Area Breast Cancer SPORE and San Francisco Mammography Registry (SFMR). Details of these studies and the study populations have been previously described.[7, 8] Incident invasive cases of breast cancer were identified by self-report, linkage to clinic and/or statewide tumor registries, or death certificates. Medical records were reviewed to further confirm disease status. Controls were selected from the underlying cohorts and were matched to cases on age, menopausal status, and year of examination (MMHS, SFMR) or blood draw (NHS, NHSII) as previously described.[7, 8] All mammograms were taken prior to case diagnosis; further cases who were diagnosed within 6 months of the study mammogram as well as their matched controls were excluded to ensure we captured only incident cases. The following risk factors were selected for analysis: current BMI, height, age at menarche, parity, age at first birth, alcohol use, family history of breast cancer, previous breast biopsy, age at menopause (postmenopausal only), and HT use (postmenopausal only). Information on these selected risk factors were obtained from self-administered questionnaires (NHS, NHSII), or both medical record review and self-administered questionnaires (MMHS,) before (NHS, NHSII) or at the time of (MMHS, SFMR) mammography. All risk factors are at time of mammography. We excluded 24 women (2 cases, 22 controls) without information on BMI. In total, these analyses included 3,392 breast cancer cases and 8,882 controls.

For all breast cancer cases, we obtained information on ER, PR, and HER2 status of breast tumors from medical records (MMHS), state and clinic cancer registries (MMHS,), state-wide Surveillance Epidemiology and End Results programs (SFMR), and pathology reports (NHS, NHSII). For a subset of cases in NHS and NHSII without receptor expression information on pathology reports, we conducted immunohistochemical staining of paraffin sections from core biopsies or tumor microarrays per standard protocols as described previously.[7, 9]

This study was approved by the Institutional Review Boards at Mayo Clinic (Rochester, MN), Brigham and Women's Hospital (Boston, MA), and the University of California, San Francisco (UCSF, San Francisco, CA). Informed consent was obtained (MMHS, SFMR) or implied by return of questionnaires (NHS, NHSII).

Mammographic density

Mammographic density measurements in the four studies have been described previously.[7, 8] Briefly, we measured dense area as well as total breast area from craniocaudal pre-diagnosis film screening mammograms using computer-assisted threshold techniques (Cumulus and UCSF custom software).[10, 11] Percent MD was calculated as dense area

divided by total breast area whereas non-dense area was calculated as total area minus dense area. In the NHS and NHSII, we averaged the MD measurements of both breasts. For the remaining studies, MD was measured in the contralateral breast for cases and for the same side in the matched controls.

Statistical analysis

Means and frequencies of anthropometric, reproductive and lifestyle risk factors were calculated by case status as well as across quartiles of percent MD among the controls stratified by menopausal status. For all analyses, MD measures were square-root transformed to improve normality. We used linear regression to estimate differences in the square-root transformed MD measures by the selected risk factors among the controls. Using the SAS macro developed by Spiegelman and colleagues (https://www.hsph.harvard.edu/donna-spiegelman/software/mediate), based on the mediation analysis method outlined by Nevo et al, we quantified the extent to which the associations between each of the exposures and breast cancer risk were mediated by MD.[12] Using data augmentation and logistic regression, we estimated the odds ratio (OR) and 95% confidence interval (CI) for a) the association between the given risk factor and breast cancer risk not adjusted for MD [i.e., the total association between the risk factor and breast cancer risk] and b) the association between the given risk factor and breast cancer risk adjusted for continuous square-root transformed MD [i.e., the association between the risk factor and breast cancer risk not through MD]. Using these estimates, we next calculated the percent of the total association (on the log odds scale) between the exposure and breast cancer risk that was mediated by MD. As percent MD is a stronger predictor of breast cancer than either absolute dense or non-dense area, our primary analysis examined percent MD as a potential mediator.[13] However, as both dense area and non-dense area have been independently associated with breast cancer risk (in opposite directions) in our prior research, we also examined mediation of the associations by these measures in secondary analyses with mutual adjustment for both measures.[8, 13]

Exposure variables were modeled continuously except for the following binary variables: nulliparity (nulliparous vs parous), age at first birth (≥30 vs <30 years of age), first degree family history of breast cancer (yes vs no), prior breast biopsy (yes vs no), and current HT use (any HT, estrogen only HT, estrogen plus progestin HT) among postmenopausal women (vs never/former use). Models were adjusted for potential confounders of the association between the exposures and breast cancer risk, potential confounders of the association between the exposures and MD, and potential confounders of the association between MD and breast cancer risk. These covariates were age (continuous), BMI (continuous), study (indicators for each study), parity/age at first birth (nulliparous, <30, ≥30), prior breast biopsy (yes, no), family history of breast cancer (yes, no), and any HT use (current, never/former). *A priori*, all analyses were conducted separately in pre- and postmenopausal women with menopausal status defined at the time of the mammogram. In a sensitivity analysis, we excluded the NHS and NHSII from the analyses for all invasive breast cancer to confirm these results were consistent in the MMHS and SFMR. Analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC) and results were considered statistically significant if $p<0.05$.

Results

Among both pre- and postmenopausal women as well as across the four studies, cases had higher mean percent MD, higher mean dense area, and lower mean non-dense area compared to controls as expected (Table 1 and Supplementary Table 1). Cases were also more likely to be nulliparous, have a later age at first birth, to have a family history of breast cancer, and to have had a prior breast biopsy. BMI and number of children among parous women were inversely associated with percent MD while age at menarche, nulliparity, prior breast biopsy, and current postmenopausal HT use were positively associated with percent MD, with a stronger association for estrogen plus progestin compared to estrogen only HT use (Supplementary Tables 2 and 3). The median time between mammogram and breast cancer diagnosis was 4.3 (IQR: 2.7, 6.3) years.

Premenopausal women

All invasive breast cancer—Among women who were premenopausal at time of mammogram, greater age at first birth (OR ≥30 vs <30 years of age = 1.32, 95%CI: 1.09, 1.60), family history of breast cancer (OR yes vs no =1.59, 95%CI: 1.30, 1.94), and prior breast biopsy (OR yes vs no =1.76, 95%CI: 1.48, 2.10) were positively associated with invasive breast cancer risk in the multivariable model (Table 2). While percent MD did not mediate the associations between age at first birth or family history of breast cancer and breast cancer risk (percent mediated =5%, p≥0.18), the association with prior breast biopsy was significantly mediated by percent MD (percent mediated=17%, p<0.01). Both dense area and non-dense area significantly mediated the association with prior breast biopsy in premenopausal women, though the percent mediated was greater for dense area (percent mediated=10%, p<0.01) compared to non-dense area (percent mediated=5%, p<0.01) (Supplemental Tables 4 and 5). Age at menarche was inversely associated with overall breast cancer risk (OR=0.86, 95%CI: 0.75, 1.00), but there was no evidence of mediation by percent MD. None of the other factors evaluated, including current BMI, height, parity (nulliparous vs parous), number of births among parous women, or alcohol use were significantly associated with invasive breast cancer risk in these analyses among premenopausal women; therefore percent MD did not significantly mediate any associations with these factors (Table 2). Results were similar when we excluded the NHS and NHSII from analyses (data not shown).

