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Plasma matrix metalloproteinase 2 levels and breast cancer risk

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Abstract

Matrix metalloproteinase 2 (MMP2) is an enzyme with important functions in breast cancer invasion and metastasis. However, it is unclear whether circulating MMP2 levels may predict breast cancer risk. We conducted a prospective nested case-control analysis in the Nurses' Health Study among 1136 cases who were diagnosed with invasive breast cancer between 1992 and 2004 and 1136 matched controls. All participants provided blood samples in 1989-1990, and a subset (170 cases, 170 controls) contributed an additional sample in 2000 – 2002. Pre-diagnostic plasma MMP2 levels were measured via immunoassay, and conditional logistic regression was performed to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs), adjusted for breast cancer risk factors. No association was observed between plasma MMP2 levels and risk of total invasive breast cancer (top vs. bottom quartile, OR = 1.0; 95% CI: 0.7, 1.2; p-trend = 0.89). Findings did not vary significantly by time since blood draw, body mass index, postmenopausal hormone use, or menopausal status at either blood draw or breast cancer diagnosis. MMP2 was associated with a greater risk of nodal metastases at diagnosis (top vs. bottom quartile, OR = 1.5; 95% CI: 1.0, 2.2;

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Author contributions

S.A.A. performed statistical analyses and drafted the manuscript. B.A.R provided statistical guidance. B.A.R, R.M.T., S.S.T., and S.E.H. contributed to data analyses and interpretation. S.S.T. and S.E.H. conceived of, designed, and supervised the study. N.B. performed MMP2 assays and assisted in manuscript writing. T.O.J. supervised laboratory measurements of MMP2. S.E.H. obtained study funding. All authors critically revised the manuscript for important intellectual content and approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

p-heterogeneity, any vs. no lymph nodes = 0.002), but no significant associations were observed with other tumor characteristics or with recurrent or fatal cancers. Plasma MMP2 levels do not appear to be predictive of total invasive breast cancer risk, although associations with aggressive disease warrant further study.

Keywords

breast cancer; matrix metalloproteinases; nested case-control; plasma; risk

INTRODUCTION

Breast cancer is the second-leading cause of cancer death among US women [1]. Matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidase enzymes [2], have been suggested as potential biomarkers of breast cancer risk based on growing biologic evidence of their involvement in breast carcinogenesis [3] and their easy detection in blood [4]. MMPs are collectively able to degrade the extracellular matrix [5], a key step in tumor invasion and metastasis. Growing evidence also supports a role for MMPs earlier in the carcinogenic process[6], and circulating MMPs might therefore be elevated among women prior to the development of clinically detectable breast cancer.

Among the more than 20 MMPs that have been identified, MMP2 (gelatinase-A) has emerged as one of the strongest potential biomarkers of breast cancer risk due to substantial *in vivo* and *in vitro* support for its involvement in breast carcinogenesis [3]. MMP2 plays a critical role in breast tumor invasion and metastasis by digesting type-IV collagen, one of the key constituents of the basement membrane separating tumors from surrounding tissue [7]. MMP2 may also facilitate tumor development via the processing of growth factors [8,6,9] and inflammatory markers [8] as well as the stimulation of angiogenesis [10-12]. A role for MMP2 in tumor initiation also has been suggested [6,13]. In addition, biologic evidence indicates that the expression and activity of MMP2 may in part be regulated by estrogen [14-16], a hormone that has well-established functions in promoting breast cancer growth [17].

Epidemiologic evidence on the relationship between circulating MMP2 and breast cancer risk is limited and inconsistent. Several small retrospective case-control studies have reported higher circulating MMP2 levels in cases than in controls [18-20], while others have observed no difference in levels [21,22]. The only prospective study to examine the relationship between pre-diagnostic MMP2 levels and subsequent breast cancer risk did not find any association with total invasive breast cancer, although there was some suggestion that higher levels may predict the risk of cancers with a worse prognosis [23]. Potential interrelationships between MMP2 and estrogen are also incompletely understood, as reported associations between estrogen and MMP2 from *in vitro* and human studies have conflicted in both magnitude and direction [24,14-16,25-28]. Further, while studies in human populations have examined postmenopausal estrogen use in relation to circulating MMP2 levels[25-28], endogenous estrogen concentrations have not been assessed.

