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Plasma matrix metalloproteinase 1, 3, and 7 levels and breast cancer risk in the Nurses' Health Study

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Abstract

Purpose—Matrix metalloproteinases (MMPs), in particular MMP1, 3, and 7, are believed to be critical to breast cancer invasion and metastasis and also may have important functions earlier in breast carcinogenesis. However, the relationship between circulating levels of MMP1, 3, and 7 and breast cancer risk is uncertain.

Methods—We examined associations between plasma MMP1, 3, and 7 and breast cancer risk in a prospective case-control study nested within the Nurses' Health Study. Blood samples were collected from 801 cases who developed breast cancer between 1992 and 2000 and 801 matched controls, and MMP levels were measured via immunofluorescence assay.

Results—No overall association was observed between any of these MMPs and breast cancer risk (top vs. bottom quintile; MMP1: odds ratio [OR] = 0.9; 95% confidence interval [CI] = 0.7, 1.3; p-trend = 0.51; MMP3: OR = 1.1; 95% CI = 0.8, 1.5; p-trend = 0.88; MMP7: OR = 1.2; 95% CI = 0.8, 1.7; p-trend = 0.18). Further, findings did not significantly vary by time since blood draw, body mass index, or postmenopausal hormone use, or by breast cancer subtypes.

Conclusions—Circulating MMP1, 3, and 7 levels do not appear to be predictive of overall breast cancer risk.

Keywords

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

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Introduction

Matrix metalloproteinases (MMPs) are a family of over 20 structurally and functionally related transmembrane and secreted zinc-dependent endopeptidases involved in a variety of normal physiologic as well as pathologic processes [1]. MMPs facilitate cancer invasion and metastasis primarily through their ability to degrade the extracellular matrix surrounding tumor cells [2]. Growing evidence suggests that MMPs may have functions earlier in the carcinogenic process [2], and thus the detection of MMPs in circulation might provide evidence of preclinical disease.

Circulating MMP1, 3, and 7 in particular hold promise as potential biomarkers of breast cancer risk, as substantial *in vivo* and *in vitro* evidence supports the involvement of these MMPs not only in tumor spread but also in earlier stages of carcinogenesis [3]. MMP1 is thought to support tumor growth by stimulating tumor cell proliferation [2] and facilitating the release of proangiogenic factors [4, 2, 5]. Evidence suggests that MMP3 and 7 may have both tumor-enhancing and -suppressing functions. MMP7 may promote carcinogenesis by increasing tumor cell survival and decreasing apoptosis [5], and both MMP3 and MMP7 may be involved in tumor initiation [6,7] and the activation of other MMPs [8,7]. However, MMP3 and MMP7 also may have antiangiogenic properties [2,5], and MMP3 may additionally inhibit tumorigenesis via apoptosis [5]. The heightened expression of MMP1 and 7 in breast cancer tissue compared with normal tissue [9,10], associations of MMP1 [11,12] and 7 [13] with adverse breast tumor prognostic factors, and correlations of MMP1 and 3 with breast cancer cell invasiveness [14] suggest that these MMPs have roles specific to breast cancer etiology.

Although biologic evidence exists for a role of MMP1, 3, and 7 in breast carcinogenesis, epidemiologic data on the relationship between circulating levels of these MMPs and breast cancer risk are limited [15–17]. The only prior prospective study examining these MMPs did not observe any associations between levels of these MMPs and breast cancer risk [17]. Additionally, one small retrospective case-control study reported lower levels of plasma MMP1 among breast cancer cases compared with controls [15], and another observed similar levels of plasma MMP3 among women with breast cancer versus fibroadenoma [16]. To further investigate associations between levels of plasma MMP1, 3, and 7 and risk of invasive breast cancer, we performed a prospective analysis in the Nurses' Health Study (NHS) with 10 years of follow-up after blood collection.