Breast cancer subtypes—When we examined the associations by breast cancer subtype defined by ER, PR, or HER2 status in premenopausal women, previous breast biopsy remained a strong, significant risk factor for all subtypes (ORs=1.68–2.07); these associations were significantly mediated by percent MD (percent mediated = 11–18%, p≤0.02) (Table 2). In addition, dense area significantly mediated the associations with prior biopsy for all breast cancer subtypes (percent mediated = 6–12%, p≤0.03), while the proportions of the associations mediated by non-dense area were lower (percent mediated = 4–6%, p≤0.09) (Supplemental Tables 4 and 5). Family history of breast cancer was positively associated with all breast cancer subtypes, though the associations were significant only for ER+, PR+, and HER2− tumors (ORs=1.59–1.68). Similar to what we observed for all invasive breast cancer, percent MD did not mediate any of the associations

with family history of breast cancer for any subtype (percent mediated≤5%, p≥0.17). In addition, though age at first birth was significantly associated with ER+ and PR+ breast cancer, percent MD did not mediate these associations (percent mediated=4%, p≥0.28). While age at menarche was inversely associated with all breast cancer subtypes, the association with risk was only significant for PR− breast cancer (OR per 2-year increase=0.74, 95%CI: 0.57, 0.96); percent MD did not mediate this association.

Postmenopausal women

All breast cancer—Among women who were postmenopausal at the time of mammogram,, BMI (OR per 5 kg/m^2 increase=1.14, 95%CI: 1.09, 1.19), nulliparity (OR nulliparous vs parous=1.23, 95%CI: 1.07, 1.41), later age at first birth (OR ≥30 vs <30 years of age=1.26, 95%CI: 1.08, 1.47), family history of breast cancer (OR yes vs no=1.58, 95%CI: 1.39, 1.80), later age at menopause (OR per category increase=1.07, 95%CI: 1.02, 1.13), previous breast biopsy (OR yes vs no=1.50, 95%CI: 1.34, 1.69), current use of any HT (OR current vs never/past=1.39, 95%CI: 1.24, 1.55), current estrogen only HT use (OR current vs never/past = 1.16, 95%CI: 1.00, 1.34), and current estrogen plus progestin (OR current vs never/past = 1.66, 95%CI: 1.45, 1.90) were significantly positively associated with all invasive breast cancer risk (Table 3). Further, height was suggestively positively associated with risk of all invasive breast cancer (OR per 3-inch increase=1.06, 95%CI: 0.99, 1.12). Percent MD did not mediate the associations with BMI, height, family history of breast cancer, or later age at menopause. However, percent MD did significantly mediate the associations between the following risk factors and all invasive breast cancer among postmenopausal women: nulliparity (percent mediated=43%, p<0.01), age at first birth (percent mediated=16%, p=0.05), prior breast biopsy (percent mediated=24%, p<0.01), current HT use (percent mediated=37%, p<0.01), and current estrogen plus progestin HT use (percent mediated =26%, p<0.01). Further, while not statistically significant, percent MD did appear to mediate the associate with current estrogen only HT use (percent mediated=69%, p=0.06). In general, both dense area and non-dense area also significantly mediated these associations, though the percent mediated was greater for dense area (Supplemental Table 6) compared to non-dense area (Supplemental Table 7). Age at menarche, number of births among parous women, and alcohol use were not significantly associated with risk of all invasive breast cancer or breast cancer subtypes in this sample of postmenopausal women; therefore percent MD did not significantly mediate any associations with these factors. Results were generally similar when we excluded the NHS and NHSII (data not shown).

Breast cancer subtypes—Among postmenopausal women, family history of breast cancer (ORs=1.52–2.01) as well as prior breast biopsy (ORs=1.47–1.68) were significantly positively associated with all breast cancer subtypes defined by ER, PR, or HER2 (Table 3). Similar to the analysis in premenopausal women, percent MD significantly mediated the associations with prior breast biopsy (percent mediated=18–27%, p<0.01), but did not mediate the associations with family history of breast cancer (percent mediated ≤2%, p≥0.44). Further, dense area significantly mediated the associations with prior biopsy for all subtypes (percent mediated=9–19%, p≤0.01), while non-dense area significantly mediated the associations with prior biopsy for ER+ (percent mediated=5%, p=0.01) and PR+ (percent

mediated=5%, p=0.01) tumors. For BMI, height, nulliparity, age at first birth, age at menopause, and current HT use, results for ER+, PR+, and HER2− breast cancer were generally similar to the results for all invasive breast cancer. Older age at first birth was also positively associated with HER2+ breast cancer (OR=1.61, 95%CI: 1.13, 2.30,), though the association was not mediated by percent MD (percent mediated=6%, p=0.09).

Discussion

Among postmenopausal women, percent MD partially mediated the associations between prior breast biopsy, age at first birth, nulliparity and current hormone therapy with risk of breast cancer and subtypes. Among premenopausal women, however, percent MD partially mediated the association with invasive breast cancer for prior breast biopsy, but did not mediate the observed association with age at first birth. Though family history was strongly associated with invasive breast cancer as well as most subtypes, percent MD did not mediate the associations in pre- or postmenopausal women. Our observations for all invasive breast cancer were consistent with a prior study in the NHS/NHSII, even after exclusion of those cohorts in this analysis.[5] In general, our results for ER+, PR+, and HER2− tumors were similar to those observed for all invasive breast cancer. While percent MD was the strongest mediator of these associations, dense area and non-dense area did mediate some of the associations. In general, the percent mediated was greater for dense-area compared to non-dense area.

Though we observed significant mediation by percent MD of the association between prior breast biopsy and breast cancer risk in pre- and postmenopausal women, this result should be cautiously interpreted. While prior breast biopsy was assessed prior to MD measurements, it is likely that women also had high percent MD at the time of their biopsy. As women with denser breasts are more likely to undergo breast biopsy, breast biopsies may be a downstream consequence of high percent MD.[14] Therefore, it is difficult to determine the temporality between these two breast cancer risk factors.

