HHS Public Access
Author manuscript
Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2017 May 01.

Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2016 May ; 25(5): 854–860. doi:10.1158/1055-9965.EPI-15-1240.

Plasma anti-Müllerian hormone concentrations and risk of breast cancer among premenopausal women in the Nurses' Health Studies

A. Heather Eliassen[1,2], **Anne Zeleniuch-Jacquotte**[3], **Bernard Rosner**[1,2,4], and **Susan E. Hankinson**[1,2,5]

[1]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

[2]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA

[3]Department of Population Health and Perlmutter Cancer Center, New York University School of Medicine, New York, NY

[4]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA

[5]Department of Biostatistics and Epidemiology, School of Public Health and Health Sciences, University of Massachusetts, Amherst, MA

Abstract

Background—Anti-Müllerian hormone (AMH) is a member of the transforming growth factor-β 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 1989–90; in NHSII, 29,611 women donated samples in 1996–99. 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. Odds ratios (ORs) and 95% confidence intervals (CIs) 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 vs. 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.

Corresponding author: A. Heather Eliassen, ScD, Channing Division of Network Medicine, 181 Longwood Avenue, Boston, MA 02115, (617) 525-2104, ; Email: heather.eliassen@channing.harvard.edu.

Conflict of Interest: The authors have no conflicts of interest to declare.

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.

Keywords

anti-Müllerian hormone; Müllerian inhibiting substance; hormones; breast cancer; premenopausal

Introduction

Anti-Müllerian hormone (AMH, also called Müllerian inhibiting substance, MIS), produced in ovarian granulosa cells, is a member of the transforming growth factor-β (TGF-β) 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) (12). AMH levels predict age at natural menopause, independent of chronological age (13–16).

While AMH has critical functions in the ovary, the AMH type II receptor also is 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 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 et al, in the Columbia MO Serum Bank study, observed a strong positive association with increasing quartiles of AMH (N=105 cases, top vs. bottom quartile odds ratio (OR)=9.8, 95% confidence interval (CI) (3.3–28.9), p-trend<0.001) (24). A positive association also was observed in the Sister Study cohort (N=452 cases, >90^{th} percentile vs. undetectable AMH levels OR=2.25, 95% CI (1.26–4.02)) (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 30–55 years, were enrolled in NHS. Biennially, participants complete mailed questionnaires on lifestyle, diet, reproductive history, and disease diagnoses. In 1989–90, 32,826 women ages 43–69 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, aged 25 to 42 years, completed and returned a questionnaire. The cohort has been followed biennially following the methods of 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 aliquotted 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 h), and fasting status (<2, 2–4, 5–7, 8–11, ≥12h) 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 h), and fasting status (<2, 2–4, 5–7, 8–11, ≥12h) 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 (Webster, TX). NHSII samples were assayed for estrogens and testosterone in five batches at either Quest Diagnostics (San Juan Capistrano, CA, USA) by radioimmunoassay preceded by organic extraction and celite chromatography (batches 1 and 2) or the Mayo Clinic (Rochester, MN, USA) by liquid chromatography-tandem mass spectrometry (batches 3 to 5) (29). NHS samples were assayed for testosterone in one batch at the Mayo Clinic (Rochester, MN, USA) 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 (CVs). 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% confidence intervals (CI) 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: 1) adjustment for age more finely than the matching allowed was important given the strong correlation between age and AMH levels, and 2) 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 to 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).

Results

Reproducibility of AMH over a 2–3 year period was good, with ICC=0.67 (95% CI (0.57–0.75). Mean age at blood collection was 43y overall (42y in NHSII, 47y in NHS) with a range of 32–53y. 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 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 (≥3406 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) <45y=2.10 (1.24–3.55), p-trend=0.01; ≥45y 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 (<50y=1.62 (0.96–2.71), p-trend=0.09; ≥50y=2.29 (1.23–4.23), p-trend=0.01; p-interaction=0.33) or time between blood collection and diagnosis (<5y=1.69 (1.00–2.87), p-trend=0.08; ≥5y=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 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) (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, suggests that AMH levels represent, or are correlated with, an aspect of biology or underlying risk other than simply a later age at menopause in an 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. While 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) (38) and 0.66 (3 years) (39) have been reported for AMH, similar to the reproducibility we observed in the NHSII population (0.67). This is comparable to the reproducibility of other biological variables such as blood pressure (ICC=0.6) (40), glucose (ICC=0.52) (41), and cholesterol (ICC=0.65) (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.

