Research Article

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Plasma Anti-Müllerian Hormone Concentrations and Risk of Breast Cancer among Premenopausal Women in the Nurses' Health Studies

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Abstract

Background: Anti-Müllerian hormone (AMH) is a member of the TGFβ family of growth and differentiation factors with a key role in regulating folliculogenesis. In experimental studies, using supraphysiologic concentrations, AMH inhibits breast cancer growth. However, high levels of AMH were associated with increased breast cancer risk in two prior prospective epidemiologic studies.

Methods: We conducted a nested case–control study of premenopausal plasma AMH and breast cancer risk within the Nurses' Health Study (NHS) and NHSII. In NHS, 32,826 women donated blood samples in 1989–1990; in NHSII, 29,611 women donated samples in 1996–1999. After blood collection and before February 2004 (NHS) or July 2010 (NHSII), 539 cases were diagnosed among women premenopausal at diagnosis, and were matched 1:1 to controls. ORs and 95% confidence intervals (CI) were calculated using unconditional logistic regression, adjusting for matching and breast cancer risk factors.

Results: Higher plasma levels of AMH were associated with increased breast cancer risk (top vs. bottom quintile multivariate OR, 2.20; 95% CI, 1.34–3.63; P trend = 0.001). The association did not vary by invasive versus *in situ* disease or by estrogen receptor status. Associations were not significantly different by age at blood or diagnosis. Further adjustment for plasma estradiol or testosterone yielded similar results.

Conclusions: Higher circulating AMH levels are associated with increased breast cancer risk among premenopausal women.

Impact: The significant positive association between premenopausal plasma AMH levels and subsequent breast cancer risk before menopause suggests AMH may be useful as a marker of breast cancer risk in younger women. *Cancer Epidemiol Biomarkers Prev; 25(5); 854–60. ©2016 AACR.*

Introduction

Anti-Müllerian hormone (AMH, also called Müllerian-inhibiting substance, MIS), produced in ovarian granulosa cells, is a member of the TGFβ family of growth and differentiation factors and plays a key role in regulating folliculogenesis (1). AMH is secreted as follicles grow from primary to small antral follicles, and through negative feedback inhibits the transition from primordial to primary follicle (2–6). It also reduces follicle sensitivity to follicle-stimulating hormone (FSH), thus further inhibiting follicle recruitment (7). AMH knockout mice undergo more rapid primordial follicle recruitment and have follicles with higher sensitivity to FSH, resulting in premature depletion of the primordial follicle pool (5, 7). Circulating levels of AMH vary over a woman's life, with low or undetectable levels at birth that rise and peak during late puberty, then decline steadily from age 25, becoming undetectable after menopause (8–11). AMH is strongly correlated with ovarian primordial follicle count (r = 0.72), even after adjustment for age (r = 0.48; ref. 12). AMH levels predict age at natural menopause, independent of chronological age (13–16).

Although AMH has critical functions in the ovary, the AMH type II receptor is also expressed in normal and tumor tissue in the breast (17). Limited laboratory data suggest a protective role of AMH in breast carcinogenesis. *In vitro*, AMH increases apoptosis (18) and decreases growth of normal mammary MCF10 cells (19), as well as that of both ER^+ and ER^- breast cancer cell lines (17). In addition, *in vivo*, AMH administration was associated with fewer palpable mammary tumors in mice, and increased apoptosis in mouse mammary ductal epithelium (19, 20). However, in most of these experimental studies, the concentrations of AMH exceeded physiologic levels; therefore, applicability to breast carcinogenesis in humans is unclear. On the other hand, the fact that higher AMH levels are associated with later age at menopause (13–16), a risk factor for breast cancer (21), suggests that higher AMH levels may be associated with higher breast cancer risk.