Materials and Methods

We conducted a prospective nested case-control study in the NHS, a cohort established in 1976 among 121,700 women. In 1989–1990, blood samples were collected from 32,826 pre- and postmenopausal women in the NHS. Details of the collection have been described previously [18]. Briefly, women arranged to have their blood collected in tubes containing heparin and shipped overnight on ice to our lab, where samples were separated into plasma, red blood cell, and white blood cell components and stored in liquid nitrogen at −130° C or colder. MMP3 and 7 levels remained stable with delayed processing up to 48 hours (Spearman rho = 0.89 for MMP3 and 0.73 for MMP7). Although the correlation between

MMP1 levels for samples processed immediately versus those processed within 48 hours was low (rho = 0.37), MMP1 levels were highly correlated for a processing delay of 24 hours (rho = 0.85), the time within which >95% of samples in our analysis were processed. We achieved a follow-up rate of 99% through 2000 among participants in the blood substudy [19]. The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

Participants in the NHS blood collection were free of cancer at the time of blood draw and followed for incident invasive breast cancer from blood draw until May 31, 2000, with the first 2 years of follow-up after blood collection excluded to preserve sample volume and to reduce the possibility that MMP levels might reflect the presence of subclinical disease. During the follow-up period, 801 breast cancer cases (548 postmenopausal, 169 premenopausal, 84 dubious/missing menopausal status) were reported by participants on biennial questionnaires. Cases were confirmed via medical record review and matched 1:1 to controls on month (±3 months) and time of day (±2 hours) of blood collection, age (±2 years), fasting status (<8 or unknown, >=8 hours), postmenopausal hormone (PMH) use (current or not), and menopausal status (pre-, postmenopausal, unknown) at blood collection.

Laboratory Analyses

Plasma samples were assayed for concentrations of MMP1, 3, and 7 in a single batch at the Natural and Medical Sciences Institute at the University of Tuebingen (Reutlingen, Germany) using the Luminex Fluorokine Multianalyte Profiling Kit (R&D Systems, Minneapolis, MN, USA). Case and control pairs were assayed together but in random order to mask the laboratory to case-control status. Overall coefficients of variation (CVs) measured via blinded split quality control samples ranged from 10% (MMP3) to 16% (MMP7), and intra-batch CVs ranged from 7% (MMP3) to 10% (MMP7). Intraclass correlation coefficients (ICCs) for within-person stability over 2–3 years ranged from 0.52 (MMP3) to 0.91 (MMP1) [20].

Outliers were detected using the extreme studentized deviate many-outlier procedure, and case-control pairs in which either the case or control had an outlying MMP value were excluded from analyses of that MMP [21]. This resulted in the removal of 5 case-control pairs from MMP1 analyses, 9 case-control pairs from MMP3 analyses, and 1 case-control pair from MMP7 analyses. We used multivariate conditional logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (95% CIs) for associations between quintiles of MMP1, 3, and 7 concentration and breast cancer risk, with quintile cutpoints based on the distribution of each MMP among controls and the lowest quintile used as the reference. Wald tests for linear trend were performed treating the median of each quintile as a continuous variable. Effect modification by time since blood draw (<5 years, ≥5 years), body mass index (BMI) (<25 kg/m^2, ≥25 kg/m^2), and postmenopausal hormone (PMH) use (current use, no current use) was assessed in stratified analyses using unconditional logistic regression with adjustment for matching factors and via Wald tests comparing linear trends between levels of each potential effect modifier. Additional cutpoints for time since blood draw and BMI were examined in sensitivity analyses. Polytomous logistic regression was

performed to evaluate whether associations varied by breast cancer subtypes (estrogen receptor (ER), progesterone receptor (PR), HER2/neu, and nodal status; ductal or lobular histology; tumor size; and grade). All analyses were adjusted for the following established or suspected breast cancer risk factors: body mass index at blood draw (BMI), age at menarche, current alcohol consumption, postmenopausal hormone (PMH) use, age at first birth/parity, family history of breast cancer, and history of benign breast disease. Covariates were measured either on a supplemental questionnaire administered at blood draw or on the 1990 main NHS study questionnaire to capture information close to the time of blood draw. We used SAS 9.3 (SAS Institute, Cary, NC), with all tests being two-sided and $p<0.05$ indicating statistical significance.

Results

Cases and controls ranged in age from 42 to 70 years, and 69% were postmenopausal at blood draw. Compared with controls, cases had a younger age at menarche and a higher prevalence of benign breast disease and family history of breast cancer (Table 1). MMP1 and MMP7 levels varied by age and menopausal status, and MMP7 additionally varied by current PMH use; both MMPs were generally unrelated to other breast cancer risk factors (Supplementary Table 1). MMP3 was not significantly associated with standard breast cancer risk factors.

