

# Anti-Müllerian Hormone Concentrations in Premenopausal Women and Breast Cancer Risk

Hazel B. Nichols<sup>1</sup>, Donna D. Baird<sup>2</sup>, Frank Z. Stanczyk<sup>3</sup>, Anne Z. Steiner<sup>4</sup>,  
Melissa A. Troester<sup>1</sup>, Kristina W. Whitworth<sup>5</sup>, and Dale P. Sandler<sup>2</sup>

## Abstract

Laboratory models support an inverse association between anti-Müllerian hormone (AMH) and breast tumor development. Human studies are lacking; one study ( $N = 105$  cases, 204 controls) with prospectively collected serum reported the opposite—an approximate 10-fold increase in breast cancer risk comparing fourth with first quartile AMH levels. We investigated the relation between serum AMH levels and breast cancer risk in a case-control ( $N = 452$  cases, 902 controls) study nested within the prospective Sister Study cohort of 50,884 women. At enrollment, participants were ages 35 to 54, premenopausal, and completed questionnaires on medical and family history, lifestyle factors, and demographics. AMH (ng/mL) was measured by ultrasensitive ELISA in serum collected at enrollment and log-transformed for analysis. Multivariate conditional logistic regres-

sion was used to calculate ORs and 95% confidence intervals (CI) to account for matching on age and enrollment year. Mean age at enrollment was 46.8 years with an average 2.9 years from blood draw to breast cancer diagnosis ( $SD = 1.9$ ). AMH concentrations were below the limit of detection (0.003 ng/mL) for approximately 25% of samples. Compared with samples below the LOD, women with AMH >2.84 ng/mL (90th percentile among controls) had a 2-fold increase in breast cancer odds (OR, 2.25; 95% CI, 1.26–4.02). For each 1-unit increase in lnAMH, overall breast cancer odds increased by 8% (OR, 1.08; 95% CI, 1.02–1.15) and odds of estrogen receptor-positive, invasive disease increased by 15% (OR, 1.15; 95% CI, 1.05–1.25). Our findings demonstrate an overall positive relation between AMH and breast cancer. *Cancer Prev Res*; 8(6); 528–34. ©2015 AACR.

## Introduction

Anti-Müllerian hormone (AMH), also called Müllerian inhibiting substance (MIS), is a peptide hormone produced by the granulosa cells of pre- and small antral ovarian follicles, and is a member of the TGF $\beta$  family (1). Named for its role in Müllerian duct regression during the embryologic development of male fetuses, AMH is clinically used in adult women as a measure of ovarian reserve. Low circulating levels of AMH can be used to inform the potential success of fertility treatments, and high levels are associated with conditions such as polycystic ovary syndrome (2).

Available evidence suggests that AMH levels peak in the mid-20s and decline thereafter, becoming nondetectable an estimated 5 to 6 years before menopause (3–5). Unlike other ovarian-produced hormones, including estrogen and progesterone, AMH levels are relatively stable across the menstrual cycle (2). Labora-

tory-based cell line and mouse models demonstrate an inverse relationship between AMH and breast tumor development and growth through activation of the NF $\kappa$ B signaling cascade (6–9). However, these models reflect a basal-like breast cancer subtype that accounts for only 15% to 20% of breast cancers—the majority of tumors are luminal subtypes characterized, in part, by expression of estrogen or progesterone receptors (10, 11). A single prospective case-control study in humans ( $N = 105$  cases, 204 controls) observed a 9.8-fold increase in breast cancer risk (95% CI, 3.3–28.9) comparing fourth with first quartile AMH levels from archived samples donated at a mean age of 45 years (12). Although the distribution of tumor subtypes was not evaluated, the mean age at diagnosis (~59 years) suggests that the majority were likely luminal tumors (12).

Because of the lower incidence rate of breast cancer among reproductive-age women (13) and the large sample sizes required for studying the effects of AMH prospectively, there has been limited available evidence to substantiate this observed association since its publication in 2009. In a 2011 cross-sectional clinical study of 30 women ages 38 to 50 undergoing breast biopsy, lower AMH levels were found among those with a coincident cancer (14). In a 2013 case-control study ( $N = 108$  cases, 99 controls), there was no overall association between AMH and breast cancer (15). For the two latter studies, AMH measurement was performed concurrent with or subsequent to cancer diagnosis and may not reflect relative levels before tumor development.

To prospectively study the relation between serum AMH levels and breast cancer risk, we conducted a case-control study ( $N = 452$  cases, 902 controls) nested within the Sister Study cohort of 50,884 women.