Two small cross-sectional studies of AMH levels and breast cancer have been conducted, with mixed results. One small study reported significantly lower AMH levels in 22 women diagnosed

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with cancer or precancerous lesions compared with 8 women with benign biopsies, but the former group was older at blood collection and age was not taken into account in the analysis (22). The other study reported no significant difference in AMH levels between 108 breast cancer cases and 99 healthy controls, adjusting for age and other covariates (23). To date, two prospective epidemiologic studies have evaluated the association between premenopausal circulating AMH levels and risk of subsequent breast cancer. Dorgan and colleagues, in the Columbia MO Serum Bank study, observed a strong positive association with increasing quartiles of AMH [$N = 105$ cases, top vs. bottom quartile OR, 9.8; 95% confidence interval (CI), 3.3–28.9, P trend < 0.001; ref. 24). A positive association was also observed in the Sister Study cohort ($N = 452$ cases, >90th percentile vs. undetectable AMH levels OR, 2.25; 95% CI, 1.26–4.02; ref. 25). In both prospective studies, the association was weaker among younger women, and unchanged with adjustment for testosterone (24, 25) or estradiol (24). Neither study had information on menopausal status at diagnosis.

Given the limited data and conflicting results between experimental studies and prospective epidemiologic studies, we examined the association between premenopausal levels of AMH and subsequent breast cancer risk in the Nurses' Health Study (NHS) and NHSII. To better understand the role of AMH before the onset of menopause, we restricted our analyses to cases (and controls) diagnosed (or matched) before menopause, and further adjusted our analyses for plasma testosterone and estradiol levels.

Materials and Methods

Study population

In 1976, 121,701 female, registered nurses, ages 30 to 55 years, were enrolled in the NHS. Biennially, participants complete mailed questionnaires on lifestyle, diet, reproductive history, and disease diagnoses. In 1989–1990, 32,826 women ages 43 to 69 years (21% premenopausal) donated blood samples (26). Briefly, each woman arranged to have her blood drawn, without regard to timing within the menstrual cycle for premenopausal women, and shipped overnight with an ice-pack to our laboratory, where it was processed and archived in liquid nitrogen freezers; 97% of samples arrived within 26 hours of collection. The follow-up rate among the women who donated blood was 97% in 2010.

The NHSII was established in 1989, when 116,430 female registered nurses, ages 25 to 42 years, completed and returned a questionnaire. The cohort has been followed biennially following the methods of the NHS. Between 1996 and 1999, 29,611 cohort members, who were cancer-free and between the ages of 32 and 54 years, provided blood and urine samples (27). Of these women, 18,521 were premenopausal participants (i.e., still having menstrual periods) who had not been pregnant, breastfed, or used oral contraceptives in the 6 months preceding collection, and provided two blood samples and one urine sample timed within the menstrual cycle (one follicular sample collected on the third to fifth day and one luteal sample collected seven to nine days before the anticipated start of their next cycle). Follicular plasma was aliquoted by the participants 8 to 24 hours after collection and stored in their home freezer until the luteal collection. The day of the luteal collection, follicular and luteal blood samples, and luteal urine samples were shipped, via overnight courier with an ice-pack, to our laboratory where the luteal blood sample was processed similarly to the NHS samples; 93% of samples arrived within 26 hours of collection. The follow-up rate among the women who donated blood was 96% in 2011.

Plasma samples from both cohorts have been stored in liquid nitrogen freezers (<130°C) since collection. The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health (Boston, MA); completion of the self-administered questionnaire and blood collection was considered to imply informed consent.

