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Circulating Anti-Müllerian Hormone and Breast Cancer Risk: A Study in Ten Prospective Cohorts

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Abstract

A strong positive association has been observed between circulating anti-Müllerian hormone (AMH), a biomarker of ovarian reserve, and breast cancer risk in three prospective studies. Confirming this association is important because of the paucity of biomarkers of breast cancer risk in premenopausal women. We conducted a consortium study including ten prospective cohorts that had collected blood from premenopausal women. A nested case-control design was implemented within each cohort. A total of 2,835 invasive (80%) and *in situ* (20%) breast cancer cases were individually matched to controls (n = 3,122) on age at blood donation. AMH was measured using

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a high sensitivity enzyme-linked immunoabsorbent assay. Conditional logistic regression was applied to the aggregated dataset. There was a statistically significant trend of increasing breast cancer risk with increasing AMH concentration (p_{trend} across quartiles < 0.0001) after adjusting for breast cancer risk factors. The odds ratio (OR) for breast cancer in the top versus bottom quartile of AMH was 1.60 (95% CI = 1.31-1.94). Though the test for interaction was not statistically significant ($p_{interaction}$ = 0.15), the trend was statistically significant only for tumors positive for both estrogen receptor (ER) and progesterone receptor (PR): ER+/PR+: OR_{Q4-Q1} = 1.96, 95% CI = 1.46-2.64, p_{trend} <0.0001; ER+/PR-: OR_{Q4-Q1} = 0.82, 95% CI = 0.40-1.68, p_{trend} = 0.51; ER-/PR+: OR_{Q4-Q1} = 3.23, 95% CI =0.48-21.9, p_{trend} = 0.26; ER-/PR-: OR_{Q4-Q1} = 1.15, 95% CI = 0.63-2.09, p_{trend} = 0.60. The association was observed for both pre- (OR_{Q4-Q1}= 1.35, 95% CI= 1.05-1.73) and post-menopausal (OR_{Q4-Q1} =1.61, 95% CI = 1.03 - 2.53) breast cancer ($p_{interaction}$ = 0.34). In this large consortium study, we confirmed that AMH is associated with breast cancer risk, with a 60% increase in risk for women in the top vs. bottom quartile of AMH.

Keywords

Breast cancer; anti-mullerian hormone; AMH; nested case-control study

Introduction

Anti-Müllerian hormone (AMH) is produced in the ovaries by the granulosa cells of pre-antral and early antral follicles [1]. Circulating AMH is present in females at birth, peaks around age 20-25, and becomes undetectable after menopause, when the ovarian follicle reserve is depleted [2]. AMH concentration has been shown to reflect the size of the follicular pool [3] and is a strong predictor of age at menopause [4-6].

The hypothesis that AMH plays a role in breast cancer development came from laboratory experiments that showed AMH stimulates apoptosis and reduces breast tumor growth [7-9], suggesting a protective role. On the other hand, the strong positive correlation of AMH with age at menopause suggests that women who have higher AMH could be at higher risk of breast cancer than women of the same age with lower AMH, because they are expected to reach menopause at a later age and thus have longer remaining duration of exposure to high concentrations of steroid sex hormones [10, 11].

A small cross-sectional study reported an inverse association of AMH concentration with breast cancer [12] and a case-control study found no association [13]. However, AMH was measured at or after diagnosis, and might not reflect the AMH concentration before cancer development. In 2009, Dorgan et al. reported a strong positive association between AMH concentration and risk of breast cancer in a case-control study nested within the Columbia, Missouri Serum Bank [14]. Subsequently, two other reports from prospective studies (the Sister Study [15] and the Nurses' Health Studies (NHS and NHSII) [16]) also reported a positive, though weaker, association. Confirming the AMH-risk association is important because of the paucity of biomarkers in premenopausal women: while sex hormones (estrogens and androgens) measured in postmenopausal women are strongly associated with breast cancer risk [17], they show only weak associations when measured in premenopausal women [18, 19].

We report here on a collaborative study that had for objectives to confirm the AMH-breast cancer risk association in a large study and to examine this association in relevant subgroups (i.e., by invasiveness, tumor receptor status, menopausal status at diagnosis, and various baseline characteristics). Ten prospective cohorts participated, including the four cohorts that previously published on this topic.

Methods

Study Design and Case and Control Selection

The ten participating cohorts are: Breakthrough Generations Study (BGS); Campaign Against Cancer and Heart Disease (CLUE II); Columbia, Missouri Serum Bank (CSB); Guernsey cohort (Guernsey); Nurses' Health Study (NHS); Nurses' Health Study II (NHSII); Northern Sweden Mammography Screening Cohort (NSMSC); New York University Women's Health Study (NYUWHS); Hormones and Diet in the Etiology of Breast Cancer (ORDET); and the Sister Study. These cohorts are briefly described in Table 1. Each cohort was approved by its institutional review board.

A nested case-control design was used. With the exception of the Sister Study, which joined this collaborative effort later [15], all cohorts used the same general selection procedures. Eligibility criteria for cases and controls were: 1) premenopausal women of any age (or age <50 years if menopausal status was unknown, for example due to hysterectomy) at blood donation; 2) no prior diagnosis of cancer (except non-melanoma skin cancer); 3) no history of bilateral oophorectomy; and 4) no current or prior use of hormone therapy. Incident cases of invasive or *in situ* breast cancer were included. Within each cohort, one control was selected for each case using incidence density sampling; matching factors included age and date at blood donation (age-matching criteria for different cohorts ranged from age ±6 mo to ±2 yrs, except ORDET which matched on age ±3 yrs and CSB which used ±5 yrs). Only 162 (6%) had a difference in age ≥ 2 years and only 30 case-control pairs (1%) had a difference in age ≥ 3 years. Some cohorts had additional matching criteria (appendix Table 1 [14-16, 19-27]). In the NHS and NHSII, cases diagnosed after menopause were not included [16]. The differences in procedures for the Sister Study [15] were: 1) in addition to being premenopausal at blood donation, women had to be between the ages of 35 and 54; 2) two controls were selected for each case; and 3) women reporting use of hormone therapy were included in the initial study but are excluded from this report.