<sup>1</sup>Department of Epidemiology, University of North Carolina Gillings School of Global Public Health, Chapel Hill, North Carolina. <sup>2</sup>Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. <sup>3</sup>Departments of Obstetrics and Gynecology, and Preventive Medicine, University of Southern California Keck School of Medicine, Los Angeles, California. <sup>4</sup>Department of Obstetrics and Gynecology, University of North Carolina School of Medicine, Chapel Hill, North Carolina. <sup>5</sup>The University of Texas School of Public Health, San Antonio Regional Campus, San Antonio, Texas.

**Corresponding Author:** Hazel B. Nichols, University of North Carolina Gillings School of Global Public Health, 135 Dauer Drive, Chapel Hill, NC 27599. Phone: 919-966-7456; Fax: 919-966-2089; E-mail: hazel.nichols@unc.edu

doi: 10.1158/1940-6207.CAPR-14-0377

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## Materials and Methods

The Sister Study prospective cohort was designed to address genetic and environmental risk factors for breast cancer. During 2003 to 2009, 50,884 U.S. and Puerto Rican women ages 35 to 74 were recruited through a national multimedia campaign and network of recruitment volunteers, breast cancer professionals, and advocates. Eligible women had a sister who had been diagnosed with breast cancer but did not have breast cancer themselves. This research was approved by the Institutional Review Boards of the National Institute of Environmental Health Sciences, NIH, and the Copernicus Group. All participants provided informed consent.

At enrollment, participants completed baseline questionnaires on medical and family history, lifestyle factors, and demographics. Blood samples were collected during a home visit by trained phlebotomists and shipped overnight to the Sister Study laboratory where they were processed to obtain serum and stored at  $-80^{\circ}\text{C}$ .

### Study design

To be eligible for selection in the current case-control study, Sister Study participants were required to be ages 35 to 54 at enrollment, have an archived serum sample from the enrollment visit, have at least 1 intact ovary, and be categorized as premenopausal. Premenopausal status was defined as reporting one or more menstrual cycles in the prior 12-month period on the enrollment questionnaire. Women whose only reason for not experiencing menses was hysterectomy (without bilateral oophorectomy) were categorized as premenopausal based on age  $\leq 55$ .

Among eligible participants, we identified 461 incident breast cancers diagnosed between enrollment and December 31, 2012. Breast cancer diagnoses were initially self-reported and then confirmed by medical record abstraction. For each identified case, two control participants who did not have a reported breast cancer diagnosis as of the corresponding case's diagnosis date were matched according to age (within 5 months) and year of study enrollment. Of the identified cases, 458 (99%) had sufficient archived serum for analysis. Of the matched controls, 4 had insufficient available sample and were replaced. In total, samples from 458 cases, and 916 controls were analyzed for AMH levels. We further excluded three cases who were determined after medical record review to have benign breast conditions, 2 cases with missing diagnosis dates, and 1 case with a low-quality sample (and their matched controls). We also excluded two controls (matched to different cases) due to prior prophylactic bilateral mastectomy or low-quality sample. Ultimately, 452 breast cancer cases and 902 controls contributed to these analyses.

### Laboratory assays

All assays were performed at the Reproductive Endocrinology laboratory at the University of Southern California Keck School of Medicine. Case-control trios were analyzed together in the same batch. AMH was measured primarily using an Ultrasensitive AMH ELISA kit (Ansh Labs). The picoAMH ELISA Kit (Ansh Labs) was used when AMH values were below the limit of detection (LOD) of the Ultrasensitive ELISA ( $<0.07$  ng/mL). The LOD of the picoAMH ELISA is 0.003 ng/mL. Of the 1,354 samples, 484 (154 cases, 330 controls) did not have detectable levels by the Ultrasensitive ELISA and were analyzed by the picoAMH ELISA. Of these, 130 (47 cases, 83 controls) had detectable concentra-

tions using the picoAMH. The manufacturer-specified interassay coefficients of variation are as follows: 4.6%, 4.8%, and 2.0% at 0.346, 0.715, and 1.85 ng/mL, respectively, for the Ultrasensitive AMH assay, and 4.5%, 2.2%, and 3.8% at 22.6, 86.5, and 373 pg/mL, for the picoAMH assays. In our study, blinded control samples (mean = 1.89 ng/mL) were run in duplicate in the first 39 batches with interassay CVs of 14.5%. Testosterone was measured by radioimmunoassay with preceding organic solvent extraction and Celite column partition chromatography (16). The assay sensitivity is 1.5 ng/dL and the interassay CVs are 8%, 12%, and 12% at 13, 30, and 96 ng/dL, respectively.

