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Plasma Matrix Metalloproteinases and Postmenopausal Breast Cancer Risk: A Nested Case-Control Study in the Multiethnic Cohort Study

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Abstract

The survival of malignant breast cells depends upon remodeling of the extracellular matrix, including complex interactions with matrix metalloproteinases (MMPs). It has been hypothesized that circulating MMPs may serve as early indicators of breast cancer development in hospital-based case-control studies. A nested case-control study of the association of pre-diagnostic plasma levels of MMPs with the subsequent risk of postmenopausal breast cancer was conducted within the Multiethnic Cohort. During the follow-up period, 713 women with incident invasive breast cancer were identified and individually (1:1) matched to controls. Four types of MMPs (1, 2, 3, and 7) were analyzed by microsphere immunofluorescence assay. Mean plasma levels of MMPs did not differ significantly between cases and controls; nor were there differences in breast cancer risk by MMP level. No difference in the risk of breast cancer by plasma level of the MMPs was found within strata of age, or ethnicity, although MMP-1 levels were positively associated with breast cancer risk in obese women and women using hormone replacement medications (*P* Values for interaction < 0.05). Few significant differences in risk by levels of the MMPs were found by any of the clinical variables. Circulating MMPs were not associated with postmenopausal breast cancer risk.

Keywords

Breast cancer; matrix metalloproteinases; body mass index; hormone replacement therapy; neoplasm staging; nested case-control study

Breast cancer is the most common malignancy among women worldwide [1], but there are presently no validated, clinically useful circulating biomarkers for breast cancer [2].

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Ethical Standards

The study was approved by the institutional review boards of the University of Hawaii and the University of Southern California.

Conflict of Interest

The authors declare that they have no conflict of interests.

Substantial interest has been generated in recent years regarding dysregulation of the extracellular matrix (ECM) as a critical component of malignant transformation and progression. Matrix metalloproteinases (MMPs) are a family of 23 known human enzymes capable of degrading essentially all macromolecules of the ECM [3, 4]. The importance of MMPs to cellular differentiation, proliferation, apoptosis, growth factor availability, tissue repair and remodeling is increasingly recognized [5]. MMPs have been linked to human disease development, chronic inflammation, and neurological disorders [3]. MMPs are more highly expressed in breast cancer tissue than in benign breast tissue [6–9]. Moreover, higher MMPs in serum or plasma have been associated with breast cancer risk [10, 11] and poor prognosis [12–18]. However, no prospective studies or randomized trials have examined the role of circulating MMPs in the etiology of breast cancer.

We conducted a nested case-control study among women who contributed to the biospecimen repository of the Multiethnic Cohort study (MEC) to examine whether pre-diagnostic levels of MMPs were associated with the risk of postmenopausal breast cancer. MMPs can be divided into four groups based on domain structure and substrate specificity of the enzyme [5]. We selected one MMP in each group, among those which were suggested to be related with breast cancer in previous literatures [10–20]. MMPs analyzed in this study were, the collagenase MMP-1, the gelatinase MMP-2, the stromelysin MMP-3, and the matrilysin MMP-7. We examined whether the associations of circulating MMPs with postmenopausal breast cancer incidence were independent of known risk factors for the disease and compared the relation of circulating MMPs levels to breast cancer risk within strata of clinical characteristics.

MATERIALS AND METHODS

Study population and data collection

A nested case-control study of postmenopausal breast cancer was conducted within the MEC, a prospective study established in Hawaii and Los Angeles between 1993 and 1996 [21]. More than 215,000 adults, ages 45 to 75 years, from five racial-ethnic groups (African-Americans, Native Hawaiians, Japanese-Americans, Latinos, and Whites), were enrolled in the cohort through completion of a detailed self-administered questionnaire regarding diet, lifestyle factors, and other potential disease determinants. A prospective biospecimen repository was developed during the follow-up period, largely between 2001 and 2006 among cohort members who agreed to provide a blood and urine specimen, along with a short interview form. Biospecimens were prospectively collected from 36,458 women. Blood samples were drawn and were processed within 4 hours of collection. Serum and plasma were processed to cryovials and stored at the vapor phase of liquid nitrogen (−186°C). About 95% of the participants contributing to the biorepository provided fasting (≥8 hours) blood samples. The MEC and the nested biospecimen study were approved by the Institutional Review Boards of the University of Hawaii and the University of Southern California.