Laboratory Assays

With the exception of the Sister Study, AMH concentration was measured using a picoAMH enzyme-linked immunoabsorbent assay (Ansh Labs, Webster, TX). NYUWHS samples were measured at Massachusetts General Hospital (MGH) and samples from the other eight cohorts were subsequently measured at Ansh Labs due to the closure of the MGH laboratory. Each batch (up to 70 samples per batch) contained 2-4 blinded quality control samples. Samples from a case and her matched control(s) were assayed together in the same batch. The samples were labeled in such a way that the laboratory was blinded with respect to case/control or quality control status. The overall cohort-specific coefficients of variation (CVs) were <10%, except for the NYUWHS (CV = 17%). The Sister Study samples were

measured at the University of Southern California using an Ultrasensitive ELISA (Ansh Labs, Webster, TX), and samples below the lower limit of detection of this assay (0.5 pmol/l) were re-measured using the picoAMH ELISA. The inter-batch CVs in the Sister Study were 14.5% [15].

We conducted a calibration study to examine how NYUWHS and Sister Study measurements compared to measurements performed at Ansh Labs, where the samples from the 8 other cohorts were analyzed. Excellent agreement (intraclass as well as Pearson correlations > 0.98, Appendix Figure 1) was found for both cohorts. Thus, we did not calibrate the AMH measurements.

Testosterone had been measured previously for 70% of the matched sets using methods described in [15, 19, 24, 25, 28-30] (see also Supplementary Methods). For the remaining 30% (all sets from CLUE II, NHS, and NSMSC plus a subset of sets from Guernsey, NYUWHS, and ORDET cohorts), testosterone was newly measured at the Mayo Clinic Endocrine Laboratory using LC-MS/MS. Intra- and inter-batch CVs were <7% and <9%, respectively. Previous testosterone measurements were calibrated to the Mayo Clinic LC-MS/MS assay (see Supplementary Methods).

Covariate Data

Each cohort sent individual data on breast cancer risk factors and factors possibly related to AMH concentration to NYU, where data harmonization was conducted. Data collected closest to blood draw were used. Data on subsequent age at menopause were also obtained (except for CSB and NSMSC which did not send follow-up questionnaires).

Statistical Analysis

Subjects whose AMH concentration was below the lowest detectable value (range <2%-18% depending on cohort, Table 3) were assigned the lowest detectable value (LDV) for their cohort (LDV differed by cohort due to different dilution factors) divided by √2. Samples with AMH above the highest detectable value (n=14) were set to the highest detectable value. AMH concentration was $\log_2$-transformed to normalize its distribution.

Conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs) for the association of AMH with breast cancer risk. Our main analyses were based on cohort-specific quartiles, defined using the controls' distribution. We also conducted analyses using consortium-wide quartiles. Restricted cubic splines were used to assess deviation from linearity [31].

Because the number of cases in some cohorts was fairly small, which was a concern for subgroup analyses, our main analyses are based on the aggregated data, i.e. combining individual data from all cohorts. We also conducted an analysis using a two-stage approach, estimating ORs within each cohort prior to pooling using a random-effects model [32].

Potential confounders included in the multivariate model were: race, education, BMI, age at menarche, parity, age at first full-term pregnancy (FTP), oral contraceptive use, partial/unilateral oophorectomy, family history of breast cancer, history of benign breast biopsy, and

smoking. For all continuous variables, only a small proportion (< 3%) of data was missing and we used the cohort-specific median for imputation. For categorical variables with missing data, an 'unknown' category was created. We also conducted analyses adjusting for total testosterone (ordered cohort-specific quartiles) in addition to these factors.

Stratified analyses were conducted to examine whether the AMH-breast cancer risk association varied according to participant or tumor characteristics. All tests for heterogeneity and effect modification were performed by comparing models with/without an interaction term between the covariate and ordered categorical AMH. The Wald test was used to assess the statistical significance of the interactions. All tests for interaction used cohort-specific AMH quartiles (coded as ordered categories 1, 2, 3, 4) and each of the other variables as categorical variables with unordered levels (as shown in the tables). For analyses stratified by age-related covariates (age at blood draw, age at diagnosis/index date (for controls, the date of diagnosis of the matched case), and menopausal status at diagnosis/index date), we used AMH quartiles based on the controls' distribution within each of four age-at-blood-draw categories (≤40, 41-44, 45-49, ≥50) within each cohort. The unconditional logistic regression model, adjusted for age at blood draw and cohort, gave results very similar to the conditional model; therefore, we used unconditional logistic regression, adjusting for age and cohort, in analyses stratified by characteristics which were not matching variables, in order to include the maximum number of subjects in the analysis.

All analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). All tests were two-sided and were considered statistically significant if $p < 0.05$.