Percent MD appeared to mediate the associations with current postmenopausal HT use for all invasive, ER+, PR+, and HER2- breast cancer, with mediation proportions ranging from 31%–37%. This is consistent with observations that percent MD increases with use of postmenopausal HT and decreases with the administration of tamoxifen, a selective estrogen receptor modulator, suggesting that the breast is susceptible to hormonally-related exogenous agents, even among postmenopausal women.[15–17] Further, this is supported by recent work in the Women’s Health Initiative which observed that the association between estrogen plus progestin HT use (compared to placebo) and breast cancer risk was entirely mediated through the change in percent MD from baseline to one year post treatment arm assignment.[6] While we observed a lower percent mediation for estrogen plus progestin use relative to this prior study, current users in our study population likely used different formulations for different lengths of time increasing heterogeneity in our exposure. Further, we assessed percent MD measured at one point in time rather than change in percent MD over time. In contrast to our observations for current HT use, we did not observe any evidence that percent MD is a mediator of the association between family history of breast cancer and risk of any breast cancer subtype. While evidence suggests that both MD and

breast cancer risk are heritable, one explanation for a lack of mediation by percent MD of the association between family history and breast cancer risk may be that heritability of MD may act through different mechanisms than heritability of breast cancer risk.[18–20] For example, studies have demonstrated that women with germline BRCA1/2 mutations do not have higher percent MD than women without these mutations.[21] However, GWAS studies suggest a shared genetic basis between MD and breast cancer as several breast cancer susceptibility loci have also been associated with MD.[22–26] This lack of consistency may be due to the fact that family history of breast cancer is a heterogeneous mix of genetic and non-genetic determinants; germline genetic information may be more appropriate for examining mediation due to heritability of breast cancer.

For some of the breast cancer risk factors studied, differences were noted in the proportion of associations mediated by percent MD by menopausal status and tumor subtype. For example, while age at first birth ≥30 years of age was associated with an approximately 30% higher risk of all invasive breast cancer in both pre- and postmenopausal women, percent MD only significantly mediated the association in postmenopausal women. Further, mediation by percent MD among postmenopausal women was significant only for all invasive and ER+ tumors; age at first birth among postmenopausal women was not associated with ER− disease. For other risk factors, associations with breast cancer risk were observed in postmenopausal women, but not premenopausal women. For example, among postmenopausal women, those who were nulliparous had a 23–27% higher risk of all invasive, ER+, PR+, and HER2− tumors, whereas the associations were weaker and non-significant in premenopausal women. This disparity may be partially explained by the transient increase in breast cancer risk post-pregnancy, which is stronger and persists for a greater number of years in women with later ages at births.[27] In fact, premenopausal women in this study were approximately twice as likely to have an age at first birth of 30 years or older compared to postmenopausal women.

Although our study included over 3,000 breast cancer cases and almost 9,000 matched controls, we did have limited power for rarer subtype and exposure combinations within strata of menopausal status (e.g., family history of breast cancer and ER− breast cancer in premenopausal women). We also did not have sufficient power to assess mediation for intrinsic molecular subtypes of breast cancer.[28] Further some of the selected risk factors were not significantly associated with breast cancer risk within strata of menopausal status in this sample, limiting our ability to assess mediation by percent MD for these risk factors (e.g., height in premenopausal women). In particular, we had a smaller number of premenopausal cases, restricting our ability to assess mediation by percent MD in younger women. Unlike other approaches,[29] our method to assess mediation did not model interaction between the selected risk factors and MD on breast cancer risk, however in our prior NHS/NHSII analysis, we observed little interaction between the selected exposures and MD.[5] Though MD measurements are highly reproducible, there may be some non-differential misclassification of MD which would likely bias our estimates of the percent mediated towards the null.[30] Further, data on some risk factors across the studies were collected through self-report, however as data were collected prior to breast cancer diagnosis for most studies, any misclassification should be non-differential. We were unable to examine former and never HT users separately due to limitations in the data for some of the

participating studies. While we were able to adjust for a large number of potential confounders, including strong predictors of MD such as BMI, we did not have information on early life body size (a risk factor for both MD and breast cancer) for two of the studies. Therefore, there may be some residual confounding, however we would expect the degree of confounding to be modest. As ER, PR, and HER2 expression are correlated, analyses are not fully independent of each other. Lastly, we did not collect data on risk factors specific to rarer subtypes of breast cancer, such as ER− related risk factors including early life body size, carotenoid intake, and breastfeeding. Strengths of this study include the large sample size, allowing for assessment of mediation by subtype of breast cancer, as well as detailed collection of data on several potential risk factors and confounders as well as detailed subtype information.

In summary, percent MD partially mediated the associations between prior breast biopsy and breast cancer risk in both pre- and postmenopausal women as well as mediated the associations with nulliparity, age at first birth, and HT use among postmenopausal women. Percent MD did not mediate the associations with breast cancer risk for some factors, such as family history of breast cancer. Results were generally similar for all invasive, ER+, PR+, and HER2− breast cancer. This study suggests that some breast cancer risk factors at least partially influence breast cancer risk through alterations in breast tissue composition, changes which are particularly evident after menopause.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev. 2006; 15(6):1159–1169. [PubMed: 16775176]
2. Boyd NF, Martin LJ, Yaffe MJ, Minkin S. Mammographic density and breast cancer risk: current understanding and future prospects. Breast cancer research: BCR. 2011; 13(6):223. [PubMed: 22114898]