No overall associations were observed with breast cancer risk for MMP1 (top vs. bottom quintile; OR = 0.9; 95% CI = 0.7, 1.3; p-trend=0.51), MMP3 (top vs. bottom quintile; OR = 1.1; 95% CI = 0.8, 1.5; p-trend=0.88), or MMP7 (top vs. bottom quintile, OR = 1.2; 95% CI = 0.8, 1.7; p-trend = 0.18) in multivariate models (Table 2). Estimates were similar in analyses limited to women who were postmenopausal at blood collection or at diagnosis (data not shown). Results did not vary significantly by time since blood draw, BMI, or current PMH use (all interaction p-values ≥0.21). No significant associations were observed by ER status (p-heterogeneity = 0.89 for MMP1, 0.64 for MMP3, and 0.50 for MMP7) (Table 2) or by any of the other breast cancer subtypes examined. However, non-significant positive associations with nodal metastases were observed for MMP3 (top vs. bottom quintile, OR = 1.4; 95% CI = 0.8, 2.5 for node positive tumors; OR = 0.9; 95% CI: = 0.6, 1.3 for node negative tumors; p-heterogeneity = 0.11) and MMP7 (top vs. bottom quintile, OR = 1.5; 95% CI = 0.9, 2.7 for node positive tumors; OR = 1.1; 95% CI = 0.8, 1.6 for node negative tumors; p-heterogeneity = 0.24) (Table 3). In addition, associations for MMP1 appeared to vary somewhat by ductal or lobular histology (top vs. bottom quintile, OR = 1.4; 95% CI = 0.8, 2.7 for lobular tumors; OR = 0.7; 95% CI: = 0.6, 1.1 for ductal tumors; p-heterogeneity= 0.08).

Discussion

In this nested case-control study, we did not observe any significant associations between plasma MMP1, 3, and 7 levels and overall breast cancer risk. Further, there were no significant associations of these MMPs with breast tumor subtypes, although a suggestive positive association with nodal metastases was observed for both MMP3 and MMP7. Associations between MMPs and breast cancer did not vary by time since blood draw or by

BMI or PMH use at blood draw, and results also were similar in analyses limited to postmenopausal women.

Consistent with our results, no significant associations were observed between pre-diagnostic plasma levels of MMP1, 3, and 7 and breast cancer risk in a nested case-control study of similar size within the Multiethnic Cohort (MEC), the only prior prospective cohort study of circulating MMPs and breast cancer risk [17]. While we hypothesized that the timing of MMP measurement might be important given the potentially changing roles of these MMPs throughout carcinogenesis [5], no associations were observed in either study in analyses stratified by time since blood draw. The MEC study reported significant positive associations between MMP1 and breast cancer risk among women with BMI ≥30 kg/m^2 and among those using PMH at blood draw, indicating that MMP1 may exert an influence on tumorigenesis only in a high estrogen environment. However, these results were based on small numbers of cases and were not replicated in our study.

Additional epidemiologic data on the relationship between circulating levels of MMP1, 3, and 7 and breast cancer risk are limited and come from small retrospective case-control studies with post-diagnostic MMP measurements. One study reported no difference in plasma levels of MMP3 among 50 women with breast cancer compared with 30 women with fibroadenoma [16], supporting our null results for MMP3. In contrast to our results, another study (n=208 cases) observed lower levels of plasma MMP1 among cases (2.01 ng/ml) compared with controls (3.45 ng/ml) [15]. To our knowledge, no other epidemiologic studies have assessed circulating MMP7 levels in relation to breast cancer risk.

Substantial biologic evidence exists for functions of MMP1, 3, and 7 in tumor growth [3,2,4,5] and potentially initiation [6,7], with evidence from breast cell lines [14,12,9,11,13,10] supporting breast cancer-specific roles of these MMPs. However, the extent to which these MMPs might be upregulated prior to the development of clinically detectable breast cancer is unclear; our lack of association for MMP1, 3, and 7 suggests that these MMPs may be primarily produced once tumors have acquired invasive potential or that tumor production of these MMPs is not reflected in circulating levels. The potentially opposing roles of MMP3 and 7 in promoting and suppressing tumor development [2,5] also may explain why no associations were observed for these MMPs in either our analysis or the MEC study. Although we are not aware of any studies that have assessed the correlation between levels of MMP1, 3, or 7 in tissue and circulation, inverse correlations between breast tumor and circulating MMP2 levels reported in one study [22] suggest that associations between circulating MMP levels and breast cancer risk may not adequately reflect associations with tissue levels. It has also been suggested that MMP activity may be more relevant to breast carcinogenesis than MMP concentrations [22], which represent a combination of the latent pro-enzyme and biologically active MMP forms.