We conducted a prospective nested case-control study to investigate the association between plasma MMP2 levels and risk of invasive breast cancer among pre- and postmenopausal women in the Nurses' Health Study (NHS). We examined associations by breast tumor characteristics and evaluated associations between circulating MMP2 and estradiol among a subset of participants with measurements of both plasma MMP2 and sex hormones.

MATERIALS AND METHODS

Study Population

We performed a case-control analysis nested in the NHS, an ongoing prospective cohort study that began with the enrollment of 121,700 female nurses (ages 30-55) in 1976. Updated information on disease occurrence and exposures is obtained via biennial questionnaires [29,30]. Blood samples were collected in 1989-1990 from 32,826 cancer-free women and again in 2000-2002 among a subset of 18,743 women in the first collection. Details of these collections have been previously described [31], [32]. Briefly, women had their blood collected in tubes containing heparin and shipped overnight to our lab on ice; 97% of samples arrived within 26 hours of collection. Upon arrival at our laboratory, samples were separated into plasma, red blood cell, and white blood cell components and stored in liquid nitrogen at −130° C or colder. Although there was some decrease in MMP2 levels with delayed processing (intraclass correlation coefficient (ICC) = 0.56, with an average decrease of 6% over 48 hours), the Spearman correlation between samples with immediate vs. delayed processing was higher (rho = 0.74), indicating that ranked sample levels were relatively unchanged. The follow-up rate to 2004 among participants in the blood substudy was 98% [33]. The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

Breast Cancer Cases and Controls

Participants were followed for incident invasive breast cancer from the first blood collection until May 31, 2004, with the first 2 years of follow-up after the first collection excluded to preserve sample volume and to reduce the possibility that MMP2 levels might reflect the presence of subclinical disease. Cases were medically confirmed and matched 1:1 to controls on month and time of day of each blood collection, age, fasting status, postmenopausal hormone (PMH) use, and menopausal status at each blood collection.

Laboratory Analyses

MMP2 concentrations, which consist of both latent pro-enzyme and biologically active MMP2, were measured in 50 μL 1:50 diluted plasma via sandwich immunoassay (Fluorokine Multianalyte Profiling (MAP) Kit (R&D Systems, Minneapolis, MN, USA)), with MMP2 concentrations quantified using a Luminex 100 analyzer system (Luminex, Austin, TX, USA). MMP2 assays were performed at the Natural and Medical Sciences Institute at the University of Tuebingen (Reutlingen, Germany) in two batches, the first in 2010 and the second in 2013. Different assay generations were used for the two batches, as the kit antibodies were reconfigured in 2012. The first batch included 801 breast cancer cases diagnosed from 1992-2000 and 801 matched controls, with all samples from the first blood collection. The second batch included an additional 335 cases diagnosed from

2000-2004 and 335 matched controls; 170 case-control pairs provided samples from both blood collections, 157 matched pairs contributed samples from only the first blood collection, and 8 pairs contributed blood from only the second collection. In total, 1136 cases and 1136 controls with 2612 blood samples were available for our analyses. Case and control pairs were assayed together but in random order. The coefficient of variation from masked quality control samples was 11% for the first batch and 14% for the second batch. No sample values were below the limit of detection, and outlier identification using the extreme studentized deviate (ESD) procedure [34] on log-transformed values detected no outliers.