### Statistical analysis

Serum AMH levels were skewed with a long tail to the right; therefore, natural log-transformed values were calculated to approximate a normal distribution for analysis as a continuous variable. When analyzed as a continuous variable, AMH samples that fell below the detectable range were imputed as 0.0015 ng/mL (the midpoint between 0 and the LOD, 0.003 ng/mL). AMH categories were defined as samples below the LOD, and at the 25th, 50th, 75th, and 90th percentiles among controls. Quartiles of total testosterone were defined on the basis of the distribution of controls with the first quartile set as the reference category.

Age-adjusted geometric means for natural log-transformed AMH were calculated using generalized linear regression models according to characteristics among control participants. We used LOESS smoothing with local quadratic regression to descriptively model the relationship between age and AMH separately for cases and controls.

To account for the matched case-control design, we used multivariable conditional logistic regression, conditioned on the matched sets, to calculate ORs and 95% confidence intervals (CI) for breast cancer. Potential confounders were identified *a priori* from the breast cancer and AMH literature and evaluated in age-controlled models. Final statistical models accounted for age and enrollment year through matching and adjusted for age at menarche (continuous), current oral contraceptive use (yes/no), smoking status (never/former/current), parity, and breastfeeding (nulliparous/parous, never breastfed/parous, breastfed), body mass index (BMI; underweight:  $<18.5$  kg/m<sup>2</sup>, normal: 18.5–24.9 kg/m<sup>2</sup>, overweight: 25.0–29.9 kg/m<sup>2</sup>, obese  $\geq 30.0$  kg/m<sup>2</sup>), and total testosterone (ln ng/dL) as potential confounders. To statistically evaluate trends, we included select variables in regression models as continuous linear terms or categorical ordinal terms, and report *P* values and the *P*<sub>trend</sub>, respectively. To test for effect modification by age at diagnosis ( $<45$  vs.  $\geq 45$ ) and estrogen receptor (ER) status (positive vs. negative), we included cross-product interaction terms in conditional logistic models and report the *P*<sub>interaction</sub>. Although ER status is an attribute of cases, to test for interaction ER status is held constant at the level of the matched set (17, 18). The interpretation of the interaction test is, therefore, whether the association between AMH and case status is differential in matched sets where the case is ER positive versus ER negative. All statistical analyses were performed with Sister Study Data Release 3.2 (August 2014) using SAS 9.3 (SAS Institute, Inc.).

## Results

Study participant characteristics according to case-control status are provided in Table 1. The average age at study enrollment

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**Table 1.** Study participant characteristics among 452 breast cancer cases and 902 controls, Sister Study, 2003–2009

|   | Breast cancer cases |       | Controls |       |
|---|---------------------|-------|----------|-------|
|   |                     |       |          |       |
| Age at enrollment, mean (y; SD)             | 46.8                | (4.4) | 46.8     | (4.4) |
| Age at menarche, y                          |                     |       |          |       |
| <12   | 86                  | 19.0  | 178      | 19.7  |
| 12  | 133                 | 29.4  | 239      | 26.5  |
| 13  | 131                 | 29.0  | 243      | 26.9  |
| ≥14   | 94                  | 20.8  | 240      | 26.6  |
| Currently using oral contraceptives, N (%)  |                     |       |          |       |
| No  | 404                 | 89.4  | 839      | 93.0  |
| Yes   | 48                  | 10.6  | 63       | 7.0   |
| Smoking status, N (%)                       |                     |       |          |       |
| Never                                       | 288                 | 63.7  | 554      | 61.4  |
| Former                                      | 138                 | 30.5  | 272      | 30.2  |
| Current                                     | 26                  | 5.8   | 76       | 8.4   |
| Parity and breastfeeding, N (%)             |                     |       |          |       |
| Nulliparous                                 | 113                 | 25.0  | 196      | 21.7  |
| Parous, never breastfed                     | 70                  | 15.5  | 132      | 14.6  |
| Parous, breastfed                           | 269                 | 59.5  | 573      | 63.5  |
| BMI (kg/m <sup>2</sup> ), N (%)             |                     |       |          |       |
| <18.5                                       | 4                   | 0.9   | 14       | 1.6   |
| 18.5–24.9                                   | 205                 | 45.4  | 397      | 44.0  |
| 25–29.9                                     | 137                 | 30.3  | 248      | 27.5  |
| ≥30   | 106                 | 23.5  | 242      | 26.8  |
| Total testosterone (ng/dL), N (%)           |                     |       |          |       |
| ≤20   | 90                  | 19.9  | 204      | 22.6  |
| 20.1–27                                     | 118                 | 26.1  | 236      | 26.2  |
| 27.1–35                                     | 119                 | 26.3  | 231      | 25.6  |
| ≥35.1                                       | 116                 | 25.7  | 219      | 24.3  |
| Undetectable/insufficient sample            | 9                   | 2.0   | 12       | 1.3   |
| Hysterectomy, N (%) <sup>a</sup>            |                     |       |          |       |
| No  | 394                 | 87.2  | 777      | 86.1  |
| Yes   | 58                  | 12.8  | 125      | 13.9  |
| Unilateral oophorectomy, N (%) <sup>a</sup> |                     |       |          |       |
| No  | 431                 | 95.4  | 848      | 94.0  |
| Yes   | 21                  | 4.6   | 54       | 6.0   |
| Polycystic ovary syndrome (PCOS), N (%)     |                     |       |          |       |
| No  | 434                 | 96.0  | 872      | 96.7  |
| Yes   | 18                  | 4.0   | 27       | 3.0   |