While no associations were observed between MMP1, 3, and 7 and overall breast cancer risk in either our study or the MEC analysis, results from tumor subtype analyses in both studies suggest that these MMPs may predict the risk of more aggressive tumors. In the MEC study, significantly or suggestively higher levels of MMP1, 3, and 7 were observed among women with distant metastases [17]. While we were unable to assess associations with distant

metastases, MMP3 and MMP7 levels were suggestively higher among women with nodal metastases in our study. Although these associations with distant and nodal metastases were based on small numbers, it is plausible that these MMPs might serve as early indicators of tumor aggressiveness given the strong evidence for a role of these MMPs in tumor invasion and metastasis. MMP1 in breast tissue has been associated with larger tumor size, higher grade, and worse overall survival [11], and greater MMP7 tissue expression has been observed among breast cancer patients with shorter relapse-free survival and greater risk of distant metastases [13]. Polymorphisms in MMP3 genes have been associated with risk of lymph node spread [23], although inverse associations between MMP3 and axillary node metastases also have been reported [24]. Further epidemiologic studies with larger case numbers are needed to better understand whether increased circulating levels of these MMPs might signal the early development of tumors with greater metastatic potential. While we also observed a suggestive positive association between MMP1 and lobular tumors, there is no clear biologic explanation for this association. This may be a chance finding given the small number of lobular tumors in our analyses, but this potential differential association by tumor histology requires confirmation in analyses better powered to examine tumor subtypes.

Our study has a number of strengths and limitations. Our measurement of MMPs prior to diagnosis and prospectively collected covariate information add to the validity of our findings. With 10 years of follow-up after blood draw, we were able to conduct detailed assessments by time since blood draw. However, both some laboratory error in measurements (overall CVs 10–16%) and having only a single blood sample per subject (ICC over 2–3 years: 0.52 – 0.91) may have attenuated our estimates. In addition, power was limited to assess potential effect modification and associations with breast tumor subtypes.

In conclusion, our results do not provide evidence that circulating MMP1, 3, and 7 are associated importantly with breast cancer risk, although these MMPs warrant further study as potential early indicators of tumor aggressiveness.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of cases and their matched controls in the Nurses' Health Study (1992 – 2000) at blood collection

	Cases	Controls
No. of participants	801	801
Median (5^{th} – 95^{th} percentile)		
Age, years[a]	57.3 (45.8 – 67.5)	57.3 (45.6 – 67.3)
Body mass index, kg/m^2	24.4 (19.8 – 34.1)	24.4 (19.6 – 33.3)
Age at menarche, years	12 (10 – 15)	13 (11 – 15)
Alcohol intake, g/day	1.5 (0 – 27.5)	1.8 (0 – 20.6)
Parity (parous only)	3 (1 – 6)	3 (1 – 6)
Age at first birth, years (parous only)	24 (21 – 32)	24 (21 – 31)
Percentage (%)		
Parous	90.6	94.4
History of benign breast disease	43.9	36.6
First degree family history of breast cancer	16.2	10.5
Current postmenopausal hormone use[a]	45.8	44.9
Postmenopausal[a]	68.4	68.8

[a] Indicates matching factor

Table 2

ORs and 95% CIs for associations between plasma MMP1, 3, and 7 quintile and breast cancer risk among women in the Nurses' Health Study, 1992 – 2000