Case–control selection

All cases were premenopausal at blood collection and diagnosis. Women were defined as premenopausal if their periods had not ceased permanently or they had at least 1 ovary remaining and were younger than 46 (for smokers) or 48 (for nonsmokers) years (28). NHS cases ($N = 144$) were diagnosed before February 2004 and were matched (1:1) to controls on age (±2 years), and month (±1), time of day (±2 hours), and fasting status (<2, 2–4, 5–7, 8–11, ≥12 hours) of blood draw. NHSII cases ($N = 395$) were diagnosed before July 2010 and were matched (1:1) to controls on age (±2 years), race, menopausal status at diagnosis, luteal day (±1 day for timed samples), and month (±2), time of day (±2 hours), and fasting status (<2, 2–4, 5–7, 8–11, ≥12 hours) of blood draw. In 27 NHSII case–control pairs, we had to loosen our matching criteria to find an appropriate match, and were not able to maintain matching on menopausal status at diagnosis (i.e., the controls were postmenopausal at the time of the case's diagnosis). In NHS, we had 41 pairs where the case was premenopausal at diagnosis, but the control was not. Because of the high correlation between premenopausal AMH levels and subsequent menopausal status, we excluded these 68 controls and maintained 539 cases and 471 controls in the analysis.

Laboratory assays

AMH and testosterone were measured in luteal or untimed samples, and estradiol was measured in both follicular and luteal samples. Case–control sets were assayed together, as well as follicular and luteal samples from the same person. Samples were ordered randomly within a set, and laboratories were masked to both case–control status and follicular and luteal samples within woman. Samples were assayed for AMH, in one batch for each cohort, by the picoAMH ELISA assay at Ansh Labs. NHSII samples were assayed for estrogens and testosterone in five batches at either Quest Diagnostics by radioimmunoassay preceded by organic extraction and celite chromatography (batches 1 and 2) or the Mayo Clinic by liquid chromatography-tandem mass spectrometry (batches 3 to 5; ref. 29). NHS samples were assayed for testosterone in one batch at the Mayo Clinic by liquid chromatography-tandem mass spectrometry. Masked replicate quality control samples (10% of the samples) were included in each batch to assess coefficients of variation (CV). CVs for AMH were 4.6% (NHS) and 9.0% (NHSII). CVs for estradiol and testosterone were ≤15%.

Reproducibility study

A subset of premenopausal NHSII participants who gave blood samples in the initial collection also provided two additional sets of samples over the following 2 to 3 years. Mid-luteal blood samples from 113 of these women, chosen randomly, were assayed for AMH at the Sluss laboratory (Massachusetts General

Hospital, Boston MA) using the AMH Gen II Elisa assay Kit (Beckman Coulter; CV = 14.5%). These data were used to assess reproducibility over time as has been published previously for other biomarkers (30, 31).

Covariate data

Information on breast cancer risk factors, including anthropometrics, reproductive history, and diet, was collected from biennial and blood collection questionnaires. Covariates included in this analysis were (year of data collection for NHS/NHSII): age at menarche (1976/1989), height and weight at age 18 [1976, 1980/1989; used to calculate body mass index (BMI), kg/m^2], parity (biennially), age at first birth (biennially), oral contraceptive use (biennially), family history of breast cancer (1976, 1982, 1988/1989, 1997; mother and/or sister), and history of benign breast disease (biennially). Values for covariates with biennial updates were taken from the closest questionnaire preceding blood collection.

Statistical analyses

Using the log-transformed AMH values, we estimated between-person and within-person variances from the three sets of measurements by random-effects models. Reproducibility of AMH over time was assessed by calculating intraclass correlation coefficients (ICC) by dividing the between-person variance by the sum of the within- and between-person variances.