Statistical Analyses

We combined data from the first and second batches in our analyses, as the association between MMP2 quartile and total invasive breast cancer risk was qualitatively similar by assay generation in a subset of participants from the first batch reassayed with the second generation assay (n=262 cases, 262 controls; top vs. bottom quartile, first generation assay: OR = 1.3 [95% CI: 0.8, 2.2]; second generation assay: OR = 1.5 [95% CI: 0.9, 2.6]). To account for moderate variation in MMP2 levels between the two batches (rho = 0.56), MMP2 values were recalibrated to represent the average MMP2 distribution across the two batches [35]. Recalibrated MMP2 values from the first and second blood collection were averaged for women with samples from both collections (except in analyses by time since blood draw, in which individual sample values were used) to better represent long-term levels among these women.

The age-standardized distribution of breast cancer risk factors was examined by MMP2 quartile among the controls. We used conditional logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (95% CIs) for the association between quartiles of MMP2 concentration and breast cancer risk, with quartile cutpoints based on the distribution of MMP2 among controls. As univariate and multivariate results were essentially identical (with minimal change in the OR or 95% CI), only multivariate results are presented. For both cross-sectional analyses and evaluation of the association between MMP2 levels and breast cancer risk, Wald tests for linear trend were performed with MMP2 modeled as the median of each quartile. Models included body mass index (BMI) at blood draw, age at menarche, current alcohol consumption, PMH use and type, age at first birth/parity, family history of breast cancer, and history of benign breast disease. Covariates were either obtained via a supplemental questionnaire administered at the time of each blood draw or from the main study questionnaire closest to each blood draw (either the 1990 or 2000 questionnaire). In addition, we assessed associations for the average of 1989-1990 and 2000-2002 MMP2 levels among women with two blood samples. As associations were similar with adjustment for covariates measured at either the time of the first or second blood draw, we adjusted for only 1990 covariates in our final models.

We assessed whether associations between MMP2 and breast cancer risk varied by time since blood draw (<5 years, 5-10 years, ≥10 years), menopausal status (premenopausal, postmenopausal), BMI (<25 kg/m^2 and ≥25 kg/m^2), and PMH use (current use, no current use) at blood draw in stratified analyses using unconditional logistic regression and via Wald

interaction tests. As women with samples from both blood draws contributed data to two categories in analyses by time since blood draw, proc genmod with a logistic link and binomial distribution was used to account for the correlated data from these women in testing for interaction by time since blood draw.

Associations by breast tumor characteristics (estrogen receptor/progesterone receptor (ER/PR) status (ER+/PR+, ER+/PR−, ER−/PR−), nodal status (no versus any nodal involvement), number of nodes involved (<4 nodes, ≥4 nodes), tumor size (<2.0 cm, ≥2.0 cm), and grade (1, 2, and 3)) were examined using polytomous logistic regression [36], with likelihood ratio tests used to assess heterogeneity of trends. In addition, we assessed risk of fatal and recurrent breast cancer using unconditional logistic regression.

We performed a subanalysis among a subset of study participants with previous plasma estradiol measurements available from the first blood collection (329 cases, 329 controls) [37], all of whom were postmenopausal and had not used PMH for at least 3 months at blood collection, to determine whether additional adjustment for estradiol altered our main associations. Estradiol was treated as a log-transformed continuous variable to improve normality. We used SAS 9.3 (SAS Institute, Cary, NC) for all analyses except those by breast tumor characteristics, for which we used STATA 12.1 (StataCorp LP, College Station, TX). All tests were two-sided, with $p<0.05$ indicating statistical significance.

RESULTS

Participants had a mean age of 57 years at the first blood collection and 68 years at the second collection. At the first blood draw, 76% of participants were postmenopausal; nearly all (98%) were postmenopausal at the second blood draw. Compared with controls, cases were less likely to be parous and more likely to have a personal history of benign breast disease or a family history of breast cancer. In cross-sectional analyses between MMP2 levels and breast cancer risk factors among controls, inverse associations were observed for current PMH use (p-trend = 0.001) and BMI (p-trend <0.0001) (Table 1). No association was observed between concentrations of plasma estradiol and MMP2 (p-trend = 0.27).