3. Harris HR, Tamimi RM, Willett WC, Hankinson SE, Michels KB. Body size across the life course, mammographic density, and risk of breast cancer. American journal of epidemiology. 2011; 174(8): 909–918. [PubMed: 21911827]
4. Boyd NF, Martin LJ, Li Q, Sun L, Chiarelli AM, Hislop G, Yaffe MJ, Minkin S. Mammographic density as a surrogate marker for the effects of hormone therapy on risk of breast cancer. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006; 15(5):961–966.
5. Rice MS, Bertrand KA, VanderWeele TJ, Rosner BA, Liao X, Adami HO, Tamimi RM. Mammographic density and breast cancer risk: a mediation analysis. Breast Cancer Res. 2016; 18(1):94. [PubMed: 27654859]
6. Byrne C, Ursin G, Martin CF, Peck JD, Cole EB, Zeng D, Kim E, Yaffe MD, Boyd NF, Heiss G, et al. Mammographic Density Change With Estrogen and Progestin Therapy and Breast Cancer Risk. J Natl Cancer Inst. 2017; 109(9)
7. Bertrand KA, Tamimi RM, Scott CG, Jensen MR, Pankratz V, Visscher D, Norman A, Couch F, Shepherd J, Fan B, et al. Mammographic density and risk of breast cancer by age and tumor characteristics. Breast Cancer Res. 2013; 15(6):R104. [PubMed: 24188089]
8. Bertrand KA, Scott CG, Tamimi RM, Jensen MR, Pankratz VS, Norman AD, Visscher DW, Couch FJ, Shepherd J, Chen YY, et al. Dense and nondense mammographic area and risk of breast cancer by age and tumor characteristics. Cancer Epidemiol Biomarkers Prev. 2015; 24(5):798–809. [PubMed: 25716949]
9. Tamimi RM, Baer HJ, Marotti J, Galan M, Galaburda L, Fu Y, Deitz AC, Connolly JL, Schnitt SJ, Colditz GA, et al. Comparison of molecular phenotypes of ductal carcinoma in situ and invasive breast cancer. Breast cancer research: BCR. 2008; 10(4):R67. [PubMed: 18681955]
10. Boyd NF, Stone J, Martin LJ, Jong R, Fishell E, Yaffe M, Hammond G, Minkin S. The association of breast mitogens with mammographic densities. British journal of cancer. 2002; 87(8):876–882. [PubMed: 12373602]
11. Shepherd JA, Kerlikowske K, Ma L, Duewer F, Fan B, Wang J, Malkov S, Vittinghoff E, Cummings SR. Volume of mammographic density and risk of breast cancer. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2011; 20(7):1473–1482.
12. Nevo D, Liao X, Spiegelman D. Estimation and Inference for the Mediation Proportion. International Journal of Biostatistics. 2017
13. Pettersson A, Hankinson SE, Willett WC, Lagiou P, Trichopoulos D, Tamimi RM. Nondense mammographic area and risk of breast cancer. Breast cancer research: BCR. 2011; 13(5):R100. [PubMed: 22017857]
14. Nelson HD, O'Meara ES, Kerlikowske K, Balch S, Miglioretti D. Factors Associated With Rates of False-Positive and False-Negative Results From Digital Mammography Screening: An Analysis of Registry Data. Ann Intern Med. 2016; 164(4):226–235. [PubMed: 26756902]
15. Cuzick J, Warwick J, Pinney E, Duffy SW, Cawthorn S, Howell A, Forbes JF, Warren RM. Tamoxifen-induced reduction in mammographic density and breast cancer risk reduction: a nested case-control study. J Natl Cancer Inst. 2011; 103(9):744–752. [PubMed: 21483019]
16. Cuzick J, Warwick J, Pinney E, Warren RM, Duffy SW. Tamoxifen and breast density in women at increased risk of breast cancer. J Natl Cancer Inst. 2004; 96(8):621–628. [PubMed: 15100340]
17. McTiernan A, Martin CF, Peck JD, Aragaki AK, Chlebowski RT, Pisano ED, Wang CY, Brunner RL, Johnson KC, Manson JE, et al. Estrogen-plus-progestin use and mammographic density in postmenopausal women: Women's Health Initiative randomized trial. J Natl Cancer Inst. 2005; 97(18):1366–1376. [PubMed: 16174858]
18. Boyd NF, Dite GS, Stone J, Gunasekara A, English DR, McCredie MR, Giles GG, Tritchler D, Chiarelli A, Yaffe MJ, et al. Heritability of mammographic density, a risk factor for breast cancer. N Engl J Med. 2002; 347(12):886–894. [PubMed: 12239257]
19. Mucci LA, Hjelmborg JB, Harris JR, Czene K, Havelick DJ, Scheike T, Graff RE, Holst K, Moller S, Unger RH, et al. Familial Risk and Heritability of Cancer Among Twins in Nordic Countries. JAMA. 2016; 315(1):68–76. [PubMed: 26746459]

20. Moller S, Mucci LA, Harris JR, Scheike T, Holst K, Halekoh U, Adami HO, Czene K, Christensen K, Holm NV, et al. The Heritability of Breast Cancer among Women in the Nordic Twin Study of Cancer. Cancer Epidemiol Biomarkers Prev. 2016; 25(1):145–150. [PubMed: 26554920]
21. Gierach GL, Loud JT, Chow CK, Prindiville SA, Eng-Wong J, Soballe PW, Giambartolomei C, Mai PL, Galbo CE, Nichols K, et al. Mammographic density does not differ between unaffected BRCA1/2 mutation carriers and women at low-to-average risk of breast cancer. Breast Cancer Res Treat. 2010; 123(1):245–255. [PubMed: 20130984]
22. Varghese JS, Thompson DJ, Michailidou K, Lindstrom S, Turnbull C, Brown J, Leyland J, Warren RM, Luben RN, Loos RJ, et al. Mammographic breast density and breast cancer: evidence of a shared genetic basis. Cancer Res. 2012; 72(6):1478–1484. [PubMed: 22266113]
23. Vachon CM, Scott CG, Fasching PA, Hall P, Tamimi RM, Li J, Stone J, Apicella C, Odefrey F, Gierach GL, et al. Common breast cancer susceptibility variants in LSP1 and RAD51L1 are associated with mammographic density measures that predict breast cancer risk. Cancer Epidemiol Biomarkers Prev. 2012; 21(7):1156–1166. [PubMed: 22454379]
24. Stone J, Thompson DJ, Dos Santos Silva I, Scott C, Tamimi RM, Lindstrom S, Kraft P, Hazra A, Li J, Eriksson L, et al. Novel Associations between Common Breast Cancer Susceptibility Variants and Risk-Predicting Mammographic Density Measures. Cancer Res. 2015; 75(12):2457–2467. [PubMed: 25862352]
25. Lindstrom S, Thompson DJ, Paterson AD, Li J, Gierach GL, Scott C, Stone J, Douglas JA, dos-Santos-Silva I, Fernandez-Navarro P, et al. Genome-wide association study identifies multiple loci associated with both mammographic density and breast cancer risk. Nat Commun. 2014; 5:5303. [PubMed: 25342443]
26. Lindstrom S, Vachon CM, Li J, Varghese J, Thompson D, Warren R, Brown J, Leyland J, Audley T, Wareham NJ, et al. Common variants in ZNF365 are associated with both mammographic density and breast cancer risk. Nat Genet. 2011; 43(3):185–187. [PubMed: 21278746]
27. Schedin P. Pregnancy-associated breast cancer and metastasis. Nat Rev Cancer. 2006; 6(4):281–291. [PubMed: 16557280]
28. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A. 2001; 98(19):10869–10874. [PubMed: 11553815]
29. Vanderweele TJ, Vansteelandt S. Odds ratios for mediation analysis for a dichotomous outcome. American journal of epidemiology. 2010; 172(12):1339–1348. [PubMed: 21036955]
30. Vachon CM, Pankratz VS, Scott CG, Maloney SD, Ghosh K, Brandt KR, Milanese T, Carston MJ, Sellers TA. Longitudinal trends in mammographic percent density and breast cancer risk. Cancer Epidemiol Biomarkers Prev. 2007; 16(5):921–928. [PubMed: 17507617]

Table 1

Selected risk factors at time of mammography by case/control status and menopausal status at time of mammogram