Results

A total of 2,835 breast cancer cases and 3,122 controls were included in the study. Participant characteristics are described in Table 2 for the whole consortium, and in Appendix Table 2 for each cohort. The majority of subjects (>65%) were between the ages of 40 and 49 at blood draw. Overall, the differences between cases and controls were as expected. Controls had a higher proportion of obese women than cases, as expected in premenopausal women. More cases than controls were nulliparous or had their first FTP after age 30. Cases were more likely to have a first-degree family history of breast cancer and a history of benign breast biopsy. The proportion of current users of oral contraceptives was small (cases: 6.2%, controls: 5.7%), reflecting the fact that this was an exclusion criterion in several cohorts.

The AMH assay results are shown in Table 3. The geometric mean AMH for controls varied by cohort, with a >4-fold difference between the lowest and highest values (0.71 pmol/l in the NSMSC and 5.21 pmol/l in NHSII). Adjusting for age, which was strongly related to AMH (Spearman correlation coefficient = -0.67), reduced these differences (2.3-fold difference: 1.36 pmol/l in Guernsey to 3.15 pmol/l in NHSII), though they remained statistically significant. In all cohorts except Guernsey, the age-adjusted geometric mean for cases was higher than for controls.

The ORs for breast cancer in relation to AMH quartiles are shown in Table 4. In univariate analysis, there was a statistically significant trend of increasing risk with increasing AMH concentration ($OR_{Q4\text{-}Q1}$ = 1.64 (95% CI= 1.35-1.98); p_{trend} < 0.0001). Results were similar after adjustment for potential confounders ($OR_{Q4\text{-}Q1}$ = 1.60, 95% CI= 1.31-1.94; p_{trend} < 0.0001). Further adjusting for testosterone did not substantially alter the ORs, nor did removing one cohort at a time (data not shown). The ORs remained statistically significant after simultaneously excluding the four cohorts that published previously ($OR_{Q4\text{-}Q1}$= 1.38, 95% CI= 1.07-1.79). Odds ratios were not appreciably different in analyses using consortium-wide AMH quartiles (Appendix Table 3). The spline analysis showed no evidence of deviation from linearity (p = 0.13). Results were similar in the two-stage analysis (multivariate-adjusted $OR_{Q4\text{-}Q1}$ = 1.66, 95% CI= 1.30-2.12; Figure 1), which showed no evidence of heterogeneity by cohort (I^2 = 22.7%, p = 0.23).

Analyses stratified by tumor characteristics are shown in Table 5. We did not see evidence of heterogeneity by invasive/*in situ* status. While several assessments of joint receptor status have supported the idea that ER-/PR+ tumors occur infrequently [33-36], others did not find this joint receptor subtype to be reproducible [37-39]. Because there has not yet been a resolution and we did not have the tumor tissues to re-assess receptor status with current IHC methods, we show analyses both by single and joint ER/PR receptor status. Although the interaction test was not statistically significant ($p_{interaction}$ = 0.21), the association between AMH and risk was statistically significant for ER+ ($OR_{Q4\text{-}Q1}$ = 1.74, 95% CI = 1.33-2.28; p_{trend} <0.0001) but not for ER- tumors ($OR_{Q4\text{-}Q1}$ = 1.17, 95% CI = 0.68-2.01; p_{trend} = 0.54). Heterogeneity was observed for PR status ($p_{interaction}$ = 0.02), with a statistically significant association for PR+ tumors ($OR_{Q4\text{-}Q1}$ = 1.97, 95% CI = 1.48-2.64; p_{trend} <0.0001) but no association for PR- tumors ($OR_{Q4\text{-}Q1}$ = 1.00, 95% CI = 0.65-1.55; p_{trend} = 0.95). Though there was no statistically significant heterogeneity ($p_{interaction}$ = 0.15) in the analysis by combined ER/PR status, the trend test was significant only for ER+/PR+. No statistically significant heterogeneity was observed between HER2+ and HER2- tumors ($p_{interaction}$ = 0.37). No association was seen for triple negative (ER-/PR-/HER2-) tumors (p_{trend} = 0.95).

No statistically significant heterogeneity of the AMH-risk association was found in analyses stratified by age at blood donation, age at diagnosis, or baseline characteristics (Appendix Tables 4 and 5), though the association appeared stronger among women ages ≥45 years at blood donation than for younger women.

Table 6 shows the results by menopausal status at diagnosis/index date. No statistically significant heterogeneity was detected ($p_{interaction}$ = 0.34). The OR comparing top vs. bottom AMH quartiles was 1.35 (95% CI= 1.05-1.73; p_{trend} = 0.03) for the premenopausal subgroup and 1.61 (95% CI= 1.03-2.53; p_{trend} = 0.03) for the postmenopausal subgroup. Further adjusting for subsequent age at menopause hardly altered the ORs in the postmenopausal subgroup.

Stratified analyses were not appreciably different in analyses using consortium-wide quartiles (data not shown).

Discussion

In this prospective study including 2,835 cases and 3,122 matched controls from ten cohorts, we found a positive association between circulating AMH concentration and breast cancer risk. Compared with women in the lowest AMH quartile, women in the top quartile had a 60% higher risk of breast cancer in analyses adjusting for potential confounders. The association appeared limited to ER+/PR+ tumors. It was observed for both premenopausal and postmenopausal breast cancer.

Our study included six new cohorts in addition to the four that previously reported a positive association between AMH and breast cancer risk. Cases from these six cohorts represented 64% of the cases included in the study. Excluding one cohort at a time did not significantly alter the results and the association was still statistically significant when the four cohorts that published previously were simultaneously excluded ($OR_{Q4\text{-}Q1}$= 1.38, 95% CI= 1.07-1.79). Thus, and given the dose-response observed, we feel confident that our results are not due to random variation.

