Supplementary Methods

Sister Study exclusions

The Sister Study conducted a study to assess the relationship between AMH and breast cancer risk prior to joining this consortium. Some inclusion criteria for this cohort differed from that of the consortium. Specifically, hormone replacement therapy use (current or prior) was not an exclusion criterion in the Sister Study but was for the consortium study. Thus, we excluded 64 cases and 141 controls from the Sister Study that were included in the initial study. All other Sister Study cases and controls (matched 1:2) from the previous study were included in this report.

Quality control samples

With the exception of the Sister Study, AMH measurements were performed in batches of up to 70 samples. Two types of blinded quality control (QC) samples were included: study-wide QC samples, which were generated from a pool of NYUWHS samples, and cohort-specific QC samples, generated from pools created by each cohort. Each of the batches included 2-4 samples of each QC sample type.

Calibration samples

We conducted a calibration study to examine how NYUWHS (measured with picoAMH at MGH laboratory) and Sister Study (measured with ultrasensitive and picoAMH assays at USC laboratory) measurements compared to measurements performed at Ansh Labs, where the samples from the 8 other cohorts were analyzed. A total of 40 samples in the NYUWHS and 35 in the Sister Study, selected from the control samples and covering the AMH distribution of each cohort, were re-measured using the picoAMH ELISA at Ansh Labs.

Testosterone measurements

Previous testosterone measurements were performed using a radioimmunoassay (RIA) with (CSB, NHSII batch 1, and Sister Study) or without (BGS, Guernsey, NYUWHS, ORDET) extraction, or with LC/MS/MS (NHSII batch 2). About 30 calibration samples each from BGS, CSB, NYUWHS, ORDET, and Sister Study across the distribution of the original measurements had good to high intra-class correlation coefficients (range: 0.71-0.95) with LC-MS/MS measurements performed at the Mayo Clinic laboratory used for testosterone measurements in this study. These calibration samples were used to calibrate previous measurements to the Mayo Clinic LC-MS/MS assay. The NHS cohorts used internal pooled samples that have been run along with each study batch for calibration to the LC-MS/MS assay. Because the original Guernsey assays were performed in several batches, years apart, we were not able to calibrate the measurements of each batch to the LC-MS/MS assay. Study variability was handled by performing analyses using cohort-specific quartiles of testosterone.

Age-adjustment sensitivity analyses

For each case, control(s) were selected from each cohort that matched the cases on age (and date of blood donation). Our analyses of AMH and risk were conducted using conditional logistic regression, to take into account the matched design when calculating odds ratios and 95% CIs. The tightness of matching on age varied somewhat by cohort (e.g. BGS matched on 5-year age groups vs. other cohorts which matched on age mostly within 1-2 years), so we repeated analyses adjusting for continuous age to see if more precise age-adjustment affected the effect estimates. Results were

not noticeably different in analyses adjusting for age nor after adjusting for age and age-squared to account for the quadratic relationship we observed for AMH with age.

Appendix Table 1: Matching factors by cohort

Cohort	Age at blood donation/ date of birth	Date of initial blood donation	Race/ ethnicity	Use of hormones at blood donation[a]	Phase/ day of cycle	Menopausal status at diagnosis	Other factors
BGS	Age in 5-year categories	± 1-3 years	✓	✓ (partially)		✓	# of days blood was in the mail, consent to access medical records
CLUE2	DOB ± 1 year	± 2 weeks	✓	✓	✓		
CSB	Age ± 2 years	± 1 year			✓		Time of day of blood donation
Guernsey	Age ± 2 years	± 1 year	All Caucasian	✓	✓		
NHS	DOB ± 1 year	± 1 month		All non-users		✓ (all premenopausal)	Time of day of blood donation, fasting status
NHSII	Age ± 2 years	± 2 months	✓		✓	✓ (all premenopausal)	Time of day of blood donation, fasting status
NSMSC	DOB ± 6 months	± 1 month	All Caucasian				Number of samples, dates of subsequent samples
NYUWHS	Age ± 6 months	± 3 months	✓	All non-users	✓		Number of samples, dates of subsequent samples
ORDET	Age ± 3 years	± 6 months	All Caucasian		All day 20-24		All fasting samples
Sister Study	Age ± 5 months	Same year					

a Current and prior users of hormone replacement therapy were excluded from our study. Hormone use is primarily oral contraceptives, but women using other hormones (e.g. infertility medications) were not excluded from BGS.