Case ascertainment and control selection

Identification of incident, invasive breast cancer cases was accomplished through linkage to the population-based cancer registries covering Hawaii and California, participants in the Surveillance, Epidemiology, and End Results Program (SEER) of the National Cancer Institute in the United States and the National Program for Cancer Registries of the Centers for Disease Control and Prevention [22]. Breast cancer diagnoses were identified using the International Classification of Diseases for Oncology, Third Edition codes C50.0–C50.9 and were restricted to invasive malignancies [23]. Deaths were identified through linkage to death certificate files in Hawaii and California, as well as to the National Death Index.

Incident breast cancer cases were verified through October, 2010, including 729 eligible postmenopausal women with a diagnosis of invasive breast cancer. Median follow-up from the date of blood draw to the date of breast cancer diagnosis was 4 years. One control per case was randomly selected from the pool of postmenopausal women in the biospecimen repository who were alive and free of breast cancer at the age of the case's diagnosis and were matched to the case within strata of geographic location (Hawaii or California), race-ethnicity, birth year (±1 y), date of blood draw (±6 mo), time of blood draw (±2 h), hours fasting prior to blood draw (0–<6, 6–<8, 8–<10, and ≥10 h), and hormone replacement therapy use (HRT; as current versus not current) at the date blood was drawn. HRT use and fasting status were used as matching criteria to ensure that matched sets would be available for the assessment of analytes requiring fasting status or non-HRT use. We excluded 16 matched sets where either the case or control had MMP measurements below the limits of detection, leaving 713 matched pairs for statistical analysis.

Laboratory assays

Frozen heparin plasma samples were retrieved from the MEC biorepository for matched case-control sets. Laboratory personnel who thawed and analyzed the matched plasma were blinded to case-control status. Plasma and quality control samples were thawed immediately prior to use and assayed in duplicate after a 10-fold dilution. Concentrations of matrix metalloproteinases collagenase 1 (MMP-1), gelatinase A (MMP-2), stromelysin 1 (MMP-3) and matrilysin (MMP-7) were assayed in 50 uL diluted (1:10) plasma employing a microsphere immunofluorescence assay using fluorokine MAP multiplex kits which were commercially available (R&D Fluorokine® MAP, Minneapolis, MN, USA). These kits measured levels of complex form of pro-, mature and tissue inhibitor of MMP-1 (TIMP-1) for MMP-1, MMP-3, and MMP-7; and measured levels of pro- and mature complex of MMP-2. Fluorescent intensities were obtained with a dual-laser analyzer (Luminex® 200TM), and median fluorescence values were quantified against a standard curve using GraphPad Prism 5 software. Multiplexed analyses were performed according to the manufacturers' instructions as previously described [24, 25]. Assays were conducted under yellow light to avoid sample and reagent degradation. Based on 47 duplicate and 23 triplet samples, between-batch coefficients of variation were 9.9% for MMP-1, 5.4% for MMP-2 and MMP-3, and 11.7% for MMP-7, and within-batch variation ranged from 3.7% to 7.9% for all analytes.

Statistical analyses

A preliminary examination of the data included comparisons of cases and controls with respect to several demographic characteristics and potential risk factors of interest. Geometric means of MMP levels were compared between cases and controls by the paired t-test. Pearson correlation coefficients were used to examine interrelations between the log-transformed plasma MMP levels. Conditional logistic regression of breast cancer incidence, with matched sets as strata, was used to explore the relationship with plasma MMPs. MMPs were modeled both as log continuous variables and as indicator variables representing quartiles based on the distribution of MMP levels among controls. Odds ratios (OR) and 95% confidence intervals (CI) were computed for a change in two standard deviations from the former model and for quartile categories from the latter model. Linear trends were evaluated by a Wald test of the parameter estimate for the log-transformed continuous variables.