	Premenopausal		Postmenopausal	
	Cases N=1105	**Controls N=3192**	**Cases N=2287**	**Controls N=5690**
Mean (SD)				
Age (years)	45.3 (4.5)	45.4 (4.5)	61.8 (8.7)	61.6 (9.2)
BMI (kg/m^2)	24.9 (4.9)	25.2 (5.4)	26.4 (5.5)	25.9 (5.4)
Height (inches)	64.7 (2.7)	64.7 (2.6)	64.1 (2.7)	64.0 (2.7)
Alcohol use (grams/day)	4.2 (7.3)	4.3 (7.3)	5.0 (9.1)	4.8 (8.6)
Percent MD	46.1 (19.3)	39.6 (19.5)	29.7 (18.9)	24.5 (17.7)
Dense area (cm^2)	62.2 (47.8)	58.9 (46.7)	37.9 (31.3)	31.6 (28.7)
Non-dense area (cm^2)	83.3 (68.0)	102.5 (78.2)	109.3 (76.6)	117.6 (80.6)
N (Percent)				
Previous breast biopsy	252 (23.0%)	468 (14.7%)	619 (28.1%)	1155 (20.8%)
Family history of breast cancer	183 (16.9%)	338 (10.6%)	472 (21.6%)	844 (14.9%)
Age at menarche (years)				
≤9	11 (1.9%)	32 (1.6%)	13 (1.1%)	22 (0.7%)
10	32 (5.6%)	107 (5.3%)	48 (4.0%)	119 (4.0%)
11	94 (16.4%)	316 (15.7%)	181 (15.1%)	448 (14.9%)
12	174 (30.4%)	583 (28.9%)	319 (26.6%)	768 (25.6%)
13	173 (30.2%)	610 (30.2%)	384 (32.1%)	930 (31.0%)
14	51 (8.9%)	207 (10.3%)	143 (11.9%)	410 (13.7%)
≥15	38 (6.6%)	163 (8.1%)	109 (9.1%)	305 (10.2%)
Nulliparous	288 (26.3%)	699 (22.0%)	423 (19.6%)	952 (17.1%)
Parity[^]				
1	67 (13.4%)	221 (13.0%)	91 (8.9%)	242 (8.9%)
2	231 (46.3%)	779 (45.9%)	297 (28.9%)	824 (30.4%)
3	145 (29.1%)	469 (27.6%)	316 (30.7%)	781 (28.8%)
4	36 (7.2%)	174 (10.3%)	170 (16.5%)	464 (17.1%)
5+	20 (4.0%)	54 (3.2%)	154 (15.0%)	402 (14.8%)
Age at first birth (years)[^]				
<30	525 (65.5%)	1786 (72.0%)	1429 (82.7%)	3937 (85.2%)
>= 30	277 (34.5%)	694 (28.0%)	298 (17.3%)	682 (14.8%)
Age at menopause (years)				
<30	--	--	109 (5.6%)	271 (5.8%)
30–39	--	--	109 (5.6%)	363 (7.8%)
40–44	--	--	183 (9.4%)	523 (11.3%)
45–49	--	--	501 (25.7%)	1235 (26.6%)
50–54	--	--	981 (50.4%)	2105 (45.3%)
≥55	--	--	65 (3.3%)	149 (3.2%)

	Premenopausal		Postmenopausal	
	Cases N=1105	Controls N=3192	Cases N=2287	Controls N=5690
Mean (SD)				
Hormone therapy use				
Never/Past	--	--	1082 (54.3%)	3077 (60.5%)
Current	--	--	911 (45.7%)	2006 (39.5%)
Type of hormone therapy use				
Never/Past			1082 (54.3%)	3077 (60.6%)
Current, E			365 (18.3%)	1000 (19.7%)
Current, E+P			546 (27.4%)	999 (19.7%)
ER status				
ER+	887 (80.3%)	--	1851 (80.9%)	--
ER−	194 (17.6%)	--	353 (15.4%)	--
Unknown	24 (2.2%)	--	83 (3.6%)	--
PR status				
PR+	824 (74.6%)	--	1578 (69.0%)	--
PR−	251 (22.7%)	--	619 (27.1%)	--
Unknown	30 (2.7%)	--	90 (3.9%)	--
HER2 status				
HER2+	161 (14.6%)	--	276 (12.1%)	--
HER2−	804 (72.8%)	--	1562 (68.3%)	--
Unknown	140 (12.7%)	--	449 (19.6%)	--

^Among parous

BMI=body mass index, E=estrogen, E+P=estrogen plus progestin

Number of individuals missing data on the following variables: height (n=1144), alcohol (n=7144), parity (n=6337), age at menarche (n=5484), age at first birth (n=2662), age at menopause (n=1463), current HT (n=1054), nulliparous (n=266), family history of breast cancer (n=144), previous breast biopsy (n=245), type of hormone therapy (N=7).

Table 2

Odds ratios (ORs) for breast cancer risk unadjusted and adjusted for percent mammographic density (MD) as well as percent of the association with breast cancer mediated by percent MD in women who were **premenopausal** at time of mammogram

	Cases/Controls	OR (95%CI)	OR (95%CI) adjusted for percent MD[±]	Percent mediated	P-value[#]
BMI (kg/m2) Per 5 unit increase					
All invasive breast cancer	1105/3192	0.98 (0.92,1.05)	1.20 (1.10,1.29)	Not mediated	N/A
ER+ breast cancer	887/3192	0.97 (0.90,1.04)	1.17 (1.08,1.28)	Not mediated	N/A
ER− breast cancer	194/3192	1.05 (0.92,1.21)	1.27 (1.09,1.48)	Not mediated	N/A
PR+ breast cancer	824/3192	0.98 (0.91,1.06)	1.20 (1.10,1.31)	Not mediated	N/A
PR− breast cancer	251/3192	0.99 (0.87,1.12)	1.17 (1.02,1.35)	Not mediated	N/A
HER2+ breast cancer	161/3192	0.99 (0.85,1.16)	1.16 (0.97,1.38)	Not mediated	N/A
HER2− breast cancer	804/3192	1.01 (0.94,1.09)	1.23 (1.13,1.35)	Not mediated	N/A
Height Per 3 inch increase					
All invasive breast cancer	1060/3018	1.02 (0.94,1.11)	1.04 (0.96,1.13)	Not mediated	N/A
ER+ breast cancer	851/3018	1.03 (0.94,1.12)	1.04 (0.95,1.14)	Not mediated	N/A
ER− breast cancer	185/3018	1.03 (0.86,1.22)	1.04 (0.88,1.23)	Not mediated	N/A
PR+ breast cancer	789/3018	1.02 (0.93,1.12)	1.03 (0.94,1.14)	Not mediated	N/A
PR− breast cancer	241/3018	1.04 (0.89,1.22)	1.06 (0.91,1.23)	Not mediated	N/A
HER2+ breast cancer	156/3018	1.00 (0.83,1.19)	1.01 (0.84,1.20)	Not mediated	N/A
HER2− breast cancer	765/3018	1.00 (0.91,1.10)	1.02 (0.93,1.12)	Not mediated	N/A
Age at menarche Per 2 year increase					
All invasive breast cancer	573/2018	0.86 (0.75,1.00)	0.84 (0.73,0.98)	Not mediated	N/A
ER+ breast cancer	456/2018	0.88 (0.75,1.03)	0.86 (0.73,1.01)	Not mediated	N/A
ER− breast cancer	101/2018	0.78 (0.58,1.05)	0.77 (0.57,1.04)	Not mediated	N/A
PR+ breast cancer	418/2018	0.90 (0.76,1.06)	0.88 (0.74,1.04)	Not mediated	N/A
PR− breast cancer	135/2018	0.74 (0.57,0.96)	0.73 (0.56,0.95)	Not mediated	N/A
HER2+ breast cancer	74/2018	0.72 (0.50,1.04)	0.71 (0.50,1.01)	Not mediated	N/A
HER2− breast cancer	399/2018	0.87 (0.74,1.03)	0.86 (0.72,1.01)	Not mediated	N/A