<sup>a</sup>All women were required to have ≥1 intact ovary for selection into the nested case-control study.

and blood draw for breast cancer cases (and matched controls) was 46.8 years. Compared with control participants, a higher proportion of breast cancer cases reported currently using oral contraceptives (10.6% vs. 7.0%) and being nulliparous (25.0% vs. 21.7%), whereas a lower proportion reported currently smoking (5.8% vs. 8.4%).

Geometric means for serum AMH levels according to select characteristics among control participants are displayed in Fig. 1. AMH levels declined with increasing age at blood draw ( $P < 0.001$ ) and were lower among women with younger ages at menarche ( $P = 0.02$ ) and current oral contraceptive users ( $P = 0.002$ ). BMI had a U-shaped association with AMH: women with underweight and obese BMIs had lower AMH than those in the normal to overweight range ( $P = 0.04$ ). Prior hysterectomy or oophorectomy was associated with lower AMH levels compared with women without a hysterectomy or unilateral oophorectomy (0.04 vs. 0.08 ng/mL for each;  $P \leq 0.02$ ). Although not statistically significant, AMH concentrations appeared lower among current cigarette smokers compared with never smokers, higher with increasing quartiles of total testosterone, and among women with polycystic ovary syndrome. Parity and breastfeeding were not associated with mean AMH.

The average time from blood draw to breast cancer diagnosis was 2.9 years (SD = 1.9; range, 1 month–8.4 years). Approximately 25% of samples had undetectable AMH levels (Table 2); increasing from ≤2% of cases and controls ages 35 to 40 to 54% to 57% of cases and controls ages 50 to 54 at blood draw. AMH levels according to age at blood draw among cases and controls, and the number of samples below the LOD by case and control status, are provided in Fig. 2.