Association with known/suggested risk factors for breast cancer and breast cancer risk were examined. The variables considered were, education level (≤12, >12 years, missing), body mass index (BMI) (<25.0, 25.0–29.9, ≥30.0 kg/m^2), tobacco smoking (never, ever, missing), alcohol drinking (no, yes, missing), family history of breast cancer (no, yes, missing), age at

menarche (≤12, >12 years, missing), age at menopause (<45, 45–49, ≥50 years, missing), and number of live births (never, 1, 2–3, ≥4, missing). Information on education, smoking, alcohol drinking, age at menarche, and number of live births were collected at the time upon cohort entry. Information on menopause, menopausal age, BMI and family history of breast cancer were updated at the time of blood collection. Based on the results, a fully adjusted statistical model was built by inclusion of potential confounders for postmenopausal breast cancer risk, which were BMI, number of live births, and family history of breast cancer. Separate estimates were created for subgroups defined by age, race/ethnicity, BMI, and HRT use at blood draw. Tests for interaction were based on the Wald statistic for cross-product terms between the corresponding variable and the logarithmic transformed MMP level. Unconditional logistic regression with adjustment for the matching variables was employed for statistical analyses in which the matched sets were broken.

Unconditional polychotomous logistic regression models, using all controls as a reference, were created to evaluate the homogeneity of the association of breast cancer with plasma MMP levels by clinical parameters, such as SEER stage, tumor size, axillary node status, grade, and estrogen and/or progesterone receptor status. Cases with clinical parameters that were unreported, unknown, or borderline for receptor status were excluded from this analysis. The test of heterogeneity from polychotomous logistic regression models were calculated using a two-sided likelihood ratio test.

All tests were two-sided and $P < 0.05$ was considered statistically significant. Analyses were conducted using SAS version 9.2 statistical software (SAS Institute, Inc., Cary, North Carolina).

RESULTS

The distribution of breast cancer cases and controls by matching variables, including age, ethnicity, and the use of hormone replacement therapy is shown in Table 1. Cases were less likely than were controls to have had ≥ 4 births; whereas controls were less likely than were cases to be overweight and obese or to have a family history of breast cancer. There were no significant differences between cases and controls in other potential risk factors for postmenopausal breast cancer.

Among cases and controls, Pearson correlations of the log-transformed MMPs ranged from 0.10 (MMP-1 and MMP-2; MMP-1 and MMP-3) to 0.34 (MMP-2 and MMP-7) (*P* Values <0.01 for all combinations, data not shown). No significant differences in the geometric mean plasma levels of any of the MMPs were found between breast cancer cases and controls (Table 2). The ORs for breast cancer were not significant and close to one whether we examined individual MMPs as continuous (log-transformed) variables or as quartiles. MMPs were not associated with breast cancer risk when the analyses were restricted to subjects followed within 2, 3, 4 or 5 years (data not shown).

The OR for the association of MMP-1 with breast cancer increased with higher BMI in stratified models (*P* Value for interaction = 0.03) (Table 3). A significant positive association of plasma MMP-1 and breast cancer risk was found among case-control pairs of HRT users, but not among pairs who were not HRT users (*P* Value for interaction = 0.04).

The plasma concentrations of MMP-1 were higher among women with metastatic breast cancer than among controls (OR: 2.62, 95% CI: 1.06–6.51, *P* Value for heterogeneity = 0.01), but this was based on only 20 cases (Table 4). Higher MMP-2, MMP-3, and MMP-7 were related with increased risks for distant metastatic breast cancer, but the associations were not statistically significant. The expression level of MMP-2 was associated with the highest grade of breast cancer (*P* Value for heterogeneity = 0.03).