	Cases/Controls	OR (95%CI)	OR (95%CI) adjusted for percent MD[±]	Percent mediated	P-value[#]
Nulliparous[*] Nulliparous vs parous					
All invasive breast cancer	1095/3180	1.14 (0.96,1.35)	1.08 (0.91,1.29)	40%	0.15
ER+ breast cancer	881/3180	1.13 (0.94,1.35)	1.07 (0.89,1.29)	44%	0.23
ER− breast cancer	190/3180	1.26 (0.89,1.78)	1.20 (0.85,1.71)	19%	0.25
PR+ breast cancer	819/3180	1.11 (0.92,1.34)	1.05 (0.86,1.27)	56%	0.31
PR− breast cancer	246/3180	1.21 (0.88,1.66)	1.16 (0.85,1.59)	21%	0.28
HER2+ breast cancer	160/3180	1.30 (0.89,1.88)	1.25 (0.86,1.83)	13%	0.27
HER2− breast cancer	795/3180	1.08 (0.89,1.30)	1.02 (0.84,1.23)	76%	0.45
Parity[^] Per 1 child increase					
All invasive breast cancer	499/1697	0.98 (0.87,1.09)	1.00 (0.89,1.12)	Not mediated	N/A
ER+ breast cancer	392/1697	1.01 (0.89,1.14)	1.03 (0.91,1.17)	Not mediated	N/A
ER− breast cancer	92/1697	0.82 (0.65,1.04)	0.84 (0.66,1.07)	10%	0.23
PR+ breast cancer	363/1697	0.98 (0.86,1.12)	1.01 (0.88,1.15)	Not mediated	N/A
PR− breast cancer	119/1697	0.91 (0.74,1.13)	0.93 (0.75,1.16)	23%	0.45
HER2+ breast cancer	68/1697	1.09 (0.83,1.42)	1.12 (0.85,1.48)	Not mediated	N/A
HER2− breast cancer	342/1697	0.90 (0.79,1.03)	0.92 (0.80,1.05)	20%	0.19
Age at first birth[^] ≥ 30 vs < 30					
All invasive breast cancer	802/2480	1.32 (1.09,1.60)	1.30 (1.08,1.58)	5%	0.29
ER+ breast cancer	648/2480	1.45 (1.19,1.78)	1.43 (1.17,1.76)	4%	0.28
ER− breast cancer	136/2480	0.85 (0.56,1.29)	0.85 (0.56,1.29)	Not mediated	N/A
PR+ breast cancer	603/2480	1.48 (1.20,1.82)	1.46 (1.18,1.80)	4%	0.34
PR− breast cancer	176/2479	0.88 (0.60,1.28)	0.87 (0.60,1.27)	Not mediated	N/A
HER2+ breast cancer	111/2480	1.55 (0.99,2.42)	1.55 (0.99,2.42)	1%	0.88
HER2− breast cancer	575/2479	1.22 (0.99,1.50)	1.20 (0.97,1.49)	6%	0.45
Alcohol use Per 10 g/day increase					
All invasive breast cancer	493/1665	0.98 (0.85,1.13)	0.97 (0.83,1.13)	Not mediated	N/A

	Cases/Controls	OR (95%CI)	OR (95%CI) adjusted for percent MD±	Percent mediated	P-value#
ER+ breast cancer	388/1665	0.95 (0.80,1.12)	0.94 (0.79,1.11)	Not mediated	N/A
ER− breast cancer	91/1665	0.98 (0.72,1.34)	0.96 (0.70,1.32)	Not mediated	N/A
PR+ breast cancer	357/1665	0.96 (0.81,1.13)	0.94 (0.80,1.12)	Not mediated	N/A
PR− breast cancer	118/1665	1.03 (0.80,1.33)	1.02 (0.79,1.33)	32%	0.84
HER2+ breast cancer	68/1665	0.98 (0.70,1.38)	0.96 (0.69,1.35)	Not mediated	N/A
HER2− breast cancer	337/1665	0.88 (0.73,1.06)	0.87 (0.72,1.06)	Not mediated	N/A
Family history of breast cancer Yes vs no					
All invasive breast cancer	1083/3190	1.59 (1.30,1.94)	1.55 (1.27,1.90)	5%	0.18
ER+ breast cancer	874/3190	1.59 (1.29,1.97)	1.56 (1.26,1.93)	5%	0.18
ER− breast cancer	185/3190	1.40 (0.92,2.14)	1.37 (0.90,2.10)	6%	0.39
PR+ breast cancer	813/3190	1.60 (1.29,1.99)	1.57 (1.26,1.96)	4%	0.23
PR− breast cancer	240/3190	1.37 (0.94,2.01)	1.35 (0.92,1.97)	6%	0.34
HER2+ breast cancer	154/3190	1.26 (0.79,2.04)	1.24 (0.77,1.99)	9%	0.42
HER2− breast cancer	789/3190	1.68 (1.36,2.09)	1.64 (1.32,2.04)	5%	0.17
Previous breast biopsy Yes vs no					
All invasive breast cancer	1098/3183	1.76 (1.48,2.10)	1.60 (1.34,1.91)	17%	<.01
ER+ breast cancer	884/3183	1.68 (1.39,2.04)	1.53 (1.26,1.86)	18%	<.01
ER− breast cancer	190/3183	2.04 (1.44,2.90)	1.85 (1.30,2.63)	14%	<.01
PR+ breast cancer	823/3183	1.79 (1.47,2.17)	1.62 (1.33,1.98)	17%	<.01
PR− breast cancer	245/3183	1.76 (1.28,2.42)	1.60 (1.16,2.20)	17%	<.01
HER2+ breast cancer	160/3183	2.07 (1.42,3.02)	1.91 (1.31,2.79)	11%	0.02
HER2− breast cancer	798/3183	1.70 (1.40,2.07)	1.54 (1.26,1.89)	18%	<.01

BMI=body mass index

Adjusted for age (continuous), current BMI (continuous), study (MMHS, NHS, NHSII, SFMR), parity/age at first birth (nulliparous, parous age at first birth <30, parous age at first birth ≥30), previous breast biopsy (no, yes, unknown) and family history of breast cancer (no, yes, unknown).