	Quintile					*P*-value, test for trend
	1	**2**	**3**	**4**	**5**	
MMP1, ng/mL	≤0.88	0.89 – 1.46	1.47 – 2.12	2.13 – 3.34	>3.34	
All breast cancer cases (796 cases, 796 controls)						
Cases/Controls	173/161	176/159	140/158	160/160	147/158	
Simple OR (95% CI)a	1.0 (ref.)	1.0 (0.8, 1.4)	0.8 (0.6, 1.1)	0.9 (0.7, 1.3)	0.9 (0.6, 1.2)	0.28
Multivariate OR (95% CI)b	1.0 (ref.)	1.1 (0.8, 1.5)	0.9 (0.6, 1.2)	1.0 (0.7, 1.3)	0.9 (0.7, 1.3)	0.51
ER+ tumors * (585 ER+ cases, 796 controls)						
Multivariate OR (95% CI)[b]	1.0 (ref.)	1.1 (0.8, 1.5)	0.9 (0.6, 1.2)	0.9 (0.6, 1.2)	0.9 (0.6, 1.3)	0.31
ER− tumors* (161 ER− cases, 796 controls)						
Multivariate OR (95% CI)[b]	1.0 (ref.)	1.0 (0.6, 1.8)	0.8 (0.5, 1.5)	1.0 (0.6, 1.6)	0.9 (0.5, 1.5)	0.61
MMP3, ng/mL	≤7.95	7.96 – 9.70	9.71 – 11.57	11.58 – 14.03	>14.03	
All breast cancer cases (792 cases, 792 controls)						
Cases/Controls	161/159	155/158	169/159	144/158	163/158	
Simple OR (95% CI)[a]	1.0 (ref.)	1.0 (0.7, 1.3)	1.1 (0.8, 1.4)	0.9 (0.7, 1.2)	1.0 (0.8, 1.4)	0.95
Multivariate OR (95% CI)[b]	1.0 (ref.)	1.0 (0.7, 1.3)	1.1 (0.8, 1.5)	0.9 (0.7, 1.3)	1.1 (0.8, 1.5)	0.88
ER+ tumors * (581 ER+ cases, 792 controls)						
Multivariate OR (95% CI)[b]	1.0 (ref.)	1.0 (0.7, 1.3)	1.0 (0.7, 1.4)	0.9 (0.6, 1.2)	1.1 (0.8, 1.6)	0.75
ER− tumors* (160 ER− cases, 792 controls)						
Multivariate OR (95% CI)[b]	1.0 (ref.)	1.3 (0.8, 2.3)	1.4 (0.8, 2.5)	1.3 (0.7, 2.3)	1.2 (0.7, 2.2)	0.50
MMP7, ng/mL	≤0.64	0.65 – 0.82	0.83 – 1.01	1.02 – 1.29	>1.29	
All breast cancer cases (800 cases, 800 controls)						
Cases/Controls	137/161	159/166	164/159	182/155	158/159	
Simple OR (95% CI)a	1.0 (ref.)	1.1 (0.8, 1.6)	1.2 (0.9, 1.7)	1.4 (1.0, 2.0)	1.2 (0.9, 1.7)	0.14
Multivariate OR (95% CI)[b]	1.0 (ref.)	1.1 (0.8, 1.6)	1.2 (0.9, 1.7)	1.4 (1.0, 2.0)	1.2 (0.8, 1.7)	0.18
ER+ tumors * (586 ER+ cases, 800 controls)						

	Quintile					*P*-value, test for trend
	1	**2**	**3**	**4**	**5**	
Multivariate OR (95% CI)[b]	1.0 (ref.)	1.2 (0.8, 1.6)	1.2 (0.9, 1.7)	1.3 (0.9, 1.8)	1.1 (0.8, 1.6)	0.40
ER− tumors [*] (163 ER− cases, 800 controls)						
Multivariate OR (95% CI)[b]	1.0 (ref.)	0.9 (0.5, 1.5)	1.3 (0.8, 2.3)	1.4 (0.8, 2.4)	1.2 (0.7, 2.2)	0.22

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; MMP, matrix metalloproteinase

[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 postmenopausal hormone use at blood draw)

[b] Multivariate polytomous logistic regression adjusting for matching factors and body mass index at blood draw (continuous), age at menarche (<12 years, 12 years, 13 years, ≥14 years), alcohol consumption in 1990 (continuous), postmenopausal hormone use (premenopausal, postmenopausal never use, past use, current use <5 years, current use ≥5 years), 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), family history of breast cancer (yes/no), and history of benign breast disease (yes/no)

[*] p-heterogeneity, ER+ vs. ER− breast cancer; MMP1: 0.89, MMP3: 0.64, MMP7: 0.50

Table 3

ORs and 95% CIs for associations between plasma MMP1, 3, and 7 quintile and breast cancer tumor size and lymph node involvement among women in the Nurses' Health Study, 1992 – 2000