DISCUSSION

Results from this nested case-control study within the Multiethnic Cohort study do not support an association of pre-diagnostic plasma levels of MMPs-1, -2, -3, or -7 with postmenopausal breast cancer risk. Only a few other studies have examined the association between MMPs and postmenopausal breast cancer risk, and these have been small, hospital-based, and retrospective in nature [10, 11, 15]. Serum levels of MMP-2 and MMP-9 had been found to be significantly higher among 60 women with breast cancer than among 40 women with benign breast disease or 60 normal 'healthy' women [10]. These results were consistent with those from an earlier study in which plasma levels of MMP-2 and MMP-9 were significantly higher in 88 breast cancer cases compared to 150 women with benign breast diseases or 107 healthy controls [11]. In a third study, serum levels of MMP-9 and TIMP-1 were significantly higher among 60 breast cancer cases compared to 18 women with benign breast diseases or 15 healthy controls [15]. It is possible that the positive results found in previous studies resulted from over-selection of advanced cases: advanced cancer cases comprised 25% (Stage III–IV) of the total in one study [15] and 54% (T4) in a second study [10], which is a higher proportion compared to that in this current study (3%). Information regarding stage among cases was not available from a third study [11].

MMPs are postulated to promote malignant invasion through degradation of the basement membrane and the interstitial connective tissue of the ECM [19]. MMP-1, the first identified matrix metalloproteinase, has been evaluated in relation to a variety of diseases because of its broad substrate specificity and its importance to the turnover of the extracellular matrix [3]. MMP-1 expression in epithelial and stromal tissues was found to be higher in breast cancer tissue than in benign breast tissue [9]. Moreover, MMP-1 expression appears elevated in the surrounding stromal cells of women with various molecular subtypes of breast cancer [6]. MMP-2, a gelatinase capable of degrading collagen and elastin, has chemotactic properties that assist in the regulation of inflammatory mediators, such as IL-1β, that may be involved in breast carcinogenesis [3]. Moreover, MMP-2, but not MMP-9 which also belongs to gelatinase, targets fibroblast growth factor receptors, degrades collagen in vascular basal membranes, and modulates mitogenic and angiogenic activities of fibroblast growth factor [3, 19, 20]. MMP-3 or stromelysin-1, degrades ECM proteins, facilitates mammary tissue involution in mice after lactation, and may enhance tumor invasiveness through shedding of E-cadherin [3, 26]. MMP-7, a matrilysin lacking the C-terminal hemopexin-like domain, has a role in human microbial defense through regulatory mechanisms within the innate and mucosal immune pathways [27]. MMP-7 is one of only a few metalloproteinases shown to be produced by tumor cells [28]. Cellular proliferation is induced by MMP-7 through a variety of pathways, including regulation of insulin-like growth factor levels via cleavage of insulin-like growth factor binding proteins [29]. Alteration of cell signaling through 'ectodomain shedding' or the proteolytic degradation of transmembrane molecules may facilitate tumor growth and metastasis by several types of MMPs [30].

Recent interest has emerged in a potential role for MMPs as blood-based or tissue-based prognostic tools for breast cancer and other malignancies [7, 8, 12, 14–16, 31–35]. Some breast cancer phenotypes appear to acquire the ability to co-opt MMP vascular remodeling functions to facilitate angiogenesis and lung metastasis [36]. Consistent with these tissue-based studies, a few retrospective investigations of breast cancer have reported significant associations of higher serologic concentrations of MMP-1 and MMP-2 with advanced stage and the presence of lymph node metastasis, higher grade, and reduced relapse-free survival [10]. In our study, although number of distant metastatic cases were small and/or the association was not statistically significant, higher MMPs (1,2,3 and 7) were related with higher risks of distant metastatic breast cancer. Nevertheless, the lack of an association of

any of the MMPs with the clinical variables for the cases should not be surprising considering the complex biological activity of the MMPs, their inhibitors, and their receptors. It is possible that specific forms of MMPs (pro-, mature or TIMP-1), not a complex form of MMPs, is associated with more aggressive types of breast cancer [12, 33].