There was no association between plasma MMP2 levels and risk of total invasive breast cancer (top vs. bottom quartile, OR = 1.0; 95% CI: 0.7, 1.2; p-trend = 0.89) (Table 2). Further adjustment for plasma estradiol in the subset with available data had minimal influence on this association. Among the smaller subset of women with measurements at both blood draws (N=170 cases, 170 controls), average MMP2 levels from the two collections displayed a marginally significant inverse association with breast cancer risk (data not shown).

No significant differences in the association between plasma MMP2 and breast cancer risk were observed by time since blood draw (p-het=0.73), current PMH use at blood draw among postmenopausal women (p-het=0.25), or menopausal status at blood draw (p-het=0.63); the association among women who were postmenopausal at diagnosis was also similar to that in the total study population. There was a suggestive inverse association among women with BMI <25 kg/m^2 (top vs. bottom quartile, OR = 0.7; 95% CI: 0.5, 1.0; p-

trend = 0.21) but no association among women with BMI ≥25 kg/m^2 (top vs. bottom quartile, OR = 1.1; 95% CI: 0.8, 1.6; p-trend = 0.52); this difference by BMI did not reach statistical significance (p-heterogeneity = 0.13) (Table 2).

In analyses by tumor characteristics, a positive association was observed between plasma MMP2 and risk of nodal metastases at diagnosis. Associations were significant for both lymph node presence (any lymph nodes: top vs. bottom quartile, OR = 1.5; 95% CI: 1.0, 2.2; p-heterogeneity, any vs. no lymph nodes = 0.002) and greater number of lymph nodes (≥4 lymph nodes: top vs. bottom quartile, OR = 1.9; 95% CI: 1.0, 3.8; p-heterogeneity, ≥4 vs. no lymph nodes = 0.008). No associations were observed by ER/PR status, tumor size, or tumor grade or for fatal or recurrent tumors (Table 3).

DISCUSSION

In this prospective analysis, we did not observe a significant association between plasma MMP2 levels and risk of total invasive breast cancer. Results were also null by time since blood draw, BMI, PMH use, and menopausal status at both blood draw and breast cancer diagnosis. Positive associations between MMP2 levels and nodal metastases at diagnosis were observed, but MMP2 was not significantly associated with any of the other tumor characteristics examined or with recurrent or fatal disease.

Our lack of association with risk of invasive breast cancer overall is consistent with the one other prospective analysis of pre-diagnostic circulating MMP2 and breast cancer risk conducted in the Multiethnic Cohort (MEC) [23]. Other epidemiologic data on the association between circulating MMP2 and breast cancer risk come from small retrospective case-control studies. Some [19,18,20], but not all [21,22], of these studies reported higher levels of MMP2 in breast cancer cases compared with controls. However, all except one of these studies measured MMP2 levels in serum, which may promote release of MMP2 from immune cells and therefore not provide an accurate representation of tumor-derived MMP2 [38]. Further, MMP2 levels were measured after breast cancer diagnosis and therefore could not distinguish whether elevated levels might be due to associations with breast cancer progression or risk.

The increased risk of nodal metastases that we observed among those with elevated plasma MMP2 levels is consistent with the known biologic roles of MMP2 in tumor invasion and metastasis [3]. Involvement of MMP2 in tumor dissemination is also supported by previously reported associations for both tumor MMP2 expression [39,40] and post-diagnostic circulating MMP2 levels [20,18] with nodal metastases, as well as an observed increased risk of metastatic breast cancer among breast cancer patients with sentinel lymph node expression of MMP2 [41]. However, our results are not entirely consistent in suggesting whether plasma MMP2 might serve as a marker for risk of aggressive tumors, as we observed no associations with fatal or recurrent breast cancer or with tumor size or grade.