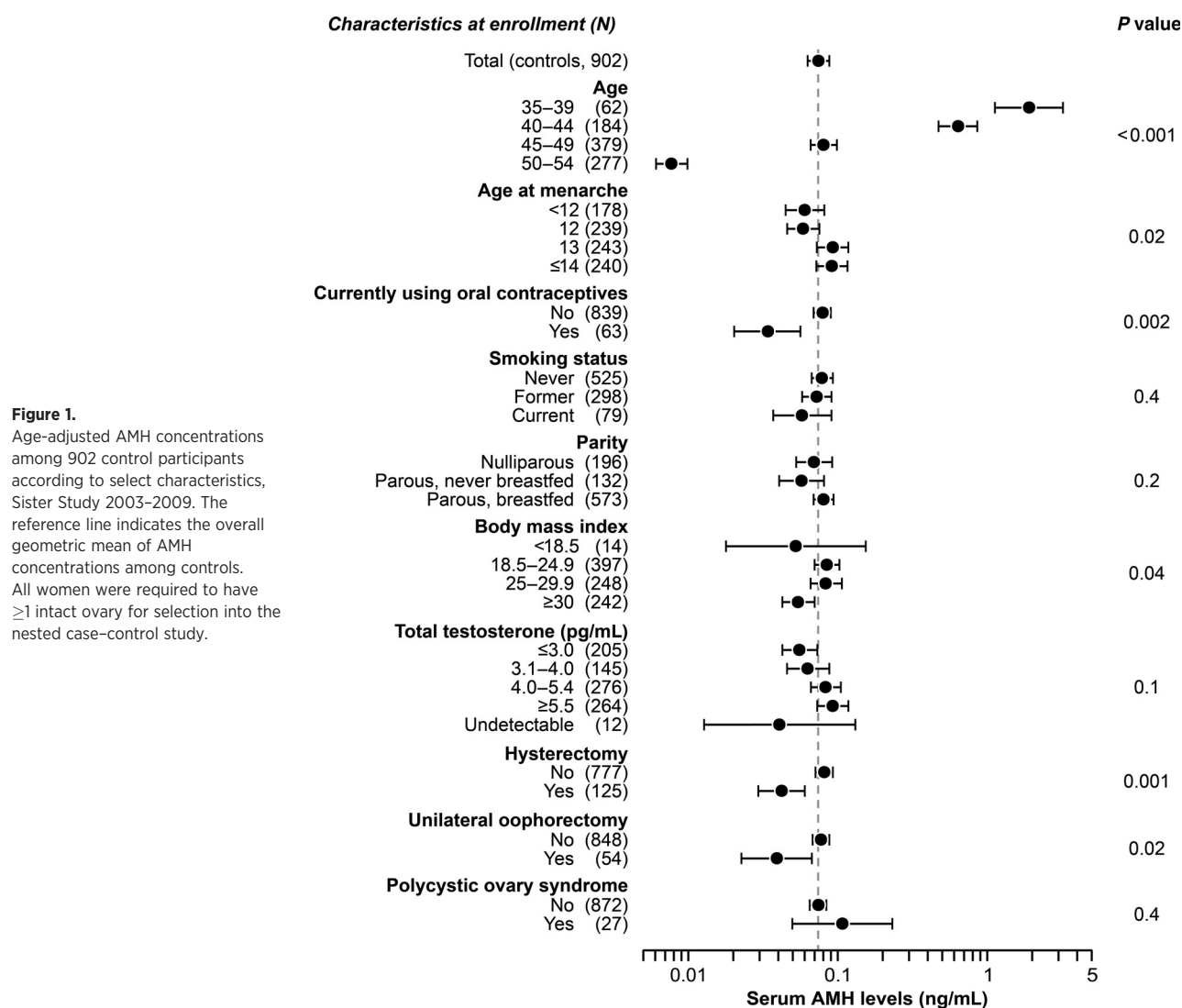
Overall, we observed a positive linear trend between increasing serum AMH levels and breast cancer odds (Table 1–3). Compared with women with AMH values below the LOD (0.003 ng/mL), women with AMH >2.84 ng/mL (the 90th percentile among controls) had an approximate 2-fold increase in breast cancer odds (OR, 2.25; 95% CI, 1.26–4.02). For each 1-unit increase in lnAMH, breast cancer odds increased by 8% (OR, 1.08; 95% CI, 1.02–1.15; Table 2–3). In sensitivity analyses restricted to women with an intact uterus ( $N = 394$  cases, 777 controls), women with intact ovaries ( $N = 431$  cases, 848 controls) or who were not currently using oral contraceptives ( $N = 404$  cases, 839 controls), effect estimates for the association between continuous lnAMH and breast cancer risk were virtually unchanged (OR, 1.09; 95% CI, 1.02–1.17; OR, 1.08; 95% CI, 1.01–1.014; and OR, 1.09; 95% CI, 1.03–1.16, respectively).

The positive trend between lnAMH and breast cancer odds was not strongly influenced by multivariate adjustment for age at menarche, current oral contraceptive use, smoking status, parity and breastfeeding, BMI, or total testosterone levels (Table 2). In breast cancer models stratified according to ER expression, the positive trend with AMH remained elevated for ER-positive tumors (OR, 1.12; 95% CI, 1.04–1.20), but not ER-negative tumors (OR, 1.02; 95% CI, 0.87–1.19). This difference in the association between AMH and breast cancer according to ER status was not statistically significant by formal interaction tests ( $P_{\text{interaction}} = 0.1$ ). Similarly, a positive trend with AMH concentrations was observed for women with breast cancer diagnosed at ages 46 to 50 and 51 to 60 (OR, 1.12; 95% CI, 1.02–1.23 and OR, 1.11; 95% CI, 1.02–1.22, respectively), but was not statistically significantly different from diagnosis ages 35 to 45 (OR, 0.95; 95% CI, 0.91–1.11;  $P_{\text{interaction}} = 0.2$ ). The association between AMH and breast cancer appeared similar for invasive (OR, 1.10; 95% CI, 1.02–1.18) and ductal carcinoma *in situ* disease (DCIS, OR, 1.06; 95% CI, 0.93–1.20; Table 3). We further evaluated the potential influence of time from blood draw to diagnosis on AMH associations with breast cancer. Estimates for tumors diagnosed within 2 years of blood draw versus >2 years were similar (Table 3).

## Discussion

In our sample of women diagnosed with breast cancer at ages 35 to 60, prediagnosis serum AMH levels were positively associated with breast cancer risk compared with age-matched controls. The positive trend appeared strongest for women diagnosed at older ages with ER-positive tumors, although interaction tests with age and ER status were not statistically significant.