*Not adjusted for parity/age at first birth

^Among parous

#P-value for percent of the total association mediated by mammographic density

± Square-root transformed

Not mediated = Percent mediated calculated to be <0% or >100%

Table 3

Odds ratios (ORs) for breast cancer risk unadjusted and adjusted for percent mammographic density (MD) as well as percent of the association with breast cancer mediated by percent MD in women who were **postmenopausal** at time of mammogram

Selected risk factor	Cases/Controls	OR (95%CI)	OR (95%CI) adjusted for percent MD±	Percent mediated	P-value#
BMI (kg/m2) Per 5 unit increase					
All invasive breast cancer	2287/5690	1.14 (1.09,1.19)	1.33 (1.26,1.40)	Not mediated	N/A
ER+ breast cancer	1851/5690	1.15 (1.10,1.21)	1.35 (1.27,1.42)	Not mediated	N/A
ER− breast cancer	353/5690	1.06 (0.96,1.18)	1.23 (1.11,1.38)	Not mediated	N/A
PR+ breast cancer	1578/5690	1.15 (1.09,1.21)	1.35 (1.27,1.43)	Not mediated	N/A
PR− breast cancer	619/5690	1.11 (1.03,1.20)	1.27 (1.17,1.38)	Not mediated	N/A
HER2+ breast cancer	276/5690	1.07 (0.96,1.20)	1.27 (1.13,1.42)	Not mediated	N/A
HER2− breast cancer	1562/5690	1.15 (1.09,1.21)	1.32 (1.24,1.40)	Not mediated	N/A
Height Per 3 inch increase					
All invasive breast cancer	2114/4938	1.06 (0.99,1.12)	1.08 (1.02,1.15)	Not mediated	N/A
ER+ breast cancer	1695/4938	1.09 (1.02,1.16)	1.11 (1.04,1.18)	Not mediated	N/A
ER− breast cancer	337/4938	0.91 (0.80,1.03)	0.93 (0.82,1.05)	28%	0.14
PR+ breast cancer	1444/4938	1.10 (1.02,1.17)	1.12 (1.04,1.20)	Not mediated	N/A
PR− breast cancer	582/4938	0.97 (0.87,1.07)	0.99 (0.90,1.10)	74%	0.51
HER2+ breast cancer	258/4938	1.06 (0.91,1.22)	1.08 (0.94,1.25)	Not mediated	N/A
HER2− breast cancer	1419/4938	1.06 (0.99,1.14)	1.09 (1.01,1.17)	Not mediated	N/A
Age at menarche Per 2 year increase					
All invasive breast cancer	1197/3002	0.96 (0.87,1.07)	0.95 (0.85,1.06)	Not mediated	N/A
ER+ breast cancer	953/3002	0.98 (0.87,1.10)	0.96 (0.85,1.08)	Not mediated	N/A
ER− breast cancer	184/3002	0.88 (0.68,1.14)	0.86 (0.66,1.13)	Not mediated	N/A
PR+ breast cancer	797/3002	1.00 (0.88,1.13)	0.98 (0.87,1.12)	Not mediated	N/A
PR− breast cancer	337/3002	0.86 (0.72,1.04)	0.85 (0.70,1.03)	Not mediated	N/A
HER2+ breast cancer	132/3002	0.92 (0.70,1.21)	0.90 (0.67,1.19)	Not mediated	N/A
HER2− breast cancer	732/3002	0.98 (0.86,1.11)	0.96 (0.84,1.10)	Not mediated	N/A

Selected risk factor	Cases/Controls	OR (95%CI)	OR (95%CI) adjusted for percent MD[±]	Percent mediated	P-value[#]
Nulliparous[*] Nulliparous vs parous					
All invasive breast cancer	2158/5575	1.23 (1.07,1.41)	1.13 (0.98,1.29)	43%	<.01
ER+ breast cancer	1748/5575	1.23 (1.07,1.43)	1.12 (0.97,1.30)	44%	<.01
ER− breast cancer	334/5575	1.11 (0.82,1.49)	1.01 (0.75,1.37)	86%	0.50
PR+ breast cancer	1488/5575	1.27 (1.09,1.49)	1.16 (0.99,1.36)	38%	<.01
PR− breast cancer	587/5575	1.10 (0.87,1.39)	1.00 (0.79,1.27)	96%	0.44
HER2+ breast cancer	256/5575	1.08 (0.77,1.52)	0.96 (0.69,1.34)	Not mediated	N/A
HER2− breast cancer	1479/5575	1.25 (1.07,1.45)	1.16 (0.99,1.35)	34%	0.01
Parity[^] Per 1 child increase					
All invasive breast cancer	1028/2713	0.99 (0.93,1.06)	1.01 (0.95,1.08)	Not mediated	N/A
ER+ breast cancer	823/2713	0.99 (0.92,1.06)	1.01 (0.94,1.09)	Not mediated	N/A
ER− breast cancer	160/2713	1.05 (0.90,1.21)	1.07 (0.92,1.24)	Not mediated	N/A
PR+ breast cancer	680/2713	1.00 (0.93,1.08)	1.02 (0.95,1.11)	Not mediated	N/A
PR− breast cancer	298/2713	1.00 (0.89,1.12)	1.02 (0.91,1.14)	Not mediated	N/A
HER2+ breast cancer	111/2713	0.91 (0.77,1.08)	0.93 (0.78,1.10)	16%	0.35
HER2− breast cancer	623/2713	1.03 (0.95,1.12)	1.05 (0.97,1.14)	Not mediated	N/A
Age at first birth[^] ≥ 30 vs < 30					
All invasive breast cancer	1727/4619	1.26 (1.08,1.47)	1.21 (1.03,1.42)	16%	0.05
ER+ breast cancer	1397/4619	1.28 (1.09,1.52)	1.23 (1.04,1.46)	16%	0.04
ER− breast cancer	270/4619	1.00 (0.70,1.43)	0.97 (0.68,1.38)	Not mediated	N/A
PR+ breast cancer	1177/4619	1.23 (1.03,1.47)	1.18 (0.98,1.41)	21%	0.08
PR− breast cancer	473/4612	1.25 (0.97,1.62)	1.21 (0.93,1.57)	14%	0.18
HER2+ breast cancer	207/4619	1.61 (1.13,2.30)	1.57 (1.10,2.23)	6%	0.09
HER2− breast cancer	1144/4612	1.24 (1.04,1.49)	1.21 (1.01,1.45)	12%	0.14
Alcohol use Per 10 g/day increase					
All invasive breast cancer	885/2087	1.03 (0.94,1.13)	1.02 (0.93,1.12)	19%	0.66