Acknowledgments

We would like to thank the participants and staff of the Nurses' Health Study and Nurses' Health Study II for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. The authors assume full responsibility for analyses and interpretation of these data.

Funding: This work was supported by grants from the National Cancer Institute: R01 CA178949 (PI: Zeleniuch-Jacquotte), R01 CA49449 (PI: Hankinson), R01 CA87969 (PI: Hankinson), UM1 CA176726, and UM1 CA186107

References

1. Gruijters MJ, Visser JA, Durlinger AL, Themmen AP. Anti-Mullerian hormone and its role in ovarian function. Mol Cell Endocrinol. 2003; 211:85–90. [PubMed: 14656480]
2. Durlinger AL, Visser JA, Themmen AP. Regulation of ovarian function: the role of anti-Mullerian hormone. Reproduction. 2002; 124:601–609. [PubMed: 12416998]
3. Visser JA, Themmen AP. Anti-Mullerian hormone and folliculogenesis. Mol Cell Endocrinol. 2005; 234:81–86. [PubMed: 15836956]
4. Grynnerup AG, Lindhard A, Sorensen S. The role of anti-Mullerian hormone in female fertility and infertility - an overview. Acta Obstet Gynecol Scand. 2012; 91:1252–1260. [PubMed: 22646322]
5. Durlinger AL, Kramer P, Karels B, de Jong FH, Uilenbroek JT, Grootegoed JA, et al. Control of primordial follicle recruitment by anti-Mullerian hormone in the mouse ovary. Endocrinology. 1999; 140:5789–5796. [PubMed: 10579345]
6. Durlinger AL, Gruijters MJ, Kramer P, Karels B, Ingraham HA, Nachtigal MW, et al. Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. Endocrinology. 2002; 143:1076–1084. [PubMed: 11861535]
7. Durlinger AL, Gruijters MJ, Kramer P, Karels B, Kumar TR, Matzuk MM, et al. Anti-Mullerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology. 2001; 142:4891–4899. [PubMed: 11606457]
8. de Vet A, Laven JS, de Jong FH, Themmen AP, Fauser BC. Antimullerian hormone serum levels: a putative marker for ovarian aging. Fertil Steril. 2002; 77:357–362. [PubMed: 11821097]
9. Kelsey TW, Wright P, Nelson SM, Anderson RA, Wallace WH. A validated model of serum anti-mullerian hormone from conception to menopause. PLoS One. 2011; 6:e22024. [PubMed: 21789206]
10. Lie Fong S, Visser JA, Welt CK, de Rijke YB, Eijkemans MJ, Broekmans FJ, et al. Serum anti-mullerian hormone levels in healthy females: a nomogram ranging from infancy to adulthood. J Clin Endocrinol Metab. 2012; 97:4650–4655. [PubMed: 22993032]
11. Lee MM, Donahoe PK, Hasegawa T, Silverman B, Crist GB, Best S, et al. Mullerian inhibiting substance in humans: normal levels from infancy to adulthood. J Clin Endocrinol Metab. 1996; 81:571–576. [PubMed: 8636269]
12. Hansen KR, Hodnett GM, Knowlton N, Craig LB. Correlation of ovarian reserve tests with histologically determined primordial follicle number. Fertil Steril. 2011; 95:170–175. [PubMed: 20522327]
13. van Disseldorp J, Faddy MJ, Themmen AP, de Jong FH, Peeters PH, van der Schouw YT, et al. Relationship of serum antimullerian hormone concentration to age at menopause. J Clin Endocrinol Metab. 2008; 93:2129–2134. [PubMed: 18334591]