Samples with AMH levels below the limit of detection (2.038 pg/mL) were set to half this value (1.019 pg/mL; $N = 14$ cases, 11 controls). As in prior analyses (29), to adjust for between-batch differences in estradiol and testosterone distributions, we used an average batch recalibration approach (32). Quintile cutpoints were defined among all the controls. Because of the smaller stratum-specific sample sizes, we used quartile categories in stratified analyses. For analyses stratified by age-related factors (age at blood collection, age at diagnosis, time between blood collection and diagnosis), given the strong association between age and AMH, we used quartile cutpoints defined among controls within age strata (age at blood collection <45, ≥45 years). Given the strong correlation between age and AMH, we maintained these age at blood collection cutpoints for analyses of age at diagnosis and time between blood collection and diagnosis. ORs and 95% CIs were calculated from multivariate unconditional logistic regression models adjusted for matching factors and breast cancer risk factors. Given we used incidence density sampling to select controls, the ORs are estimates of the incidence rate ratios (33). We chose to use an unconditional logistic regression model for two reasons: (i) adjustment for age more finely than the matching allowed was important given the strong correlation between age and AMH levels, and (ii) an unconditional model allowed inclusion of the cases whose matched controls were excluded. We used likelihood ratio tests to compare different approaches to adjusting for age and determined the best adjustment was with age as a continuous variable plus age-squared as a continuous variable. Tests for trend were conducted by a Wald test on quintile (or quartile) medians, modeled continuously. Wald tests for interaction between stratification variables and AMH levels compared the slope of the quartile medians between groups. To test whether associations differed by tumor characteristics (ER status, invasiveness), we used polychotomous logistic regression (34) with a likelihood ratio test comparing a model with separate slopes for AMH in each case group with one with a common slope. All P values were based on two-sided tests and were considered statistically significant if ≤0.05. Analyses were conducted using SAS version 9 (SAS Institute) or STATA version 11.0 (StataCorp).

Table 1. Characteristics of cases and controls, NHS and NHSII, mean (SD) or %

	Cases ($n = 539$)	Controls ($n = 471$)
Age at blood draw, y	43.9 (4.3)	43.4 (4.0)
Age at menarche, y	12.5 (1.8)	12.5 (1.4)
BMI at age 18, kg/m^2	20.8 (2.6)	21.1 (2.8)
BMI at blood draw, kg/m^2	24.6 (4.6)	25.2 (5.5)
Parous	82.7%	83.4%
Parity, children	2.3 (0.9)	2.4 (0.9)
Age at first birth, y	26.1 (4.7)	25.8 (4.3)
Past oral contraceptive use	82.0%	82.6%
Current oral contraceptive use	1.1%	1.1%
Family history of breast cancer	15.4%	9.3%
History of benign breast disease	28.8%	18.5%
AMH levels, pg/mL: median (10th–90th)	947 (63.2–4,213)	763 (69.9–3,406)

Results

Reproducibility of AMH over a 2- to 3-year period was good, with ICC = 0.67 (95% CI, 0.57–0.75). Mean age at blood collection was 43 years overall (42 years in NHSII, 47 years in NHS) with a range of 32 to 53 years. Cases had slightly lower BMI at age 18 and blood collection and had fewer children (2.3 vs. 2.4; Table 1). Cases were more likely to have a family history of breast cancer (15.4% vs. 9.3%) and a history of benign breast disease (28.8% vs. 18.5%). AMH levels were higher in cases (median, 947 pg/mL) than controls (763 pg/mL). Among controls, higher AMH levels were associated with younger age at blood collection, later age at subsequent menopause, and more years from blood collection to menopause (Table 2). Levels were not strongly associated with age at menarche, BMI, or parity-related variables. Family history of breast cancer was more common in women with higher AMH levels.

Higher plasma AMH levels were associated with an increased odds of breast cancer (simple model, top vs. bottom quartile OR, 2.38; 95% CI, 1.46–3.88; P trend = 0.0004; Table 3). Adjustment

Table 2. Characteristics of controls by quintile of AMH (pg/mL), NHS and NHSII, mean (SD) or %