The relationship between MMP2 and risk of aggressive disease was similarly equivocal in the MEC; higher MMP2 levels were suggestively associated with risk of distant metastases

(2 standard deviation change in MMP2 levels, OR = 1.84; 95% CI = 0.83, 4.09) and modestly associated with grade (grade 3, 2 standard deviation change in MMP2 levels, OR = 1.17; 95% CI = 0.83, 1.63; p-trend for grade = 0.03), but no associations were seen with nodal status or tumor size [23]. As a number of studies using breast tumor tissue [42,39,18,43,40,20,41,44-46] and post-diagnostic blood samples [41,18,47] have observed associations between MMP2 and aggressive tumor phenotypes, associations with pre-diagnostic circulating MMP2 levels warrant further examination in larger studies.

The inverse cross-sectional relationships we observed between plasma MMP2 and both BMI and PMH, factors associated with increased circulating estrogen levels [37,48] suggest a potential inhibitory effect of estrogen on plasma MMP2 levels. However, a similar inverse association was not observed with plasma estradiol among postmenopausal women not using PMH. Given the relatively small number of controls with estradiol measurements, it is possible that we failed to detect a modest association between estradiol and MMP2; however, it is unlikely that a strong association exists. As associations between estradiol and MMP2 have varied in magnitude and direction in prior studies [24,14-16,25-28], it is not entirely surprising that our results do not clearly support an association between estrogen and MMP2. We also observed no evidence of confounding or effect modification by estrogen, as adjustment for plasma estradiol had little impact on estimates, and results did not vary significantly by BMI or PMH use.

Our null findings may reflect the inability of circulating MMP2 levels to adequately capture expression or activity of MMP2 in breast tissue, as suggested by one study that compared tumor expression of MMP2 with circulating active and pro-enzymatic MMP2 levels [49]. A discordance between tumor and circulating MMP2 levels also may explain why associations between post-diagnostic circulating levels of MMP2 and breast cancer prognostic indicators have varied [50-55,43,18,41,47] despite more consistent associations for tumor levels [51,39,56,57,47,43].

There are several limitations to our study. Most women in our study had only a single blood sample, which may have weakened estimates. However, attenuation of estimates is likely to be modest, as MMP2 demonstrated high within-person stability over ten years in our cohort [ICC, 10 years = 0.61 (95% CI: 0.55, 0.67)]. In addition, our assays measured MMP2 concentrations and thus did not distinguish between the active and pro-enzymatic MMP2 forms. It has been speculated that these MMP2 forms, as well as their interactions with MMP2 inhibitors, may be more directly related to breast carcinogenesis than MMP2 concentrations [49]. Also, case numbers were limited to evaluate effect modification and examine associations by tumor characteristics, and therefore suggestive findings from these analyses require replication in studies with larger case numbers. This study has a number of notable strengths. Our measurement of MMP2 prior to diagnosis and prospectively collected covariate information add to the validity of our findings. Importantly, we had two blood specimens collected ten years apart from a subset of women, as well as up to 15 years of follow-up after blood draw, which allowed detailed temporal analyses. In addition, our collection of both plasma MMP2 and estrogens from the same women is unique and made it possible to examine potential interrelationships between MMP2 and estrogen.

In conclusion, our results do not support the utility of plasma MMP2 as a biomarker of breast cancer risk, although MMP2 warrants further study as a potential early indicator of tumor aggressiveness.

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Role of the Sponsor

The funding organizations had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

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Highlights

- In this nested case-control study, pre-diagnostic plasma MMP2 levels were not associated with total invasive breast cancer risk.
- MMP2 levels were positively associated with risk of nodal metastases at diagnosis.
- No associations were observed with other tumor characteristics or with fatal or recurrent cancers.
- MMP2 warrants further study as a potential early indicator for risk of aggressive breast cancer.