14. Freeman EW, Sammel MD, Lin H, Gracia CR. Anti-mullerian hormone as a predictor of time to menopause in late reproductive age women. J Clin Endocrinol Metab. 2012; 97:1673–1680. [PubMed: 22378815]
15. Tehrani FR, Solaymani-Dodaran M, Tohidi M, Gohari MR, Azizi F. Modeling age at menopause using serum concentration of anti-mullerian hormone. J Clin Endocrinol Metab. 2013; 98:729–735. [PubMed: 23316087]
16. Broer SL, Eijkemans MJ, Scheffer GJ, van Rooij IA, de Vet A, Themmen AP, et al. Anti-mullerian hormone predicts menopause: a long-term follow-up study in normoovulatory women. J Clin Endocrinol Metab. 2011; 96:2532–2539. [PubMed: 21613357]
17. Segev DL, Ha TU, Tran TT, Kenneally M, Harkin P, Jung M, et al. Mullerian inhibiting substance inhibits breast cancer cell growth through an NFkappa B-mediated pathway. J Biol Chem. 2000; 275:28371–28379. [PubMed: 10874041]
18. Hoshiya Y, Gupta V, Kawakubo H, Brachtel E, Carey JL, Sasur L, et al. Mullerian inhibiting substance promotes interferon gamma-induced gene expression and apoptosis in breast cancer cells. J Biol Chem. 2003; 278:51703–51712. [PubMed: 14532292]
19. Segev DL, Hoshiya Y, Stephen AE, Hoshiya M, Tran TT, MacLaughlin DT, et al. Mullerian inhibiting substance regulates NFkappaB signaling and growth of mammary epithelial cells in vivo. J Biol Chem. 2001; 276:26799–26806. [PubMed: 11356848]
20. Gupta V, Carey JL, Kawakubo H, Muzikansky A, Green JE, Donahoe PK, et al. Mullerian inhibiting substance suppresses tumor growth in the C3(1)T antigen transgenic mouse mammary carcinoma model. Proc Natl Acad Sci U S A. 2005; 102:3219–3224. [PubMed: 15728372]
21. Collaborative Group on Hormonal Factors in Breast Cancer. Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. Lancet Oncol. 2012; 13:1141–1151. [PubMed: 23084519]
22. McCoy AC, Kliethermes B, Zhang K, Qin W, Sticca R, Bouton M, et al. Serum Mullerian inhibiting substance levels are lower in premenopausal women with breast precancer and cancer. BMC Res Notes. 2011; 4:152. [PubMed: 21615907]
23. Su HI, Flatt SW, Natarajan L, DeMichele A, Steiner AZ. Impact of breast cancer on anti-mullerian hormone levels in young women. Breast Cancer Res Treat. 2013; 137:571–577. [PubMed: 23224236]
24. Dorgan JF, Stanczyk FZ, Egleston BL, Kahle LL, Shaw CM, Spittle CS, et al. Prospective case-control study of serum mullerian inhibiting substance and breast cancer risk. J Natl Cancer Inst. 2009; 101:1501–1509. [PubMed: 19820206]
25. Nichols HB, Baird DD, Stanczyk FZ, Steiner AZ, Troester MA, Whitworth KW, et al. Anti-Mullerian hormone concentrations in premenopausal women and breast cancer risk. Cancer Prev Res (Phila). 2015; 8:528–534. [PubMed: 25873369]
26. Hankinson SE, Willett WC, Manson JE, Hunter DJ, Colditz GA, Stampfer MJ, et al. Alcohol, height, and adiposity in relation to estrogen and prolactin levels in postmenopausal women. J Natl Cancer Inst. 1995; 87:1297–1302. [PubMed: 7658481]
27. Eliassen AH, Missmer SA, Tworoger SS, Spiegelman D, Barbieri RL, Dowsett M, et al. Endogenous steroid hormone concentrations and risk of breast cancer among premenopausal women. J Natl Cancer Inst. 2006; 98:1406–1415. [PubMed: 17018787]
28. Eliassen AH, Chen WY, Spiegelman D, Willett WC, Hunter DJ, Hankinson SE. Use of aspirin, other nonsteroidal anti-inflammatory drugs, and acetaminophen and risk of breast cancer among premenopausal women in the Nurses' Health Study II. Arch Intern Med. 2009; 169:115–121. discussion 21. [PubMed: 19171806]
29. Fortner RT, Eliassen AH, Spiegelman D, Willett WC, Barbieri RL, Hankinson SE. Premenopausal endogenous steroid hormones and breast cancer risk: results from the Nurses' Health Study II. Breast Cancer Res. 2013; 15:R19. [PubMed: 23497468]
30. Missmer SA, Spiegelman D, Bertone-Johnson ER, Barbieri RL, Pollak MN, Hankinson SE. Reproducibility of plasma steroid hormones, prolactin, and insulin-like growth factor levels among premenopausal women over a 2- to 3-year period. Cancer Epidemiol Biomarkers Prev. 2006; 15:972–978. [PubMed: 16702379]