Plasma MMP levels were modestly correlated in the controls, consistent with results from 1,678 participants in the Atherosclerosis Risk in Communities cohort (ARIC) [37]. Nonetheless, circulating levels of MMPs may be sensitive to a variety of host and environment characteristics, including genetics, BMI, oxidative stress, and tobacco smoke exposure [37, 38]. Correlates of plasma MMPs-1, -2, -3, and -7 among participants in the Atherosclerosis Risk in Communities included components of the metabolic syndrome, such as cholesterol, BMI, C-reactive protein, hypertension, and diabetes mellitus [37]. Little is known about the relevance of steroid hormones to MMPs, although *in vitro* studies suggest that estrogens and progestogens up-regulate MMP-2 expression in breast cancer cell lines [39]; and MMP-2 and MMP-3 are known to influence rodent mammary development [27, 40]. Modest interactions of BMI and HRT on the association of MMP-1 with breast cancer risk are likely chance observations, but may also suggest interactions with estrogen levels [39, 41, 42], providing conditional leads for further research.

The study is strengthened by its multiethnic composition and relatively large size; however, no heterogeneity in breast cancer risk associated with circulating MMP levels was identified by race or ethnic group. Our power was limited to examine association in subgroup analysis of clinical characteristics.

In conclusion, results from this large nested case-control study within the Multiethnic Cohort study do not support an association of pre-diagnostic plasma levels of MMPs with overall postmenopausal breast cancer risk. The search for circulating biomarkers for breast cancer has yielded few clinically relevant candidates. The critical role of the MMPs in tissue remodeling provided the basis for this analysis. As release of MMPs into the circulation from incipient breast tumors may be poor or undetectable, the substantial biological rationale for an association of serologic level of MMPs with postmenopausal breast cancer risk prompts caution in the interpretation of our null results. Considerable advancement in our knowledge of the biology of breast cancer will be required to understand the potential role of MMPs in aggressive phenotypes.

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Table 1

Baseline characteristics of postmenopausal breast cancer cases and controls [a]

	Cases (n=713)		Controls (n=713)			
	No.	**%**	**No.**	**%**	**OR**	**(95% CI) [b]**
Matching variables						
Mean age at blood draw, years (Mean (SD))	67.9	(7.5)	67.9	(7.5)	-	
Ethnicity					-	
White	151	21.2	151	21.2		
African-American	107	15.0	107	15.0		
Native Hawaiian	68	9.5	68	9.5		
Japanese-American	254	35.6	254	35.6		
Latino	133	18.7	133	18.7		
Hormone replacement therapy use at blood draw	241	33.8	241	33.8	-	
Non-matching variables [c]						
Years of education, years						
≤12	283	39.7	276	38.7	1.00	-
>12	427	59.9	434	60.9	0.95	(0.76 – 1.20)
Body mass index, kg/m^2						
<25.0	320	44.9	382	53.6	1.00	-
25.0–29.9	250	35.1	199	27.9	1.60	(1.24 – 2.08)
≥30.0	129	18.1	122	17.1	1.37	(1.00 – 1.88)
Tobacco smoking						
Never	388	54.4	408	57.2	1.00	-
Ever	318	44.6	298	41.8	1.13	(0.91 – 1.40)
Alcohol drinking						
No	415	58.2	441	61.9	1.00	-
Yes	281	39.4	256	35.9	1.19	(0.95 – 1.50)
Family history of breast cancer						
No	571	80.1	596	83.6	1.00	-
Yes	109	15.3	84	11.8	1.38	(1.00 – 1.89)

	Cases (n=713)		Controls (n=713)			
	No.	%	No.	%	OR	(95% CI) [b]
Age at menarche, years						
≤12	366	51.3	360	50.5	1.00	-
>12	336	47.1	351	49.2	0.93	(0.75 – 1.15)
Age at menopause, years						
<45	191	26.8	191	26.8	1.00	-
45–49	143	20.1	170	23.8	0.85	(0.62 – 1.15)
≥50	379	53.2	352	49.4	1.09	(0.84 – 1.42)
Live births						
Never	87	12.2	71	10.0	1.00	-
1 birth	79	11.1	79	11.1	0.80	(0.51 – 1.25)
2–3 births	343	48.1	321	45.0	0.86	(0.60 – 1.22)
≥4 births	200	28.1	238	33.4	0.67	(0.46 – 0.97)

Abbreviation: SD, standard deviation; OR, odds ratio; CI, confidence interval.

[a] Data are presented as number and percentage unless otherwise indicated.