	Quintile 1	Quintile 3	Quintile 5
N	94	94	95
AMH, pg/mL	85.2 (73.6)	779 (137)	4,206 (2,755)
Age at blood draw, y	47.1 (2.6)	43.6 (3.4)	40.3 (3.3)
Age at menarche, y	12.3 (1.4)	12.1 (1.2)	12.5 (1.3)
Subsequent age at menopause[a], y	51.4 (2.2)	52.3 (2.6)	53.1 (2.8)
Years to menopause, y	4.4 (2.6)	8.5 (2.5)	10.9 (2.4)
BMI at age 18, kg/m^2	21.4 (3.0)	21.1 (3.0)	21.1 (2.7)
BMI at blood draw, kg/m^2	25.9 (5.9)	25.1 (5.4)	25.0 (5.4)
Parous	81.7%	87.2%	86.3%
Parity, children	2.6 (1.1)	2.3 (0.8)	2.5 (1.0)
Age at first birth, y	25.8 (4.7)	26.6 (4.6)	26.1 (3.8)
Past oral contraceptive use	79.8%	85.1%	81.1%
Current oral contraceptive use	2.1%	1.1%	1.1%
Family history of breast cancer	5.3%	7.5%	14.7%
History of benign breast disease	14.9%	19.2%	16.8%

[a]With continued follow-up, after cases' diagnoses.

Table 3. ORs (95% CIs) of breast cancer by quintile of prediagnostic plasma AMH (pg/mL), NHS and NHSII

	Q1	Q2	Q3	Q4	Q5	*P* trend	
Cases/controls (*N*)	102/94	99/94	81/94	124/94	133/95		
Cutpoints (pg/mL)	<225	225–<575	575–<1,053	1,053–<2,213	≥2,213		
Simple[a] OR (95% CI)	1.00 (ref.)	1.41 (0.91–2.20)	1.24 (0.78–1.97)	2.06 (1.29–3.29)	2.38 (1.46–3.88)	0.0004	
Multivariate[b] OR (95% CI)	1.00 (ref.)	1.29 (0.82–2.03)	1.17 (0.73–1.88)	1.92 (1.19–3.10)	2.20 (1.34–3.63)	0.001	
							P het
Invasive (*N* = 369 cases)	1.00 (ref.)	1.29 (0.80–2.10)	0.93 (0.55–1.56)	1.71 (1.01–2.89)	2.30 (1.34–3.95)	0.001	
In situ (*N* = 150 cases)	1.00 (ref.)	1.49 (0.69–3.20)	1.54 (0.71–3.32)	2.59 (1.19–5.62)	2.08 (0.90–4.82)	0.21	0.27
ER^+ (*N* = 292 cases)	1.00 (ref.)	1.55 (0.91–2.64)	1.18 (0.67–2.08)	2.13 (1.21–3.77)	2.78 (1.54–5.02)	0.001	
ER^- (*N* = 64 cases)	1.00 (ref.)	0.89 (0.37–2.17)	0.41 (0.14–1.18)	0.99 (0.37–2.68)	1.62 (0.57–4.58)	0.14	0.33
Triple negative[c] (*N* = 30 cases)	1.00 (ref.)	1.02 (0.26–3.99)	0.43 (0.08–2.44)	2.16 (0.54–8.59)	2.05 (0.45–9.29)	0.20	

[a]Simple model adjusted for matching factors: age at blood (continuous), year of blood (1989, 1990, 1996, 1997, 1998, 1999), time of blood (1–8 am, 9 am–noon, 1 pm–midnight), fasting (yes/no), luteal day (3–5, 6–7, 8–9, 10–28, untimed), race (Caucasian, other), and age at blood squared (continuous).

[b]Multivariate model additionally adjusted for the following variables (categories): age at menarche (<12, 12, 13, ≥14 years), parity/age at first birth (nulliparous, 1–2 children/<25 years, 1–2 children/≥25 years, ≥3 children/<25 years, ≥3 children/≥25 years), BMI at age 18 (<21, 21–<23, ≥23), oral contraceptive use (ever/never), family history of breast cancer (yes/no), and history of benign breast disease (yes/no).