Selected risk factor	Cases/Controls	OR (95%CI)	OR (95%CI) adjusted for percent MD[±]	Percent mediated	P-value[#]
ER+ breast cancer	701/2087	1.04 (0.95,1.15)	1.04 (0.94,1.15)	10%	0.66
ER− breast cancer	137/2087	0.96 (0.76,1.22)	0.95 (0.74,1.21)	Not mediated	N/A
PR+ breast cancer	574/2087	1.05 (0.94,1.17)	1.04 (0.94,1.16)	9%	0.67
PR− breast cancer	261/2087	1.00 (0.85,1.17)	0.99 (0.84,1.16)	Not mediated	N/A
HER2+ breast cancer	93/2087	1.04 (0.81,1.34)	1.03 (0.80,1.34)	15%	0.82
HER2− breast cancer	504/2087	0.98 (0.87,1.11)	0.98 (0.86,1.11)	Not mediated	N/A
Family history of breast cancer Yes vs no					
All invasive breast cancer	2188/5669	1.58 (1.39,1.80)	1.58 (1.39,1.80)	1%	0.72
ER+ breast cancer	1774/5669	1.60 (1.39,1.83)	1.59 (1.39,1.83)	1%	0.79
ER− breast cancer	336/5669	1.64 (1.24,2.16)	1.62 (1.23,2.14)	2%	0.44
PR+ breast cancer	1515/5669	1.61 (1.39,1.86)	1.59 (1.37,1.85)	2%	0.5
PR− breast cancer	588/5669	1.57 (1.27,1.95)	1.57 (1.26,1.95)	0%	0.98
HER2+ breast cancer	258/5669	2.01 (1.49,2.70)	2.00 (1.48,2.69)	1%	0.67
HER2− breast cancer	1503/5669	1.52 (1.32,1.76)	1.52 (1.31,1.77)	0%	0.97
Previous breast biopsy Yes vs no					
All invasive breast cancer	2202/5546	1.50 (1.34,1.69)	1.36 (1.21,1.53)	24%	<.01
ER+ breast cancer	1786/5546	1.48 (1.31,1.68)	1.34 (1.18,1.52)	26%	<.01
ER− breast cancer	339/5546	1.66 (1.29,2.13)	1.51 (1.18,1.95)	18%	<.01
PR+ breast cancer	1520/5546	1.47 (1.28,1.67)	1.32 (1.15,1.51)	27%	<.01
PR− breast cancer	598/5546	1.59 (1.31,1.92)	1.46 (1.20,1.77)	19%	<.01
HER2+ breast cancer	263/5546	1.68 (1.28,2.22)	1.51 (1.14,2.00)	21%	<.01
HER2− breast cancer	1513/5546	1.48 (1.30,1.69)	1.35 (1.18,1.55)	23%	<.01
Age at menopause Per category increase[†]					
All invasive breast cancer	1948/4646	1.07 (1.02,1.13)	1.07 (1.02,1.13)	1%	0.9
ER+ breast cancer	1564/4646	1.09 (1.03,1.15)	1.09 (1.03,1.15)	2%	0.67
ER− breast cancer	308/4646	1.00 (0.90,1.10)	1.00 (0.90,1.10)	Not mediated	N/A
PR+ breast cancer	1328/4646	1.09 (1.03,1.15)	1.09 (1.03,1.15)	1%	0.85

Selected risk factor	Cases/Controls	OR (95%CI)	OR (95%CI) adjusted for percent MD[±]	Percent mediated	P-value[#]
PR− breast cancer	538/4646	1.04 (0.96,1.13)	1.04 (0.95,1.13)	6%	0.64
HER2+ breast cancer	238/4646	1.11 (0.98,1.26)	1.11 (0.98,1.26)	Not mediated	N/A
HER2− breast cancer	1303/4646	1.09 (1.03,1.16)	1.09 (1.03,1.15)	2%	0.71
Hormone therapy use Current vs never/former use					
All invasive breast cancer	1993/5083	1.39 (1.24,1.55)	1.23 (1.10,1.37)	37%	<.01
ER+ breast cancer	1612/5083	1.42 (1.26,1.60)	1.25 (1.11,1.42)	36%	<.01
ER− breast cancer	311/5083	1.15 (0.91,1.46)	1.03 (0.81,1.31)	78%	0.25
PR+ breast cancer	1366/5083	1.49 (1.31,1.69)	1.32 (1.16,1.50)	31%	<.01
PR− breast cancer	551/5083	1.12 (0.93,1.34)	1.00 (0.83,1.20)	Not mediated	N/A
HER2+ breast cancer	237/5083	1.24 (0.94,1.64)	1.09 (0.83,1.44)	60%	0.13
HER2− breast cancer	1361/5083	1.36 (1.20,1.55)	1.22 (1.07,1.39)	36%	<.01
Hormone therapy use Current E vs never/former use					
All invasive breast cancer	1447/4077	1.16 (1.00,1.34)	1.05 (0.90,1.22)	69%	0.06
ER+ breast cancer	1164/4077	1.16 (0.99,1.36)	1.05 (0.89,1.23)	71%	0.07
ER− breast cancer	230/4077	1.03 (0.75,1.42)	0.94 (0.69,1.29)	Not mediated	N/A
PR+ breast cancer	963/4077	1.15 (0.97,1.37)	1.03 (0.87,1.23)	76%	0.13
PR− breast cancer	428/4077	1.10 (0.87,1.40)	1.01 (0.80,1.28)	91%	0.41
HER2+ breast cancer	172/4077	1.12 (0.76,1.64)	1.01 (0.69,1.48)	93%	0.57
HER2− breast cancer	982/4077	1.09 (0.92,1.30)	0.99 (0.83,1.18)	Not mediated	N/A
Hormone therapy use Current E+P vs never/former use					
All invasive breast cancer	1628/4076	1.66 (1.45,1.90)	1.46 (1.27,1.67)	26%	<.01
ER+ breast cancer	1324/4076	1.74 (1.50,2.01)	1.52 (1.31,1.76)	24%	<.01
ER− breast cancer	254/4076	1.27 (0.95,1.69)	1.13 (0.85,1.51)	48%	0.12
PR+ breast cancer	1133/4076	1.87 (1.60,2.17)	1.64 (1.40,1.91)	21%	<.01
PR− breast cancer	440/4076	1.16 (0.92,1.47)	1.05 (0.83,1.32)	70%	0.21
HER2+ breast cancer	194/4076	1.38 (0.99,1.91)	1.21 (0.87,1.68)	40%	0.07
HER2− breast cancer	1135/4076	1.66 (1.43,1.94)	1.48 (1.26,1.73)	23%	<.01

Adjusted for age (continuous), current BMI (continuous), study (MMHS, NHS, NHSII, SFMR), parity/age at first birth (nulliparous, parous age at first birth <30, parous age at first birth ≥30), previous biopsy (no, yes, unknown), family history of breast cancer (no, yes, unknown), and any HT use (current vs past/never).

[*] Not adjusted for parity/age at first birth

[^] Among parous

[†] Age at menopause categories: <30, 30–39, 40–44, 45–49, 50–54, ≥55

[#] P-value for percent of the total association mediated by mammographic density

[±] Square-root transformed

Not mediated = Percent mediated calculated to be <0% or >100%