A statistically significant trend of increasing risk with increasing AMH was observed for ER +, PR+, and ER+/PR+ tumors. This suggests that estrogens and progesterone, whose binding to their respective receptors results in increased breast epithelial cell proliferation [11, 40], are involved in the mechanism underlying the AMH-breast cancer association. AMH is not strongly correlated with estradiol (follicular r = 0.02; luteal r = 0.17; untimed r = 0.12) [14, 16], but is strongly predictive of age at menopause and is thus an indicator of remaining duration of exposure to the high levels of estrogens and progesterone observed prior to menopause. We also observed that ORs and dose-response trends were strongest for women who were ≥45 years of age at blood draw and thus approaching menopause. This suggests that AMH concentration during perimenopause may be particularly informative regarding breast cancer risk. Perimenopause is characterized by an increase in the number of anovulatory cycles, which lack the surge in progesterone observed in the luteal phase of ovulatory cycles, in addition to changes in patterns of estrogen concentrations. AMH concentration, as a marker of perimenopausal progression, would be expected to reflect ovarian sex hormone exposure during this life stage. These observations support the hypothesis that the AMH-breast cancer risk association may be explained, in part, by AMH acting as a marker of time to menopause.

However, other observations from our study suggest that the association of AMH with risk is not explained entirely by its role as a marker of remaining years before menopause. First, we observed a positive association for premenopausal breast cancer. Also, the association of AMH with postmenopausal breast cancer was not attenuated by adjusting for age at menopause. We therefore cannot exclude an effect of AMH through other mechanisms, including a direct action of AMH, given the presence of AMH receptors in the breast [41].

Because experimental studies have shown a protective effect of AMH against breast tumors related to basal-like histology, Nichols et al. hypothesized that AMH could protect against this tumor subtype [15]. We did not observe a positive association with AMH for triple-negative tumors, a subgroup that substantially overlaps with the subgroup of basal-like

tumors [42, 43]. The number of cases in this subgroup, was small (115 cases) though, and additional studies specifically in the basal-like subgroup would be of interest.

Besides its prospective design and large sample size, another strength of our study was that detailed data on breast cancer risk factors were available. Odds ratios were not much altered when we adjusted for these factors, suggesting that they do not confound the AMH-risk association. We also adjusted for testosterone, which has been consistently associated with risk of breast cancer in both pre- and post-menopausal women [19, 44]. These two hormones were not correlated (age-adjusted Spearman correlation coefficient = 0.12) and ORs did not change substantially, suggesting that these two hormones act through different mechanisms. We used only one blood sample per participant, but AMH has been shown to vary little both within [45-47] and between [48, 49] menstrual cycles and also for repeat measurements (intra-class correlation coefficients of 0.88 for measurements 1 year apart, 0.67 for measurements taken 2-3 years apart, and correlation of 0.66 for measurements taken 4 years apart) [16, 50, 51]. Further, using only one measurement in biomarker studies usually tends to attenuate true associations [52]. Because neither biological/lifestyle variables (e.g. age, smoking, parity), nor the technical factors on which we had data (time in storage, type of sample (serum/plasma), time between collection and processing, and storage temperature) explained the differences in AMH concentrations we observed between cohorts, we do not know whether these differences reflect true differences between populations or technical artifacts. This is why we chose to conduct our analyses using cohort-specific quartiles, and our results should be interpreted on the relative scale (i.e. risk associated with levels in a specific quartile relative to women of the same age with levels in the lowest quartile) and not on the absolute scale (risk associated with absolute AMH concentration).

We note some implications of our results. First, the protective effect of AMH against breast and gynecological cancers in laboratory studies has led to the suggestion that AMH could be used in the treatment of these cancers [53]. Our results, however, indicate an opposite effect of AMH in women than observed in laboratory studies, which may be due to the use of supraphysiologic doses of recombinant AMH in those studies [7, 9, 41, 54]. The second implication regards breast cancer risk prediction models. Information on absolute risk is needed for younger women because guidelines regarding the age to start mammographic screening are not consistent [55-57] and because younger women tend to benefit most from preventive pharmacologic intervention [58]. Current risk prediction models, though, have shown limited discriminatory accuracy [59]. Our results suggest that AMH could improve breast cancer risk prediction models for younger women.

In conclusion, we found that women with high AMH concentrations were at higher risk of breast cancer than women of the same age with lower AMH concentrations in a large prospective study. The association was statistically significant only for ER+/PR+ tumors, which suggests that the association is due, at least in part, to the role of AMH as an indicator of exposure to estrogens and progesterone. The association with postmenopausal breast cancer is also consistent with AMH reflecting remaining time to menopause; however, because this association was not attenuated with adjustment for age at menopause and because we also observed an association of AMH with pre-menopausal breast cancer, our results suggest that additional mechanisms are at play.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nurses' Health Study (NHS): NCI UM1 CA186107; R01 CA49449

Nurses' Health Study II (NHSII): NCI UM1 CA176726; R01 CA67262

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Abbreviations

AMH	Anti-Müllerian Hormone
BGS	Breakthrough Generations Study
BMI	body mass index
CLUE II	Campaign Against Cancer and Heart Disease
CSB	Columbia, Missouri Serum Bank
CV	coefficient of variation
FTP	full-term pregnancy
NHS	Nurses' Health Study
NHSII	Nurses' Health Study II
NSMSC	Northern Sweden Mammography Screening Cohort
NYUWHS	New York University Women's Health Study
ORDET	Hormones and Diet in the Etiology of Breast Cancer

Novelty and Impact

Information on their individual risk of breast cancer can help women make decisions about breast cancer screening and prevention but current risk prediction models lack discriminatory accuracy. In this large prospective study, premenopausal women with AMH concentration in the top quartile had a 60% greater risk of breast cancer than women of the same age with AMH concentration in the bottom quartile. AMH is thus a candidate for inclusion in breast cancer risk prediction models for younger women.