	Quintile 1	2	3	4	5	*P*-value, test for trend	*P*-heterogeneity
MMP1, ng/mL	≤0.88	0.89 –1.46	1.47 –2.12	2.13 –3.34	>3.34		
Tumor size							
≤2.0 cm (572 cases, 796 controls)							
Multivariate OR (95% CI)[a]	1.0 (ref.)	1.0 (0.7, 1.4)	0.8 (0.6, 1.2)	0.9 (0.6, 1.2)	0.9 (0.6, 1.3)	0.39	
							0.89
>2.0 cm (186 cases, 796 controls)							
Multivariate OR (95% CI)[a]	1.0 (ref.)	1.1 (0.7, 1.8)	1.0 (0.6, 1.6)	0.9 (0.5, 1.5)	0.9 (0.5, 1.5)	0.47	
Lymph node involvement							
No lymph nodes (524 cases, 796 controls)							
Multivariate OR (95% CI)[a]	1.0 (ref.)	1.1 (0.8, 1.6)	0.8 (0.6, 1.2)	0.9 (0.6, 1.3)	0.8 (0.6, 1.2)	0.19	
							0.37
Any lymph nodes (183 cases, 796 controls)							
Multivariate OR (95% CI)[a]	1.0 (ref.)	0.9 (0.5, 1.5)	0.9 (0.5, 1.5)	0.8 (0.5, 1.4)	1.1 (0.6, 1.8)	0.97	
MMP3, ng/mL	≤7.95	7.96 – 9.70	9.71 – 11.57	11.58 – 14.03	>14.03		
Tumor size							
≤2.0 cm (567 cases, 792 controls)							
Multivariate OR (95% CI)[a]	1.0 (ref.)	0.9 (0.7, 1.3)	1.0 (0.7, 1.4)	0.9 (0.6, 1.3)	1.0 (0.7, 1.4)	0.97	
							0.74
>2.0 cm (186 cases, 792 controls)							
Multivariate OR (95% CI)[a]	1.0 (ref.)	1.1 (0.7, 1.9)	1.7 (1.0, 2.7)	1.0 (0.6, 1.7)	1.2 (0.7, 2.0)	0.71	
Lymph node involvement							
No lymph nodes (518 cases, 792 controls)							
Multivariate OR (95% CI)[a]	1.0 (ref.)	0.9 (0.6, 1.3)	1.0 (0.7, 1.4)	0.8 (0.6, 1.2)	0.9 (0.6, 1.3)	0.52	
							0.11
Any lymph nodes (185 cases, 792 controls)							

	Quintile 1	2	3	4	5	*P*-value, test for trend	*P*-heterogeneity
Multivariate OR (95% CI)[a]	1.0 (ref.)	1.1 (0.7, 2.0)	1.7 (1.0, 2.8)	1.1 (0.6, 2.0)	1.4 (0.8, 2.5)	0.23	
MMP7, ng/mL	≤0.64	0.65 – 0.82	0.83 – 1.01	1.02 – 1.29	>1.29		
Tumor size							
≤2.0 cm (575 cases, 800 controls)							
Multivariate OR (95% CI)[a]	1.0 (ref.)	1.2 (0.9, 1.8)	1.3 (0.9, 1.9)	1.4 (1.0, 2.0)	1.3 (0.9, 1.9)	0.10	
							0.32
>2.0 cm (186 cases, 800 controls)							
Multivariate OR (95% CI)[a]	1.0 (ref.)	1.0 (0.6, 1.6)	1.2 (0.7, 2.0)	1.2 (0.7, 2.0)	0.9 (0.5, 1.6)	0.93	
Lymph node involvement							
No lymph nodes (527 cases, 800 controls)							
Multivariate OR (95% CI)[a]	1.0 (ref.)	1.2 (0.8, 1.7)	1.2 (0.8, 1.7)	1.3 (0.9, 1.9)	1.1 (0.8, 1.6)	0.52	
							0.24
Any lymph nodes (184 cases, 800 controls)							
Multivariate OR (95% CI)[a]	1.0 (ref.)	1.3 (0.7, 2.2)	1.7 (1.0, 2.9)	1.6 (0.9, 2.7)	1.5 (0.9, 2.7)	0.10	

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; MMP, matrix metalloproteinase

[a]Multivariate polytomous logistic regression adjusting for matching factors and body mass index at blood draw (continuous), age at menarche (<12 years, 12 years, 13 years, ≥14 years), alcohol consumption in 1990 (continuous), postmenopausal hormone use (premenopausal, postmenopausal never use, past use, current use <5 years, current use ≥5 years), 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)