Table 1

Age and age-standardized 1990 characteristics according to MMP2 quartile among controls in the Nurses' Health Study (1992 - 2004)[a]

Quartile (ng/mL)	Q1 (<187.9)	Q2 (188.0 – 214.8)	Q3 (214.9 – 246.1)	Q4 (>246.1)	*P*-trend*
No. of controls	284	284	284	284	
Mean (Standard Deviation)					
Age, years	56.3(6.7)	57.6(6.8)	56.3(6.9)	57.3(7.2)	0.32
Body mass index, kg/m^2	26.2(4.1)	25.6(3.6)	24.7(3.5)	24.5(3.3)	<0.0001
Age at menarche, years	12.7(1.2)	12.5(1.4)	12.4(1.3)	12.6(1.2)	0.22
Alcohol intake, g/day	5.2(8.0)	4.9(6.6)	5.7(8.3)	3.7(4.9)	0.30
Parity[b]	3.2(1.3)	3.3(1.2)	3.3(1.2)	3.2(1.2)	0.83
Age at first birth, years[b]	24.8(2.7)	24.5(2.3)	24.5(2.4)	25.5(2.9)	0.02
Plasma estradiol (pg/ml)[c]	8.1(3.3)	7.2(2.8)	7.1(3.5)	7.2(3.8)	0.27
Percentage					
Parous	96.4	91.2	94.9	93.0	0.34
History of benign breast disease	41.9	39.1	32.3	40.6	0.59
First-degree family history of breast cancer	9.2	13.6	6.3	12.6	0.95
Postmenopausal at blood draw	80.9	78.8	73.1	77.2	0.01
Current postmenopausal hormone use[d]	65.4	61.7	50.9	49.0	0.001
Current estrogen alone[d]	29.7	28.3	21.5	24.8	0.06
Current estrogen + progesterone[d]	27.4	25.3	20.3	19.1	0.17

[a] Unless otherwise specified, all variables refer to the time of first blood draw. All factors except age were age-standardized to the age distribution of the controls at first blood draw.

[b] Among parous controls only

[c] Among controls with available hormone data and not using postmenopausal hormones (PMH) at blood draw (N=329). Estradiol was log transformed in tests of linear trend.

[d] Among postmenopausal controls only; percentage of women with current use of estrogen alone and estrogen + progesterone does not sum to total percentage with current PMH use due to the exclusion of controls with other PMH use or with missing data on PMH use

* Calculated from Wald tests with MMP2 modeled as the median level of each quartile

Table 2

ORs and 95% CIs for associations between MMP2 levels and breast cancer risk among women in the Nurses' Health Study, 1992 – 2004