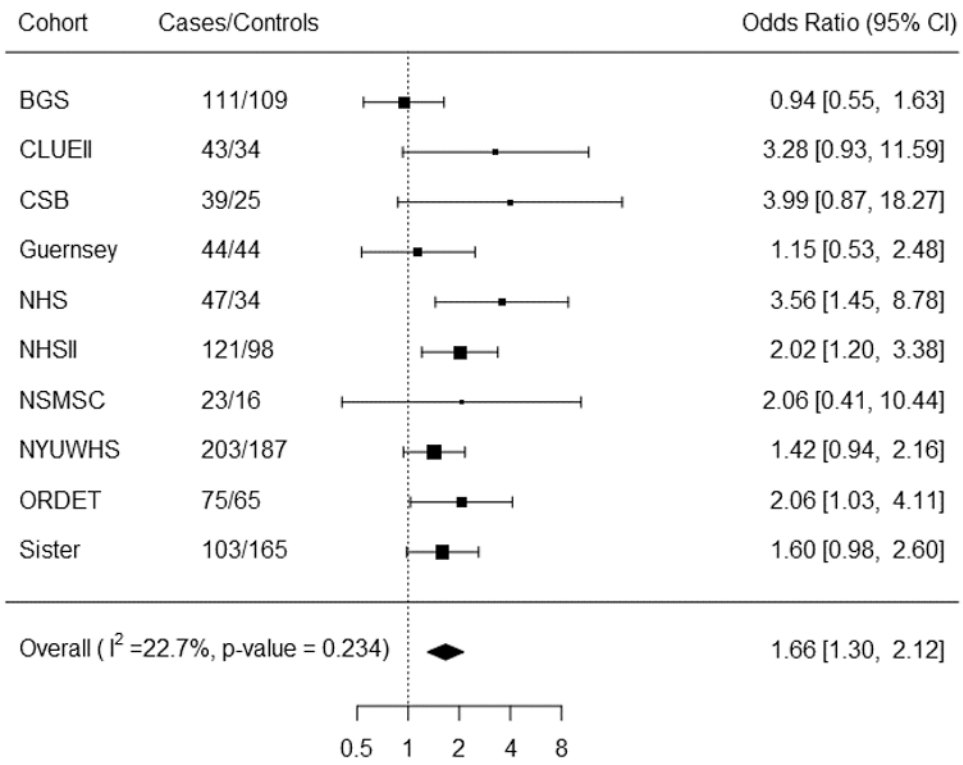

Figure 1. Cohort-specific associations between AMH and breast cancer risk (ORs and 95% CIs for the 4th quartile vs. 1st quartile)[1]

Table 1

Participating cohorts, sample collection and storage, and number and characteristics of cases and controls

Cohort[1]	Country	Source population	Years of blood draw	Sample type used in study	Storage temperature	Effective cohort size[2]	Cases/ Controls	Median age at blood donation in controls yr (min-max)	Median time to diagnosis, yr (min-max)
BGS [1]	UK	General population	2003-2010	Plasma	-180°C	46,344	439/439	44.0 (21.0-57.0)	3.0 (0.0-9.0)
CLUE II [2, 3]	USA	Residents of Washington County, MD	1989	Plasma	-70°C	2,899	136/136	40.0 (22.0-49.0)	13.5 (0.7-23.5)
CSB [4, 5]	USA	Attendees of breast cancer screening centers in Columbia, Missouri	1977-1987	Serum	-70°C	2,459	101/101	44.6 (33.3-54.7)	16.6 (0.2-23.3)
Guernsey [6, 7]	UK	General population	1977-1990	Serum	-20°C	3,120	176/176	40.1 (32.0-53.5)	16.7 (0.6-30.4)
NHS [8]	USA	Nurses	1989-1990	Plasma	-130°C	6,926	136/136	46.7 (43.0-53.8)	4.6 (0.1-13.8)
NHSII [9, 10]	USA	Nurses	1996-1999	Plasma	-130°C	22,000	395/395	42.8 (33.1-52.2)	4.9 (0.1-13.3)
NSMSC [11, 12]	Sweden	Attendees of a population-based screening program in Västerbotten	1995-2006	Plasma	-80°C	3,569	66/66	49.5 (39.6-53.3)	6.1 (0.0-13.6)
NYUWHS [13, 14]	USA	Attendees of a breast cancer screening center, NYC	1985-1991	Serum	-80°C	7,222	749/749	44.2 (34.3-56.5)	12.8 (0.6-24.5)
ORDET [15]	Italy	Residents in Varese Province	1987-1992	Serum	-80°C	5,942	263/263	44.4 (35.2-54.1)	9.7 (0.3-19.2)
Sister Study [16]	USA	Sisters of women with breast cancer	2003-2009	Serum	-180°C	14,772	374/661	46.5 (35.1-54.6)	2.8 (0.0-8.4)

[1] Cohort abbreviations: BGS: Breakthrough Generations Study; CLUE II: Campaign Against Cancer and Heart Disease; CSB: Columbia, Missouri Serum Bank; NHS: Nurses' Health Study; NHSII: Nurses' Health Study II; NSMSC: Northern Sweden Mammography Screening Cohort; NYUWHS: New York University Women's Health Study; ORDET: Hormones and Diet in the Etiology of Breast Cancer.

[2] Participants who would have been eligible if diagnosed with breast cancer during follow-up (i.e. female participants with blood collected prior to menopause).