[b] Modeled through conditional logistic regression.

[c] Sum of the number of subjects is not consistent with the total number because of missing information.

Table 2

Adjusted odds ratio (OR) and 95% confidence intervals (CI) for postmenopausal breast cancer by plasma MMP levels

	Geometric Mean (pg/ml) (Mean±SD)			Across 2 SD of the log-transformed MMP values [b]		Quartiles (Q) [d]							
						Q1	Q2		Q3		Q4		
	Cases (n=713)	Controls (n=713)	P-value [a]	OR	(95% CI) [c]	OR	OR	(95% CI) [c]	OR	(95% CI) [c]	OR	(95% CI) [c]	P_{trend} [c,e]
MMP-1	81.7±0.81	78.7±0.82	0.37	1.07	(0.93 – 1.23)	1.00	0.80	(0.59 – 1.09)	0.93	(0.70 – 1.25)	1.04	(0.77 – 1.41)	0.32
MMP-2	20.4±1.79	20.5±0.21	0.58	0.82	(0.44 – 1.53)	1.00	1.06	(0.78 – 1.42)	0.87	(0.63 – 1.20)	0.87	(0.63 – 1.21)	0.53
MMP-3	1.22±0.46	1.22±0.49	0.86	0.93	(0.62 – 1.39)	1.00	1.01	(0.74 – 1.37)	0.97	(0.71 – 1.32)	1.02	(0.75 – 1.38)	0.72
MMP-7	167.0±0.63	163.4±0.68	0.50	1.04	(0.87 – 1.25)	1.00	1.07	(0.79 – 1.47)	1.07	(0.78 – 1.47)	1.00	(0.72 – 1.39)	0.64

Abbreviation: MMP, matrix metalloproteinase; SD, standard deviation.

[a] P-value for the difference between cases and controls based on paired t-test.

[b] Two SD of the log-transformed MMPs: 1.60 for MMP-1; 0.37 for MMP-2; 0.55 for MMP-3; 1.29 for MMP-7.

[c] Modeled through conditional logistic regression after adjustment for body mass index, number of live births, and family history of breast cancer.

[d] Medians for the quartiles: 3.53, 4.14, .4.59, 5.27 for MMP-1; 2.88, 3.02, 3.12, 3.25 for MMP-2; 0.56, 0.72, 0.86, 1.09 for MMP-3; 4.51, 4.92, 5.25, 5.71 for MMP-7.

[e] P value for the continuous log-transformed MMP level.

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Table 3

Odds ratio (OR) and 95% confidence intervals (CI) for the association of postmenopausal breast cancer with plasma MMP levels by subgroup of participant characteristics [a]

Variables	Numbers		MMP-1			MMP-2			MMP-3			MMP-7		
	Cases	Controls	OR	(95% CI)	P*int* [d]	OR	(95% CI)	P*int* [d]	OR	(95% CI)	P*int* [d]	OR	(95% CI)	P*int* [d]
Age, y [b]					0.85			0.69			0.62			0.22
<65	282	277	1.17	(0.79–1.72)		0.86	(0.57–1.30)		0.83	(0.57–1.22)		1.30	(0.88–1.93)	
≥65	431	436	1.01	(0.76–1.34)		0.93	(0.69–1.25)		1.00	(0.75–1.34)		0.95	(0.71–1.28)	
Ethnicity [b]					0.98			0.96			0.33			0.81
White	151	151	1.29	(0.78–2.10)		1.11	(0.66–1.90)		0.88	(0.55–1.42)		0.91	(0.54–1.52)	
African-American	107	107	0.92	(0.52–1.64)		0.57	(0.28–1.18)		1.58	(0.78–3.18)		1.25	(0.63–2.47)	
Native Hawaiian	68	68	0.96	(0.44–2.07)		1.16	(0.57–2.34)		0.93	(0.50–1.75)		0.96	(0.49–1.87)	
Japanese-American	254	254	0.99	(0.68–1.44)		0.94	(0.63–1.41)		0.89	(0.60–1.30)		1.18	(0.79–1.77)	
Latino	133	133	1.43	(0.81–2.52)		0.88	(0.48–1.60)		0.78	(0.44–1.39)		1.06	(0.61–1.86)	
Body mass index [c]					0.03			0.71			0.68			0.64
<25.0	320	382	0.91	(0.66–1.24)		1.00	(0.72–1.39)		0.96	(0.71–1.32)		1.01	(0.73–1.40)	
25.0–29.9	250	199	1.08	(0.74–1.60)		0.66	(0.43–1.02)		0.76	(0.50–1.15)		0.99	(0.67–1.47)	
≥30.0	129	122	1.77	(1.02–3.06)		1.47	(0.85–2.57)		1.16	(0.67–2.01)		1.17	(0.68–2.02)	
Hormone replacement therapy use [b]					0.04			0.62			0.64			0.96
No	472	472	0.92	(0.70–1.20)		0.90	(0.67–1.21)		1.00	(0.76–1.33)		1.10	(0.82–1.47)	
Yes	241	241	1.73	(1.16–2.59)		1.01	(0.69–1.50)		0.91	(0.63–1.32)		0.98	(0.67–1.42)	