Quartile (ng/mL)	**Q1 (≤187.9)**	**Q2 (188.0 – 214.8)**	**Q3 (214.9 – 246.1)**	**Q4 (>246.1)**	***P*-trend***	***P*-het****
Overall						
Cases/Controls	309/284	264/284	265/284	298/284		
OR (95% CI)[a]	1.0 (ref.)	0.8 (0.7, 1.1)	0.9 (0.7, 1.1)	1.0 (0.7, 1.2)	0.89	
Menopausal status at blood draw						
Premenopausal						
Cases/Controls	63/55	55/51	59/74	72/66		
OR (95% CI)[b]	1.0 (ref.)	0.8 (0.5, 1.5)	0.6 (0.3, 1.0)	0.8 (0.5, 1.4)	0.33	
Postmenopausal						0.63
Cases/Controls	204/191	185/203	179/183	202/196		
OR (95% CI)[b]	1.0 (ref.)	0.8 (0.6, 1.1)	0.9 (0.7, 1.3)	1.0 (0.7, 1.3)	0.96	
Time since blood draw						
<5 years						
Cases/Controls	131/117	99/98	95/96	96/110		
OR (95% CI)[b]	1.0 (ref.)	0.9 (0.6, 1.3)	0.9 (0.6, 1.3)	0.8 (0.6, 1.2)	0.34	
5 - <10 years						
Cases/Controls	115/115	127/135	120/136	149/126		
OR (95% CI)[b]	1.0 (ref.)	0.9 (0.6, 1.3)	0.9 (0.6, 1.3)	1.2 (0.8, 1.7)	0.39	0.73
≥10 years						
Cases/Controls	132/109	70/86	75/77	97/101		
OR (95% CI)[b]	1.0 (ref.)	0.6 (0.4, 0.9)	0.8 (0.5, 1.2)	0.8 (0.5, 1.2)	0.43	
BMI at blood draw						
<25 kg/m²						
Cases/Controls	158/128	126/154	155/179	172/179		
OR (95% CI)[b]	1.0 (ref.)	0.6 (0.4, 0.9)	0.7 (0.5, 1.0)	0.7 (0.5, 1.0)	0.21	
						0.13
≥25 kg/m²						
Cases/Controls	151/156	138/130	110/105	126/105		
OR (95% CI)[b]	1.0 (ref.)	1.1 (0.8, 1.5)	1.0 (0.7, 1.5)	1.1 (0.8, 1.6)	0.52	
PMH use at blood draw[c]						
No current use						
Cases/Controls	74/70	76/87	82/91	116/105		
OR (95% CI)[b]	1.0 (ref.)	0.9 (0.6, 1.4)	0.9 (0.6, 1.4)	1.1 (0.7, 1.7)	0.45	
						0.25
Current use						

Quartile (ng/mL)	**Q1 (≤187.9)**	**Q2 (188.0 – 214.8)**	**Q3 (214.9 – 246.1)**	**Q4 (>246.1)**	***P*-trend***	***P*-het****
Cases/Controls	129/118	109/115	94/92	83/90		
OR (95% CI)[b]	1.0 (ref.)	0.8 (0.6, 1.2)	0.9 (0.6, 1.4)	0.8 (0.5, 1.2)	0.43	

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; MMP, matrix metalloproteinase; BMI, body mass index; PMH, postmenopausal hormone

[a] Conditional logistic regression conditioning on matching factors (age at blood draw, date and time of blood draw, fasting status at blood draw, menopausal status at blood draw, and PMH use at blood draw) and adjusted for BMI at blood draw (continuous), age at menarche (<12 years, 12 years, 13 years, ≥14 years), alcohol consumption in 1990 (continuous), PMH type (current estrogen alone, current estrogen plus progesterone, current other PMH), and age at first birth/parity (nulliparous, 1-4 children with age at first birth <25 years, 1-4 children with age at first birth 25-29 years, 1-4 children with age at first birth ≥30 years, ≥5 children with age at first birth <25 years, ≥5 children with age at first birth ≥25 years), family history of breast cancer (yes/no), and history of benign breast disease (yes/no)

[b] Unconditional logistic regression adjusted for matching factors and above covariates

[c] Among postmenopausal women only

* Calculated from Wald tests with MMP2 modeled as the median level of each quartile

** Calculated from Wald tests with a cross-product term between MMP2 quartile medians and each potential effect modifier

Table 3

ORs and 95% CIs for associations between MMP2 levels and breast tumor characteristics among women in the Nurses' Health Study, 1992 – 2004