[c]Triple-negative model adjusts for reduced set of matching factors only: age at blood (continuous), age at blood squared (continuous), and luteal day (3–5, 6–7, 8–9, 10–28, untimed).

for multiple breast cancer risk factors did not substantially alter the results (multivariate OR, 2.20; 95% CI, 1.34–3.63; *P* trend = 0.001). In sensitivity analyses restricted to matched pairs, results were comparable between conditional (multivariate top vs. bottom quintile OR, 2.11; 95% CI, 1.22–3.64; *P* trend = 0.005) and unconditional (OR, 1.97; 95% CI, 1.18–3.31; *P* trend = 0.01) models. Expanding the analysis to deciles of AMH, the association appeared linear [top (≥3,406 pg/mL) vs. bottom (<70.0 pg/mL) decile OR, 2.88; 95% CI, 1.48–5.62, *P* trend = 0.0001].

Results were not significantly different (*P* heterogeneity = 0.27) between invasive (*N* = 369, top vs. bottom quintile OR, 2.30; 95% CI, 1.34–3.95; *P* trend = 0.001) and *in situ* (*N* = 150, OR, 2.08; 95% CI, 0.90–4.82; *P* trend = 0.21) cases (Table 3). Results appeared stronger for ER^+ (*N* = 292, OR, 2.78; 95% CI, 1.54–5.02; *P* trend = 0.001) than ER^- (*N* = 64, OR, 1.62; 95% CI, 0.57–4.58, *P* trend = 0.14) tumors, but the difference was not significant (*P* heterogeneity = 0.33).

The association between AMH and breast cancer did not differ significantly by age at blood collection [top vs. bottom quartile OR (95% CI) <45 years = 2.10 (1.24–3.55), *P* trend = 0.01; ≥45 y, 1.65 (0.87–3.11), *P* trend = 0.11; *P* interaction = 0.39; Table 4]. Results were also not significantly different by age at diagnosis [<50 years = 1.62 (0.96–2.71), *P* trend = 0.09; ≥50 years = 2.29 (1.23–4.23), *P* trend = 0.01; *P* interaction = 0.33] or time between blood collection and diagnosis [<5 years = 1.69 (1.00–2.87), *P* trend = 0.08; ≥5 years = 2.16 (1.16–4.01), *P* trend = 0.01; *P* interaction = 0.53).

Results were not significantly different by BMI at blood collection (*P* interaction = 0.18; data not shown). We had too few current users of oral contraceptives at blood collection to examine this subgroup. Although most women were past users, the association with AMH appeared stronger in never users [*N* = 91 cases, top vs. bottom quartile OR (95% CI), 5.71 (1.41–23.2)] than in past users [1.40 (0.87–2.26)], but this difference was not significant (*P* interaction = 0.10). There was a significant difference in the association between AMH and breast cancer risk by family history of breast cancer (*P* interaction = 0.01), with a positive association observed among women without a family history (top vs. bottom quartile OR, 2.02; 95% CI, 1.26–3.23; *P* trend = 0.0001) and a suggested inverse association among women with a family history (OR, 0.35; 95% CI, 0.07–1.71; *P* trend = 0.05), though the numbers in this analysis were limited (83 cases, 44 controls). There was no statistically significant interaction by cohort (*P* interaction = 0.58).

Plasma testosterone measures were available for 530 cases and 469 controls. Among controls, AMH levels were modestly correlated with testosterone (*r* = 0.39) levels. The association between AMH levels and breast cancer risk was unchanged with additional adjustment for plasma testosterone (top vs. bottom quintile OR, 2.22; 95% CI, 1.32–3.71; *P* trend = 0.002). The association

Table 4. ORs[a] (95% CIs) of breast cancer by quartile (with age-specific cutpoints) of prediagnostic plasma AMH (pg/mL), by tumor and participant characteristics, NHS and NHSII