Abbreviation: MMP, matrix metalloproteinase; P*int*, *P* Value for interaction.

[a] Odds ratios and 95% confidence intervals were based on a change in two standard deviation of the log-transformed plasma MMPs (pg/mL).

[b] Modeled through conditional logistic regression after adjustment for body mass index, number of live births, and family history of breast cancer.

[c] Modeled through unconditional logistic regression model after adjustment for body mass index, number of live births, family history of breast cancer, study area, ethnicity, birth year, date of blood draw, fasting time, and use of hormone replacement therapy.

[d] Tests for interaction based on the Wald statistic for cross-product terms between the corresponding variable and the log-transformed plasma MMP level.

Table 4

Odds ratio (OR) and 95% confidence intervals (CI) for the association of postmenopausal breast cancer with plasma MMP levels by clinical characteristics of the cases

Characteristics	No. Cases [a]	MMP1			MMP2			MMP3			MMP7		
		Geometric Mean (pg/ml)	OR	(95% CI) [b]	Geometric Mean (pg/ml)	OR	(95% CI) [b]	Geometric Mean (pg/ml)	OR	(95% CI) [b]	Geometric Mean (pg/ml)	OR	(95% CI) [b]
SEER Stage													
Localized	468	84.4	1.17	(0.92– 1.49)	20.3	0.90	(0.70– 1.15)	1.22	0.93	(0.73– 1.20)	168.4	1.05	(0.82– 1.35)
Regional	161	73.9	0.90	(0.63– 1.29)	20.1	0.90	(0.63– 1.30)	1.21	0.99	(0.69– 1.41)	156.5	0.88	(0.61– 1.28)
Distant metastasis	20	107.4	2.62	(1.06– 6.51)	21.6	1.84	(0.83– 4.09)	1.48	1.57	(0.80– 3.10)	232.7	2.12	(0.94– 4.80)
P Value [c]		*0.55*	*0.01*		*0.68*	*0.08*		*0.38*	*0.17*		*0.74*	*0.05*	
Size (cm)													
<2.0	302	85.3	1.21	(0.91– 1.62)	20.3	0.92	(0.69– 1.24)	1.24	1.03	(0.77– 1.38)	166.4	1.12	(0.84– 1.51)
2.0–5.0	128	83.7	1.10	(0.74– 1.64)	20.3	1.00	(0.68– 1.49)	1.15	0.81	(0.52– 1.25)	150.7	0.88	(0.60– 1.31)
>5.0	20	104.2	1.82	(0.76– 4.28)	20.1	0.64	(0.27– 1.51)	1.24	0.81	(0.30– 2.18)	182.2	1.09	(0.43– 2.77)
P Value [c]		*0.62*	*0.07*		*0.88*	*0.26*		*0.30*	*0.26*		*0.51*	*0.24*	
Axillary node status													
N0	470	85.2	1.21	(0.95– 1.54)	20.4	0.91	(0.72– 1.17)	1.22	0.94	(0.73– 1.20)	169.1	1.08	(0.85– 1.38)
N1	158	75.0	0.94	(0.66– 1.35)	20.1	0.91	(0.63– 1.32)	1.21	1.00	(0.70– 1.44)	157.8	0.89	(0.61– 1.29)
P Value [c]		*0.22*	*0.08*		*0.09*	*0.43*		*0.28*	*0.59*		*0.03*	*0.29*	
Grade													
I	158	86.8	1.24	(0.87– 1.77)	20.1	0.73	(0.51– 1.05)	1.24	0.96	(0.66– 1.40)	169.8	1.12	(0.78– 1.62)
II	278	85.1	1.21	(0.91– 1.62)	20.3	0.90	(0.67– 1.22)	1.23	0.98	(0.73– 1.32)	164.7	1.05	(0.78– 1.41)
III	179	74.5	0.91	(0.64– 1.28)	20.5	1.17	(0.83– 1.63)	1.19	0.94	(0.67– 1.32)	159.1	0.84	(0.60– 1.19)
P Value [c]		*0.08*	*0.06*		*0.23*	*0.03*		*0.44*	*0.70*		*0.35*	*0.19*	
Receptor status													
ER+	503	82.1	1.08	(0.85– 1.37)	20.2	0.83	(0.65– 1.06)	1.21	0.89	(0.70– 1.13)	167.4	1.05	(0.82– 1.34)
ER−	122	83.1	1.26	(0.84– 1.88)	20.6	1.15	(0.78– 1.69)	1.19	0.95	(0.63– 1.42)	160.7	0.96	(0.64– 1.42)
P Value [c]		*0.31*	*0.23*		*0.88*	*0.06*		*0.54*	*0.34*		*0.11*	*0.63*	
PR+	417	81.5	1.06	(0.82– 1.36)	20.2	0.84	(0.65– 1.09)	1.21	0.88	(0.68– 1.15)	168.3	1.07	(0.83– 1.39)