Quartile (ng/mL)	Q1 (<187.9)	Q2 (188.0 – 214.8)	Q3 (214.9 – 246.1)	Q4 (>246.1)	*P*-trend*	*P*-het**
Controls[a]	284	284	284	284		
ER/PR status						
ER+/PR+ tumors (667 cases)						
OR (95% CI)[b]	1.0 (ref.)	0.8 (0.6, 1.0)	0.8 (0.7, 1.1)	0.9 (0.7, 1.2)	0.94	
ER+/PR− tumors (137 cases)						
OR (95% CI)[b]	1.0 (ref.)	0.6 (0.3, 1.0)	0.9 (0.5, 1.4)	1.1 (0.7, 1.8)	0.38	0.37
ER−/PR− tumors (163 cases)						
OR (95% CI)[b]	1.0 (ref.)	0.8 (0.6, 1.0)	0.8 (0.7, 1.1)	0.9 (0.7, 1.2)	0.43	
Tumor size						
<2.0 cm (824 cases)						
OR (95% CI)[b]	1.0 (ref.)	0.9 (0.7, 1.2)	0.9 (0.7, 1.2)	1.0 (0.8, 1.3)	0.996	
						0.76
≥2.0 cm (262 cases)						
OR (95% CI)[b]	1.0 (ref.)	0.7 (0.5, 1.1)	0.8 (0.5, 1.1)	0.9 (0.6, 1.3)	0.75	
Tumor grade						
Grade 1 (225 cases)						
OR (95% CI)[b]	1.0 (ref.)	0.7 (0.5, 1.1)	0.8 (0.5, 1.1)	1.0 (0.6, 1.4)	0.98	
Grade 2 (399 cases)						
OR (95% CI)[b]	1.0 (ref.)	0.7 (0.5, 1.0)	0.7 (0.5, 1.0)	0.8 (0.6, 1.1)	0.21	0.39
Grade 3 (244 cases)						
OR (95% CI)[b]	1.0 (ref.)	1.0 (0.6, 1.4)	0.8 (0.6, 1.3)	1.0 (0.7, 1.6)	0.87	
Nodal involvement						
No nodal involvement (752 cases)						
OR (95% CI)[c]	1.0 (ref.)	0.8 (0.6, 1.0)	0.8 (0.6, 1.0)	0.8 (0.6, 1.1)	0.19	
Any nodal involvement (262 cases)						
OR (95% CI)[c]	1.0 (ref.)	1.0 (0.7, 1.5)	1.1 (0.7, 1.7)	1.5 (1.0, 2.2)	0.02	0.002
<4 lymph nodes (188 cases)						
OR (95% CI)[c]	1.0 (ref.)	1.0 (0.6, 1.6)	1.1 (0.7, 1.8)	1.3 (0.9, 2.1)	0.16	0.03
≥4 lymph nodes (74 cases)						
OR (95% CI)[c]	1.0 (ref.)	1.0 (0.5, 2.1)	1.1 (0.5, 2.3)	1.9 (1.0, 3.8)	0.03	0.008
Fatal/recurrent tumors *(218 cases)*						
OR (95% CI)[d]	1.0 (ref.)	0.8 (0.5, 1.2)	0.8 (0.5, 1.3)	0.8 (0.5, 1.3)	0.49	

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; MMP, matrix metalloproteinase; ER/PR, estrogen receptor/progesterone receptor

[a] All controls (n=1136) used in analyses by tumor characteristics

[b] Polytomous logistic regression adjusted for matching factors (age at blood draw, date and time of blood draw, fasting status at blood draw, menopausal status at blood draw, and postmenopausal hormone (PMH) use at blood draw) and BMI at blood draw (continuous), alcohol consumption in 1990 (continuous), PMH type (current estrogen alone, current estrogen plus progesterone, current other PMH), and age at first birth/parity (nulliparous, 1-4 children with age at first birth <25 years, 1-4 children with age at first birth 25-29 years, 1-4 children with age at first birth ≥30 years, ≥5 children with age at first birth <25years, ≥5 children with age at first birth ≥25 years)

[c] Separate polytomous logistic regression models were fit for any vs. no nodal involvement, <4 lymph nodes vs. no nodal involvement, and >4 lymph nodes vs. no nodal involvement; p-het compares test of trend for each lymph node category vs. no lymph nodes

[d] Unconditional logistic regression adjusted for matching factors and the above covariates

[*] Calculated from Wald tests with MMP2 modeled as the median level of each quartile

[**] Calculated from likelihood ratio tests comparing models with separate slopes (based on MMP2 quartile medians) for each outcome category with models constraining the slopes to be the same