	Number of cases/controls	Q1	Q2	Q3	Q4	*P* trend	*P* intxn
Overall, age-specific quartiles	539/471	1.00 (ref.)	1.22 (0.83–1.79)	1.40 (0.95–2.05)	1.78 (1.22–2.60)	0.002	
Age at blood <45	302/294	1.00 (ref.)	1.34 (0.79–2.27)	2.02 (1.21–3.38)	2.10 (1.24–3.55)	0.01	
Age at blood ≥45	237/177	1.00 (ref.)	1.22 (0.66–2.25)	0.82 (0.42–1.59)	1.65 (0.87–3.11)	0.11	0.39
Age at diagnosis <50	291/281	1.00 (ref.)	1.25 (0.74–2.10)	1.77 (1.05–3.01)	1.62 (0.96–2.71)	0.09	
Age at diagnosis ≥50	248/190	1.00 (ref.)	1.34 (0.72–2.49)	1.06 (0.56–1.98)	2.29 (1.23–4.23)	0.01	0.33
Time from blood to diagnosis <5 years	282/252	1.00 (ref.)	1.31 (0.80–2.15)	1.05 (0.62–1.78)	1.69 (1.00–2.87)	0.08	
Time from blood to diagnosis ≥5 years	257/219	1.00 (ref.)	1.31 (0.68–2.53)	2.01 (1.06–3.80)	2.16 (1.16–4.01)	0.01	0.53

[a]Multivariate model adjusted for: age at blood (continuous), age at blood squared (continuous), year of blood (1989, 1990, 1996, 1997, 1998, 1999), time of blood (1–8 am, 9 am–noon, 1 pm–midnight), fasting (yes/no), luteal day (3–5, 6–7, 8–9, 10–28, untimed), race (Caucasian, other), age at menarche (<12, 12, 13, ≥14 years), parity/age at first birth (nulliparous, 1–2 children/<25 years, 1–2 children/≥25 years, ≥3 children/<25 years, ≥3 children/≥25 years), BMI at age 18 (<21, 21–<23, ≥23), oral contraceptive use (never/ever), family history of breast cancer (yes/no), and history of benign breast disease (yes/no).

Age-specific cutpoints: <45 years: <580/580–<1,237/1,237–<2,631/≥2,631; ≥45 years: <87/87–<347/347–<706/≥706 pg/mL.

also was not significantly different (P interaction = 0.23) by testosterone level [top vs. bottom quartile OR (95% CI), <median = 2.13 (1.05–4.31), P trend = 0.005; ≥median = 1.57 (0.83–2.98), P trend = 0.10].

NHSII women with blood samples timed in the menstrual cycle had measures of early follicular and mid-luteal plasma estradiol (344 cases, 320 controls). Plasma AMH levels were not correlated with estradiol (follicular $r = 0.02$; luteal $r = 0.17$), and additional adjustment for plasma estradiol levels did not alter the association [e.g., top vs. bottom quartile AMH OR (95% CI) without adjustment = 2.07 (1.23–3.47), P trend = 0.004; with adjustment for luteal estradiol = 2.10 (1.22–3.62), P trend = 0.005]. The association of AMH levels with breast cancer was not significantly different stratified by either follicular or luteal estradiol [e.g., top vs. bottom quartile OR (95% CI) luteal estradiol <median = 2.53 (1.13–5.67), P trend = 0.02; ≥median = 1.84 (0.84–4.02), P trend = 0.10; P interaction = 0.37).

Discussion

In this large, prospective analysis of plasma levels of AMH and subsequent breast cancer risk in premenopausal women, women in the top 20% of AMH levels were at twice the risk of women in the bottom 20%. Results were unchanged with adjustment for estradiol or testosterone and were not significantly different by age.

Our study confirms the positive associations between AMH levels and subsequent breast cancer risk observed in the prior Columbia MO (24) and Sister Study (25) cohorts. Although the magnitude of the association was higher in the Columbia MO cohort (top vs. bottom quartile OR, 9.8; 95% CI, 3.3–28.9; P trend < 0.001; ref. 24), the small number of cases (n = 105) yielded wide CIs. Our results are more similar to those of the Sister Study (25), where the top 10% of women were at more than twice the odds of those whose AMH levels were undetectable (OR, 2.25; 95% CI, 1.26–4.02). Although we observed a significant interaction with family history of breast cancer, with a suggested inverse association among those with a family history, the positive association observed in the Sister Study, where all participants have a family history (25), suggests ours may be a chance finding.