31. Kotsopoulos J, Tworoger SS, Campos H, Chung FL, Clevenger CV, Franke AA, et al. Reproducibility of plasma and urine biomarkers among premenopausal and postmenopausal women from the Nurses' Health Studies. Cancer Epidemiol Biomarkers Prev. 2010; 19:938–946. [PubMed: 20332276]
32. Rosner B, Cook N, Portman R, Daniels S, Falkner B. Determination of blood pressure percentiles in normal-weight children: some methodological issues. Am J Epidemiol. 2008; 167:653–666. [PubMed: 18230679]
33. Greenland S, Thomas DC. On the need for the rare disease assumption in case-control studies. Am J Epidemiol. 1982; 116:547–553. [PubMed: 7124721]
34. Marshall RJ, Chisholm EM. Hypothesis testing in the polychotomous logistic model with an application to detecting gastrointestinal cancer. Stat Med. 1985; 4:337–344. [PubMed: 4059720]
35. MacLaughlin DT, Donahoe PK. Mullerian inhibiting substance/anti-Mullerian hormone: a potential therapeutic agent for human ovarian and other cancers. Future Oncol. 2010; 6:391–405. [PubMed: 20222796]
36. Key T, Appleby P, Barnes I, Reeves G, Endogenous H. Breast Cancer Collaborative G. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst. 2002; 94:606–616. [PubMed: 11959894]
37. Endogenous Hormones Breast Cancer Collaborative Group. Sex hormones and risk of breast cancer in premenopausal women: a collaborative reanalysis of individual participant data from seven prospective studies. Lancet Oncol. 2013; 14:1009–1019. [PubMed: 23890780]
38. Dorgan JF, Spittle CS, Egleston BL, Shaw CM, Kahle LL, Brinton LA. Assay reproducibility and within-person variation of Mullerian inhibiting substance. Fertil Steril. 2010; 94:301–304. [PubMed: 19409547]
39. van Rooij IA, Broekmans FJ, Scheffer GJ, Looman CW, Habbema JD, de Jong FH, et al. Serum antimullerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study. Fertil Steril. 2005; 83:979–987. [PubMed: 15820810]
40. Rosner B, Hennekens CH, Kass EH, Miall WE. Age-specific correlation analysis of longitudinal blood pressure data. Am J Epidemiol. 1977; 106:306–313. [PubMed: 910798]
41. Gordon T, Shurtleff D. The Framingham Study: an epidemiologic investigation of cardiovascular disease. Section 29: Means at each examination and inter-examination variation of specified characteristics: Framingham Study Exam 1 to Exam 10. DHEW Pub No (NIH) 74-478.
42. Shekelle RB, Shryock AM, Paul O, Lepper M, Stamler J, Liu S, et al. Diet, serum cholesterol, and death from coronary heart disease. The Western Electric study. N Engl J Med. 1981; 304:65–70. [PubMed: 7442730]

Table 1

Characteristics of cases and controls, Nurses' Health Study and Nurses' Health Study II, 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/m2	20.8 (2.6)	21.1 (2.8)
BMI at blood draw, kg/m2	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 (63.2–4213)	763 (69.9–3406)

Table 2

Characteristics of controls by quintile of AMH (pg/mL), Nurses' Health Study and Nurses' Health Study II, mean (SD) or %

	Quintile 1	Quintile 3	Quintile 5
N	94	94	95
AMH, pg/mL	85.2 (73.6)	779 (137)	4206 (2755)
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*, 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%

*With continued follow-up, after cases' diagnoses

Table 3

Odds ratios (95% confidence intervals) of breast cancer by quintile of prediagnostic plasma anti-Mullerian hormone (pg/mL), Nurses' Health Study and Nurses' Health Study II

	Q1	Q2	Q3	Q4	Q5	p-trend	
N cases/controls	102/94	99/94	81/94	124/94	133/95		
Cutpoints (pg/mL)	<225	225–<575	575–<1053	1053–<2213	≥2213		
Simple* 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^ 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** (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	

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

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

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

Table 4

Odds ratios* (95% confidence intervals) of breast cancer by quartile (with age-specific cutpoints) of prediagnostic plasma anti-Mullerian hormone (pg/mL), by tumor and participant characteristics Nurses' Health Study and Nurses' Health Study II

	N 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 <5y	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 ≥5y	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

*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 (1–8am, 9am-noon, 1pm-midnight), fasting (yes/no), luteal day (3–5, 6–7, 8–9, 10–28, untimed), race (Caucasian, other) age at menarche (<12, 12, 13, ≥14y), parity/age at first birth (nulliparous, 1–2 children/<25y, 1–2 children/≥25y, ≥3 children/<25y, ≥3 children/≥25y), BMI at age 18 (<21, 21–<23, ≥23), oral contraceptive use (never/ever), family history of breast cancer (yes/no), history of benign breast disease (yes/no)

Age-specific cutpoints: <45y <580/580–<1237/1237–<2631/≥2631; ≥45y: <87/87–<347/347–<706/≥706 pg/mL