Table 2
Baseline characteristics of cases and controls

Characteristic[1]	Cases (N = 2835)	Controls (N = 3122)	P-value[2]
	N (%)	N (%)	
Age at blood draw, years			Matched
<35	108 (3.8%)	111 (3.6%)	
35-39	534 (18.8%)	535 (17.1%)	
40-44	897 (31.6%)	999 (32.0%)	
45-49	966 (34.1%)	1117 (35.8%)	
50-54	318 (11.2%)	349 (11.2%)	
55+	12 (0.4%)	11 (0.4%)	
Race/ethnicity[1]			0.75
White	2562 (93.7%)	2800 (93.9%)	
Black/African American	118 (4.3%)	120 (4.0%)	
Other	53 (1.9%)	61 (2.0%)	
Education[1]			0.02
High school or less	759 (30.2%)	873 (30.8%)	
Some college/university, vocational training or more	1758 (69.8%)	1963 (69.2%)	
BMI[1], kg/m^2			0.04[3]
<18.5	51 (1.8%)	57 (1.8%)	
18.5-24.9	1702 (60.4%)	1779 (57.4%)	
25-29.9	710 (25.2%)	777 (25.0%)	
30+	353 (12.5%)	489 (15.8%)	
Age at menarche, years			0.44[3]
<12	603 (21.7%)	659 (21.6%)	
12	788 (28.3%)	803 (26.3%)	
13	786 (28.2%)	903 (29.5%)	
14+	606 (21.8%)	692 (22.6%)	
Parity[1]			0.05[3]
0	680 (24.6%)	710 (23.3%)	
1	400 (14.5%)	435 (14.3%)	
2	1028 (37.2%)	1138 (37.4%)	
3+	653 (23.7%)	758 (24.9%)	
Age at first full-term pregnancy[1], years			0.003[3]
<20	161 (7.5%)	226 (9.4%)	
21-24	696 (32.4%)	825 (34.4%)	
25-29	784 (36.5%)	834 (34.8%)	
≥30 or nulliparous	506 (23.6%)	515 (21.5%)	
Oral contraceptive use[1]			0.15
Never user	736 (26.9%)	772 (25.5%)	

Characteristic[1]	Cases (N = 2835)	Controls (N = 3122)	P-value[2]
	N (%)	N (%)	
Former user	1830 (66.9%)	2083 (68.8%)	
Current user	171 (6.2%)	174 (5.7%)	
Partial oophorectomy[1]			0.02
No	2747 (97.3%)	2989 (96.1%)	
Yes	76 (2.7%)	120 (3.9%)	
Family history of breast cancer[4]			<0.001
No	1984 (80.6%)	2143 (87.1%)	
Yes	477 (19.4%)	318 (12.9%)	
Benign breast biopsy[1]			<0.001
No	2096 (75.8%)	2511 (82.3%)	
Yes	669 (24.2%)	541 (17.7%)	
Smoking status[1]			0.02
Never	1576 (58.8%)	1847 (62.5%)	
Former	752 (28.1%)	751 (25.4%)	
Current	352 (13.1%)	359 (12.1%)	

[1]Missing data: race/ethnicity: 4.1%; education: 10.1%; BMI: 0.7%; age at menarche: 2.0%; parity: 2.6%; age at first full-term pregnancy: 0.2%; oral contraceptive use: 3.2%; partial oophorectomy: 0.4%; benign breast biopsy: 2.4%; smoking status: 5.4%.

[2]p-value from conditional logistic regression model

[3]p for trend from conditional logistic regression model for ordered categorical variable

[4]Calculated after excluding the Sister Study (all participants in this study have a family history of breast cancer).

Table 3

AMH assay, lowest detected value (LDV) and AMH geometric means (95% CIs) for cases and controls

Cohort[1]	Assay[2]	LDV[3], pmol/l	< LDV, %		Geometric mean[4] (95% CI), pmol/l		Age-adjusted geometric mean[4] (95% CI), pmol/l	
			Cases	Controls	Cases	Controls	Cases	Controls
BGS	picoAMH ELISA	0.0165	4.1	5.9	2.57 (2.12, 3.11)	2.33 (1.91, 2.86)	2.31 (2.00,2.67)	1.95 (1.68,2.27)
CLUE II	picoAMH ELISA	0.0165	3.7	2.9	4.71 (3.29, 6.75)	4.14 (2.91, 5.90)	1.85 (1.41,2.42)	1.52 (1.14,2.01)
CSB	picoAMH ELISA	0.0330	5.0	12.9	2.52 (1.67, 3.81)	1.39 (0.91, 2.13)	2.90 (2.15,3.92)	1.61 (1.17,2.20)
Guernsey	picoAMH ELISA	0.0264	5.7	2.8	3.12 (2.33, 4.17)	3.68 (2.84, 4.78)	1.29 (1.03,1.63)	1.36 (1.07,1.73)
NHS	picoAMH ELISA	0.0165	4.4	10.3	2.03 (1.45, 2.83)	1.03 (0.71, 1.52)	4.21 (3.24,5.46)	2.22 (1.69,2.92)
NHSII	picoAMH ELISA	0.0165	1.5	1.5	6.77 (5.83, 7.87)	5.21 (4.47, 6.06)	4.55 (3.90,5.30)	3.15 (2.68,3.70)
NSMSC	picoAMH ELISA	0.0165	6.1	7.6	1.00 (0.58, 1.70)	0.71 (0.43, 1.18)	2.98 (2.05,4.33)	2.23 (1.51,3.31)
NYUWHS	picoAMH ELISA	0.143	15.4	15.6	2.54 (2.21, 2.92)	2.32 (2.02, 2.67)	2.76 (2.47,3.08)	2.40 (2.14,2.70)
ORDET	picoAMH ELISA	0.0264	3.8	9.5	2.84 (2.25, 3.58)	1.93 (1.48, 2.51)	2.79 (2.31,3.36)	1.93 (1.59,2.34)
Sister Study	Ultrasensitive & picoAMH ELISA[5]	0.0214	16.0	18.5	1.20 (0.93, 1.54)	1.03 (0.85, 1.25)	2.30 (1.96,2.70)	1.80 (1.59,2.05)