The contrast in the results of experimental studies, in which AMH inhibits breast cancer growth, and those of epidemiologic studies, in which AMH is associated with higher breast cancer risk, is not easily explained. Several experimental studies have focused on breast cancer cell lines and models that reflect basal-like tumors (19, 35), and one hypothesis is that AMH may only reduce the risk of basal-like breast cancer (25). However, other studies have included ER^+ cell lines where reduced growth also has been observed (17). Further, our results and those of the Sister Study (25) do not suggest significant differences in the association with ER^+ and ER^- breast cancers. However, numbers of ER^- breast cancers have been small in these studies (N = 64 in each), and further research with more ER^- or basal-like tumors is warranted. Another possible explanation for the discrepant results is that the concentrations of AMH are not comparable between experimental and human studies, with experimental studies far exceeding physiologic equivalent levels (24).

Although AMH may be associated with breast cancer as a marker of later menopause, which is itself a confirmed breast cancer risk factor (21), our study was restricted to women who were still premenopausal at the time of diagnosis to understand the role of AMH prior to the onset of menopause. Had we not restricted our matched controls to be premenopausal at the time of the case's diagnosis, and allowed controls to be postmenopausal, it is likely we would have observed even higher estimates of the association between AMH and breast cancer risk. The observed associations with AMH levels in a population of women premenopausal at both blood collection and at the time of the case's diagnosis suggest that AMH levels represent, or are correlated with, an aspect of biology or underlying risk other than simply a later age at menopause in and of itself. For instance, AMH levels may be a marker of preclinical menopausal decline of ovarian function that is perhaps representative of the lifetime hormonal milieu. Our results offer intriguing possibilities of using AMH as an independent biomarker of risk in premenopausal women, for whom there are few established biomarkers of breast cancer risk. Although the associations between circulating estrogens and androgens and breast cancer risk are well established in postmenopausal women (36), they are less consistent in premenopausal women (37). Further, in our study and prior studies, the association with AMH appears to be independent of estradiol (24) and testosterone (24, 25) levels.

Strengths of this study include the measurement of AMH prior to breast cancer diagnosis, the large sample size, and detailed covariate information, including estradiol and testosterone measures. Despite this being the largest study of AMH and breast cancer to date, we were still limited in our investigation of breast cancer subtypes, and larger studies are warranted. Further, we were limited in our ability to determine whether the association varies across key subgroups. Although we only had one measure of AMH, previous work has shown one level to be reproducible over time. ICCs of 0.87 (1 year; ref. 38) and 0.66 (3 years; ref. 39) have been reported for AMH, similar to the reproducibility we observed in the NHSII population (0.67). This is comparable with the reproducibility of other biologic variables such as blood pressure (ICC = 0.6; ref. 40), glucose (ICC = 0.52; ref. 41), and cholesterol (ICC = 0.65; ref. 42), all exposures considered to be reasonably well-measured and consistent predictors of disease.

In conclusion, in the largest study to date, we observed a significant positive association between premenopausal plasma AMH levels and subsequent breast cancer risk before menopause. Our results, confirming the positive associations observed in two prior prospective epidemiologic studies, suggest that AMH may be useful as a marker of breast cancer risk in younger women.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The authors assume full responsibility for analyses and interpretation of these data.

Authors' Contributions

Conception and design: A.H. Eliassen, A. Zeleniuch-Jacquotte, S.E. Hankinson
Development of methodology: A.H. Eliassen, S.E. Hankinson
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.H. Eliassen, S.E. Hankinson
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.H. Eliassen, A. Zeleniuch-Jacquotte, B. Rosner, S.E. Hankinson

Writing, review, and/or revision of the manuscript: A.H. Eliassen, A. Zeleniuch-Jacquotte, B. Rosner, S.E. Hankinson
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.H. Eliassen

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