Characteristics	No. Cases [a]	MMP1			MMP2			MMP3			MMP7		
		Geometric Mean (pg/ml)	OR	(95% CI) [b]	Geometric Mean (pg/ml)	OR	(95% CI) [b]	Geometric Mean (pg/ml)	OR	(95% CI) [b]	Geometric Mean (pg/ml)	OR	(95% CI) [b]
PR−	187	86.7	1.36	(0.97– 1.90)	20.4	1.04	(0.75– 1.45)	1.19	0.93	(0.66– 1.30)	163.6	1.01	(0.72– 1.41)
P Value [c]		*0.75*	*0.08*		*0.78*	*0.15*		*0.53*	*0.34*		*0.90*	*0.59*	
ER+ or PR+	512	81.7	1.07	(0.85– 1.36)	20.2	0.84	(0.66– 1.07)	1.21	0.89	(0.69– 1.13)	166.9	1.03	(0.81– 1.32)
ER− and PR−	113	84.9	1.34	(0.88– 2.02)	20.6	1.15	(0.77– 1.71)	1.19	0.97	(0.64– 1.46)	162.2	1.00	(0.66– 1.50)
P Value [c]		*0.32*	*0.16*		*0.82*	*0.08*		*0.34*	*0.32*		*0.10*	*0.78*	

Abbreviation: MMP, matrix metalloproteinase; SEER, Surveillance, Epidemiology, and End Results Program.

[a] Sum of number of subjects is not consistent with the total number because of unrecorded, unknown, or borderline cases (for receptors) who were excluded from the analyses.

[b] Modeled through unconditional polychotomous logistic regression using all controls in each analysis after adjustment for body mass index, number of live births, family history of breast cancer, study area, ethnicity, birth year, date of blood draw, time fasting, and use of hormone replacement therapy. Odds ratios and 95% confidence intervals were based on a change of two standard deviations in the log-transformed plasma MMPs (pg/mL).

[c] *P* Value for the difference of geometric mean between subgroups based on analysis of variance; P for heterogeneity for the odds ratio between subgroups based on two-sided likelihood ratio test from unconditional polychotomous logistic regression models.