Previous experimental studies have suggested that AMH may play an important role in breast cancer risk. AMH receptors (MIS type II) are expressed in both normal and cancerous breast tissue. Contrary to our findings, several *in vitro* and *in vivo* studies have shown AMH can inhibit tumor cell growth and migration through NFκB-mediated pathways (8, 9, 19). However, these studies have



focused on basal-like breast cancer models such as the C3Tag mouse model or the MCF10A cell line, and therefore may not be comparable with a study examining all breast cancers. Basal-like breast cancers represent only 15% to 20% of breast cancers overall (10, 11, 20), and distinct effects in those tumors would not be readily detectable in our sample, which included a majority of ER-positive breast cancers

Although basal-like breast cancer represents the minority of tumors overall, basal-like subtypes are proportionally more common among younger women (e.g., 26.5% of tumors at ages 40–49 vs. 17.5% at ages 60–69 in Sweeney and colleagues; ref. 21). Epidemiologic studies of AMH and breast cancer diagnosed at reproductive ages are lacking. A cross-sectional study of 30 women ages 38 to 50 undergoing breast biopsies found lower AMH levels ( $P = 0.0009$ ) among women with a cancer finding versus a benign diagnosis (14). A case-control study ( $N = 108$  cases, 99 controls) using post-diagnosis, pretreatment serum samples reported no overall association between mean AMH and breast cancer, but an inverse association among women diagnosed after age

37 ( $\beta = -0.85$ , 95% CI,  $-1.48$  to  $-0.22$ ). These findings are consistent with the laboratory data, but the small sample sizes and use of AMH measures collected concurrently or after breast cancer diagnosis are problematic for evaluating AMH as a predictive factor. Other elements of the case-control study design also limit interpretation: Cases were identified from oncology clinics in Pennsylvania and California, whereas controls were North Carolina residents participating in a fecundability study. Furthermore, the median age at enrollment was 40.2 years among cases and 33.9 among controls (15).

One prior epidemiologic study analyzed prediagnosis AMH concentrations in relation to breast cancer risk, and unlike the other reports, observed a positive association between AMH and breast cancer risk (12). Using a nested case-control design ( $N = 108$  cases, 99 controls), AMH concentrations were measured in archived blood samples donated at an average age of 45 years. After a mean follow-up of 14 years, 105 breast cancers were diagnosed. The authors reported a 9.8-fold increase in breast cancer odds comparing fourth versus first quartile AMH (OR,

**Table 2.** ORs and 95% CI breast cancer for incident breast cancer according to prediagnosis AMH concentrations

|   | Cases<br>(N = 452) | Controls<br>(N = 902) | OR (95% CI) <sup>a</sup> | OR (95% CI) <sup>b</sup> |
|---|--------------------|-----------------------|--------------------------|--------------------------|
| AMH (ng/mL)                                 |                    |                       |                          |                          |
| Nondetectable                               | 107                | 247                   | 1                        | 1                        |
| 0.003–0.10                                  | 78                 | 166                   | 1.16 (0.81–1.67)         | 1.23 (0.84–1.81)         |
| 0.10–0.37                                   | 88                 | 166                   | 1.39 (0.94–2.06)         | 1.56 (1.04–2.35)         |
| 0.38–1.17                                   | 85                 | 162                   | 1.44 (0.96–2.15)         | 1.56 (1.02–2.39)         |
| 1.17–2.84                                   | 52                 | 96                    | 1.60 (0.98–2.64)         | 1.81 (1.08–3.05)         |
| >2.84                                       | 42                 | 65                    | 1.96 (1.13–3.40)         | 2.25 (1.26–4.02)         |
| <i>P</i> <sub>trend</sub> (categorical AMH) |                    |                       | 1.13 (1.03–1.25)         | 1.16 (1.05–1.29)         |
| <i>P</i> (continuous lnAMH)                 |                    |                       | 1.06 (1.01–1.13)         | 1.08 (1.02–1.15)         |

<sup>a</sup>Adjusted for age and enrollment year through matching.  
<sup>b</sup>Additionally adjusted for age at menarche, current oral contraceptive use, smoking status, parity, breastfeeding, BMI, and total testosterone.