[1] Cohort abbreviations: BGS: Breakthrough Generations Study; CLUE II: Campaign Against Cancer and Heart Disease; CSB: Columbia, Missouri Serum Bank; NHS: Nurses' Health Study; NHSII: Nurses' Health Study II; NSMSC: Northern Sweden Mammography Screening Cohort; NYUWHS: New York University Women's Health Study; ORDET: Hormones and Diet in the Etiology of Breast Cancer.

[2] Assays were conducted at Ansh Labs, except for the NYUWHS (Core Laboratory, Massachusetts General Hospital Pathology Service) and the Sister Study (Reproductive Endocrinology Laboratory, University of Southern California).

[3] LDV varied depending on the dilution factor used.

[4] Subjects with AMH measurement below the LDV were assigned the value of LDV divided by the square root of 2. Age-adjusted means adjusted for age and age-squared. Samples with AMH above the highest detectable value (n=14 total, 3 from CLUE II and 11 from NYUWHS) were set to the highest detectable value.

[5] All samples were measured using the Ultrasensitive assay; samples with AMH concentration < the LDV of the ultrasensitive assay (0.500 pmol/l) were re-measured using the picoAMH ELISA assay.

Table 4

Odds ratios (ORs) and 95% confidence intervals (95% CIs) for breast cancer associated with AMH concentration

	AMH quartiles[1]				P_{trend}[5]
	Q1	Q2	Q3	Q4	
Cases/Controls	631/789	684/777	711/779	809/777	.
Unadjusted OR[2] (95% CI)	1.00 (Referent)	1.20 (1.02, 1.41)	1.35 (1.14, 1.61)	1.64 (1.35, 1.98)	<.0001
Adjusted OR[3] (95% CI)	1.00 (Referent)	1.18 (1.00, 1.39)	1.32 (1.10, 1.58)	1.60 (1.31, 1.94)	<.0001
Adjusted OR[3] (95% CI), among women with testosterone measurements	1.00 (Referent)	1.18 (0.99, 1.40)	1.34 (1.11, 1.61)	1.62 (1.32, 1.98)	<.0001
Adjusted OR[4] (95% CI), including adjustment for testosterone	1.00 (Referent)	1.17 (0.99, 1.40)	1.33 (1.10, 1.60)	1.58 (1.29, 1.93)	<.0001

[1] Defined using cohort-specific cutpoints.

[2] Estimated using conditional logistic regression (cohort and age are adjusted for through matching).

[3] Estimated using conditional logistic regression and adjusting for race/ethnicity (white, black, other or unknown), education (high school or less, some college or higher, unknown), BMI (ordered categorical, <18.5, 18.5-25, 25-30, 30+ kg/m^2), age at menarche (ordered categorical, <12, 12, 13, 14+ years), parity (ordered categorical, 0, 1, 2, 3+), age at 1^{st} FTP (ordered categorical, <=20, 21-25, 26-30, 30+ years or nulliparous), oral contraceptive use (never, former, current, unknown), partial oophorectomy (no, yes, unknown), family history of breast cancer (no, yes), benign breast biopsy (no, yes, unknown), and smoking status (never, former, current, unknown).

[4] Estimated using conditional logistic regression and adjusting for variables in footnote 2 and testosterone (cohort-specific quartiles, with measurements from previous studies calibrated to the Mayo LC-MS/MS assay).

[5] P_{trend} was calculated using ordered-categorical AMH.

Table 5

Odds ratios[1] (ORs) and 95% confidence intervals (95% CIs) for breast cancer associated with AMH concentration by tumor characteristics