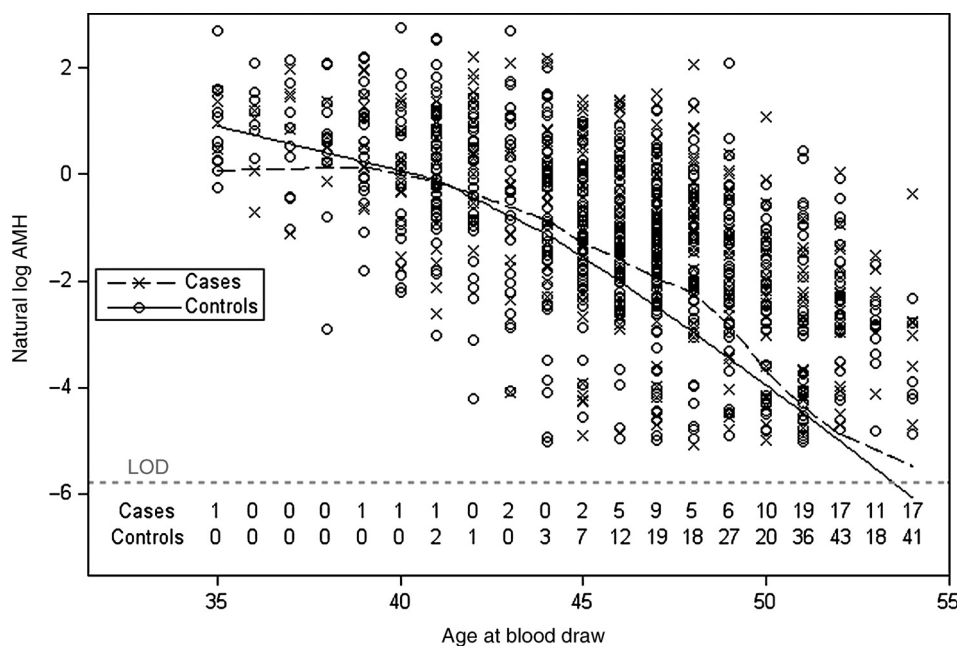
9.8; 95% CI, 3.3–28.9). This effect was most apparent among women diagnosed at ages ≥55 years (12).

On the basis of the laboratory models and few human studies, we hypothesized that the direct effect of circulating AMH on breast tissue would be antitumorigenic such that higher levels of AMH would be associated with decreased risk for premenopausal breast cancer, whereas higher relative levels of AMH before menopause would be positively associated with older-onset breast cancer as a marker of delayed menopause, an established breast cancer risk factor. In our analysis, we were unable to identify the hypothesized inverse association except by the weak, nonsignificant association among women diagnosed with breast cancer at ages 35 to 45. However, only 64 cases were characterized as ER-negative, and only a subset of these would likely meet the full definition of the basal-like subtype (10, 11).

The increased risk of breast cancer among older women with higher AMH may reflect an older age at menopause. As noted above, AMH is produced by the granulosa cells of pre- and small antral ovarian follicles. As the number of ovarian follicles is depleted over the life course, AMH levels decline until they become

undetectable before menopause. In the Penn Ovarian Aging Study, women ages 35 to 48 with undetectable AMH concentrations (lower LOD = 0.10 ng/mL) had a median time to menopause of 6 years (3). Similarly, the longitudinal Michigan Bone Health and Metabolism study demonstrated undetectable (<0.17 ng/mL) AMH concentrations approximately 5 years before the final menstrual period (3). In these studies and others (22, 23), higher relative AMH concentration was a marker for delayed menopause (2, 24) within a given age group. Older menopausal age confers a small, but consistently observed, increase in breast cancer odds. In Norwegian registry data, a 1-year difference in menopausal age was estimated to confer a 4% increase in breast cancer odds (25).

Overall, our findings agree with the prior report by Dorgan and colleagues, although the magnitude of the positive association is smaller in our study. In the Dorgan study, control participants were matched 2:1 to the cases on age and other factors and were selected from a pool of premenopausal women based on menstrual status and serum follicle-stimulating hormone and estradiol concentrations. In contrast, our study was not restricted to women with hormone levels consistent with premenopausal status. Instead, we relied upon self-report of one or menstrual cycles in the previous 12 months, a common definition for menopausal status in large epidemiologic studies (26). Furthermore, for women who reported a prior hysterectomy without removal of both ovaries, we applied an age-based cutoff of <55 years to identify potentially premenopausal women. These criteria may lead to some misclassification of menopausal status and the possible inclusion of peri- or post-menopausal women. On the basis of our definition of menopause, our analytic population likely had a lower distribution of AMH levels compared with the study population in the Dorgan and colleagues report. Consistent with Dorgan and colleagues, we observed a stronger effect estimate among women diagnosed at older ages. Furthermore, we observed the same pattern of association in sensitivity analyses that excluded women with a prior hysterectomy where the misclassification of menopausal status is likely greatest.



**Figure 2.** AMH values according to age at blood draw among breast cancer cases and controls, Sister Study, 2003–2009. The number of samples below the LOD are shown according to case and control status (e.g., 1 case and 0 controls below the LOD at age 35 and 17 cases and 41 controls below the LOD at age 54).