		AMH quartiles[2]			P_{trend}[3]	$P_{interaction}$[4]	
		Q1	Q2	Q3	Q4		
Invasiveness							0.41
Invasive	Cases/Controls	508/636	547/619	564/595	636/606		
	Adjusted OR (95% CI)	1.00 (Referent)	1.19 (0.99, 1.43)	1.39 (1.14, 1.70)	1.67 (1.34, 2.09)	<.0001	
In situ	Cases/Controls	122/153	136/156	147/184	172/169		
	Adjusted OR (95% CI)	1.00 (Referent)	1.19 (0.79, 1.79)	1.10 (0.72, 1.69)	1.35 (0.85, 2.13)	0.25	
ER status							0.21
ER+	Cases/Controls	324/438	353/424	377/411	441/439		
	Adjusted OR (95% CI)	1.00 (Referent)	1.27 (1.01, 1.60)	1.52 (1.19, 1.96)	1.74 (1.33, 2.28)	<.0001	
ER-	Cases/Controls	84/90	93/108	91/109	112/110		
	Adjusted OR (95% CI)	1.00 (Referent)	0.95 (0.60, 1.52)	1.02 (0.62, 1.69)	1.17 (0.68, 2.01)	0.54	
PR status							0.02
PR+	Cases/Controls	266/374	304/372	334/369	405/390		
	Adjusted OR (95% CI)	1.00 (Referent)	1.29 (1.00, 1.65)	1.61 (1.23, 2.11)	1.97 (1.48, 2.64)	<.0001	
PR-	Cases/Controls	142/154	142/160	134/151	148/159		
	Adjusted OR (95% CI)	1.00 (Referent)	0.96 (0.67, 1.39)	0.99 (0.66, 1.49)	1.00 (0.65, 1.55)	0.95	
HER2 status							0.37
HER2+	Cases/Controls	44/60	44/55	38/62	80/57		
	Adjusted OR (95% CI)	1.00 (Referent)	1.11 (0.58, 2.11)	1.17 (0.57, 2.44)	3.39 (1.55, 7.42)	0.002	
HER2-	Cases/Controls	182/275	227/280	244/263	266/279	.	
	Adjusted OR (95% CI)	1.00 (Referent)	1.36 (1.01, 1.83)	1.80 (1.31, 2.48)	2.05 (1.45, 2.92)	<.0001	
Joint receptor status							
ER+/PR+	Cases/Controls	259/360	288/358	317/354	386/371		0.15
	Adjusted OR (95% CI)	1.00 (Referent)	1.26 (0.97, 1.62)	1.58 (1.20, 2.08)	1.96 (1.46, 2.64)	<.0001	
ER+/PR-	Cases/Controls	65/78	65/66	60/57	55/68		
	Adjusted OR (95% CI)	1.00 (Referent)	1.25 (0.68, 2.28)	1.13 (0.58, 2.19)	0.82 (0.40, 1.68)	0.51	
ER-/PR+	Cases/Controls	7/14	16/14	17/15	19/19		
	Adjusted OR (95% CI)	1.00 (Referent)	3.10 (0.60, 15.9)	3.53 (0.60, 20.8)	3.23 (0.48, 21.9)	0.26	

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	AMH quartiles[2]				P_{trend}[3]	$P_{interaction}$[4]
	Q1	**Q2**	**Q3**	**Q4**		
ER-/PR-	Cases/Controls	77/76	77/94	74/94	93/91	
	Adjusted OR (95% CI)	1.00 (Referent)	0.83 (0.50, 1.39)	0.90 (0.51, 1.58)	1.15 (0.63, 2.09)	0.60
Triple-negative (ER-/PR-/HER2-) tumors						
	Cases/Controls	29/28	25/35	28/29	33/42	
	Adjusted OR (95% CI)	1.00 (Referent)	0.84 (0.31, 2.28)	1.17 (0.41, 3.37)	1.02 (0.34, 3.04)	0.95

[1] Estimated using conditional logistic regression model and adjusting for race/ethnicity (white, black, other or unknown), education (high school or less, some college or higher, unknown), BMI (<18.5, 18.5-25, 25-30, 30+ kg/m^2), age at menarche (ordered categorical, <12, 12, 13, 14+ years), parity (ordered categorical, 0, 1, 2, 3+), age at 1st FTP (ordered categorical, <=20, 21-25, 26-30, 30+ years or nulliparous), oral contraceptive use (never, former, current, unknown), partial oophorectomy (no, yes, unknown), family history of breast cancer (no, yes), benign breast biopsy (no, yes, unknown), and smoking status (never, former, current, unknown).

[2] Defined using cohort-specific cutpoints.

[3] P_{trend} was calculated using ordered categorical AMH.

[4] $P_{interaction}$ was calculated by including an interaction term between AMH (ordered categorical) and each tumor characteristic.

Table 6

Odds ratios (ORs) and 95% confidence intervals (95% CIs) for breast cancer associated with AMH concentration by menopausal status at diagnosis

	AMH quartiles[1]				P_{trend}[4]	$P_{interaction}$[5]
	Q1	Q2	Q3	Q4		
Matched sets with both case and control(s) pre-menopausal at diagnosis/index date						0.34
Cases/Controls	222/292	282/339	327/369	369/374		
Adjusted OR[2] (95% CI)	1.00 (Referent)	1.21 (0.93, 1.56)	1.17 (0.91, 1.50)	1.35 (1.05, 1.73)	0.03	
Matched sets with both case and control(s) post-menopausal at diagnosis/index date						
Cases/Controls	161/176	90/116	96/94	100/75		
Adjusted OR[2] (95% CI)	1.00 (Referent)	0.88 (0.60, 1.30)	1.14 (0.74, 1.76)	1.61 (1.03, 2.53)	0.03	
Adjusted OR[3] (95% CI)	1.00 (Referent)	0.88 (0.59, 1.30)	1.13 (0.72, 1.79)	1.59 (0.96, 2.63)	0.06	

[1] Defined using cohort- and age-specific cutpoints.

[2] Estimated using conditional logistic regression model, adjusting for race/ethnicity (white, black, other or unknown), education (high school or less, some college or higher, unknown), BMI (<18.5, 18.5-25, 25-30, 30+ kg/m^2), age at menarche (ordered categorical, <12, 12, 13, 14+ years), parity (ordered categorical, 0, 1, 2, 3+), age at 1st FTP (ordered categorical, <=20, 21-25, 26-30, 30+ years or nulliparous), oral contraceptive use (never, former, current, unknown), partial oophorectomy (no, yes, unknown), family history of breast cancer (no, yes), benign breast biopsy (no, yes, unknown), and smoking status (never, former, current, unknown). Analyses were performed among women with known age at menopause.

[3] Estimated using conditional logistic regression model and adjusting for variables in footnote 2 and age at menopause.

[4] P_{trend} was calculated using ordered categorical AMH.

[5] $P_{interaction}$ was calculated by including an interaction term between AMH (ordered categorical) and menopausal status at diagnosis.