**Table 3.** ORs and 95% CI breast cancer for incident breast cancer according to prediagnosis AMH concentrations (continuous ln AMH); stratified by sub-classifications of breast cancer

| AMH (ln ng/mL)                        | Cases | Controls | OR (95% CI) <sup>a</sup> |
|---------------------------------------|-------|----------|--------------------------|
| All breast cancer                     | 443   | 890      | 1.08 (1.02-1.15)         |
| ER expression                         |       |          |                          |
| ER-positive                           | 332   | 665      | 1.12 (1.04-1.20)         |
| ER-negative                           | 64    | 129      | 1.02 (0.87-1.19)         |
| Extent of disease                     |       |          |                          |
| Invasive                              | 302   | 603      | 1.10 (1.02-1.18)         |
| DCIS                                  | 110   | 225      | 1.06 (0.93-1.20)         |
| Age at diagnosis                      |       |          |                          |
| 35-45                                 | 81    | 165      | 0.95 (0.81-1.11)         |
| 46-50                                 | 167   | 335      | 1.12 (1.02-1.23)         |
| 51-60                                 | 194   | 388      | 1.11 (1.02-1.22)         |
| Time between blood draw and diagnosis |       |          |                          |
| ≤2 years                              | 80    | 163      | 1.10 (1.00-1.20)         |
| >2 years                              | 160   | 315      | 1.07 (1.00-1.16)         |
| ER-positive invasive breast cancer    | 240   | 478      | 1.15 (1.05-1.25)         |

<sup>a</sup>Adjusted for age and enrollment year (through matching), age at menarche, current oral contraceptive use, smoking status, parity, breastfeeding, BMI, and total testosterone.

Newly developed, ultrasensitive AMH assays allow for high-quality AMH measurement at older reproductive ages and were used in this analysis (27, 28). Nevertheless, we observed an increasing proportion of samples with nondetectable levels with increasing age from 35 to 54–50% of women 50 and older had nondetectable values. AMH levels are relatively stable across the menstrual cycle (2), and therefore are amenable to measurement in large epidemiologic cohorts, like the Sister Study, where blood draws are not timed to the menstrual cycle. However, one study of 27 women reported greater variability in AMH across the menstrual cycle at ages 45 to 55 years compared with 18 to 35 years (29); if variability is greater at older reproductive ages, our effect estimates could be biased toward the null due to exposure misclassification.

Testosterone levels have less variability across the menstrual cycle compared with estradiol and progesterone and are positively associated with pre- and post-menopausal breast cancer (30). Testosterone concentrations are also positively correlated with AMH levels (31, 32) and were controlled for in the current analysis. We did not measure estradiol levels because the blood draw was not timed to the menstrual cycle and premenopausal estradiol has a weaker association with breast cancer risk compared with postmenopausal levels (30, 33, 34). In the Dorgan and

colleagues report, additional adjustment for estradiol did not influence the association between AMH and breast cancer (12).

In our study, where 75% of breast cancers were diagnosed after age 45 and 81% were ER positive, we observed a positive association between prospectively collected serum AMH and breast cancer risk. A larger sample of women with a younger age distribution or higher proportion of basal-like tumors is needed to conclusively address possible protective effects of AMH on breast cancer. Quantifying expression of AMH receptors in breast tissue and polymorphisms in the AMH and AMH type II receptor genes may also provide a more complete picture of the underlying biology in future studies.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Authors' Contributions

**Conception and design:** H.B. Nichols, D.D. Baird, F.Z. Stanczyk, A.Z. Steiner, M.A. Troester, K.W. Whitworth, D.P. Sandler

**Development of methodology:** H.B. Nichols, D.D. Baird, F.Z. Stanczyk, D.P. Sandler

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** H.B. Nichols, F.Z. Stanczyk

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** H.B. Nichols, D.D. Baird, F.Z. Stanczyk, M.A. Troester, K.W. Whitworth

**Writing, review, and/or revision of the manuscript:** H.B. Nichols, D.D. Baird, F.Z. Stanczyk, A.Z. Steiner, M.A. Troester, K.W. Whitworth, D.P. Sandler

**Study supervision:** D.P. Sandler

### Acknowledgments

The authors appreciate the helpful comments of Drs. Anne Marie Jukic and Matthew Longnecker, laboratory and data support from Cynthia Kleeberger and Melissa House, and statistical advice from Dr. Mark Weaver.

### Grant Support

This research was supported in part by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences (Z01-ES044005; to D.P. Sandler), the Avon Foundation (02-2012-085; to H.B. Nichols and D.P. Sandler), and by the National Center for Advancing Translational Sciences (KL2-TR001109 and UL1-TR001111; to H.B. Nichols).

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Received October 23, 2014; revised March 27, 2015; accepted April 1, 2015; published OnlineFirst April 14, 2015.

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*Cancer Prev Res* 2015;8:528-534. Published OnlineFirst April 14, 2015.

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