Appendix Table 2. Baseline characteristics of cases and controls by cohort

Cohort[1,2]		Median age (range), years	White %	More than high school education %	Median BMI, kg/m^2	Median age at menarche, years	Nulli-parous[3] %	Hyster ectomy %	Current oral contraceptive user, %	Partial oophor ectomy %	First degree family history of breast cancer %	Benign breast biopsy %	Current smoker %
BGS	Case	44.0 (21.0-57.0)	98.9	40.8	23.7	13.0	21.0	2.3	16.9	3.0	25.5	8.4	7.1
	Control	44.0 (21.0-57.0)	98.9	41.9	24.0	13.0	22.3	1.6	13.9	2.7	16.2	3.6	7.1
CLUE II	Case	40.0 (22.0-49.0)	100	50.7	23.3	13.00	14.1	.	14.7	.	15.4	17.6	12.5
	Control	40.0 (22.0-49.0)	100	44.9	24.2	12.00	19.9	0.7	14.7	3.2	12.5	10.3	15.4
CSB	Case	44.4 (31.4-56.1)	99.0	.	24.8	13.0	8.9	13.9	3.0	.	18.8	12.9	15.8
	Control	44.6 (33.3-54.7)	100	.	24.0	13.0	6.9	13.9	6.9	.	5.0	4.0	24.8
Guernsey	Case	39.8 (31.8-54.0)	100	14.8	23.9	13.0	11.9	6.3	8.0	.	13.1	15.3	23.2
	Control	40.1 (32.0-53.5)	100	20.5	24.0	13.0	8.0	5.7	8.0	.	5.1	11.9	20.5
NHS	Case	46.9 (42.7-53.8)	98.5	100	23.5	12.0	7.4	3.7	.	1.5	11.8	47.1	12.5
	Control	46.7 (43.0-53.8)	99.3	100	23.8	12.0	6.7	6.6	.	2.2	5.9	30.9	14.0
NHSII	Case	42.8 (32.5-52.3)	97.7	100	23.3	12.0	20.5	3.3	1.5	1.5	17.0	23.8	8.6
	Control	42.8 (33.1-52.2)	97.7	100	24.0	12.0	19.7	1.3	1.3	3.5	10.4	17.2	4.8
NSMSC	Case	49.5 (39.7-53.1)	100	28.6	25.1	13.0	1.5	1.5	15.2	1.5	9.1	.	.
	Control	49.5 (39.6-53.3)	100	18.2	25.6	13.0	6.3	.	16.7	.	6.1	.	.
NYUWHS	Case	44.1 (34.1-56.0)	83.5	82.3	23.0	12.0	44.4	4.7	.	4.0	25.0	23.0	19.2
	Control	44.2 (34.3-56.5)	83.0	79.0	23.0	13.0	42.7	4.8	.	5.5	18.4	16.4	16.7
ORDET	Case	44.0 (35.0-54.7)	100	38.8	23.9	13.0	12.9	3.4	0.4	6.1	9.9	40.2	22.1
	Control	44.4 (35.2-54.1)	100	27.0	24.3	13.0	9.9	2.7	-.	6.5	9.5	34.1	22.1
Sister Study	Case	46.9 (35.2-54.5)	87.4	90.6	25.2	13.0	29.9	5.9	11.5	2.1	97.9	35.8	4.8
	Control	46.5 (35.1-54.6)	90.6	87.9	25.6	13.0	24.2	7.6	8.5	4.4	96.5	24.9	7.3

[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] Missing data: race/ethnicity: 4.0%; education: 10.0% (data unavailable for CSB); age at menarche: 1.9%; BMI: 0.7%, smoking: 5.3% (current smoking status was unavailable for NSMSC); nulliparity: 2.6%; partial oophorectomy: 0.4%; history of benign breast biopsy: 2.3% (data unavailable for NSMSC).
[3] Women were defined as parous if they had at least one live birth (CLUEII), at least one pregnancy lasting ≥ 24 weeks (BGS) or at least one pregnancy lasting ≥37 weeks (CSB, Guernsey, NSMSC, NYUWHS, ORDET, Sister Study).

Appendix Table 3: Odds ratios (ORs) and 95% confidence intervals (95% CIs) for breast cancer associated with AMH concentration, consortium-wide quartile cutpoints

	AMH quartiles[1]				
	Q1 <LDV-0.64 pmol/L	Q2 0.65-3.43 pmol/L	Q3 3.44-10.5 pmol/L	Q4 10.6-165 pmol/L	P_{trend}[5]
Cases/Controls	621/783	686/778	677/781	851/780	
Unadjusted OR[2] (95% CI)	1.00 (Referent)	1.19 (1.01, 1.41)	1.23 (1.03, 1.47)	1.62 (1.33, 1.97)	<.0001
Adjusted OR[3] (95% CI)	1.00 (Referent)	1.16 (0.98, 1.37)	1.22 (1.01, 1.47)	1.60 (1.30, 1.96)	<.0001
Adjusted OR[3] (95% CI), among women with testosterone measurements	1.00 (Referent)	1.19 (1.00, 1.42)	1.23 (1.01, 1.49)	1.65 (1.34, 2.04)	<.0001
Adjusted OR[4] (95% CI), including adjustment for testosterone	1.00 (Referent)	1.18 (0.99, 1.42)	1.21 (1.00, 1.47)	1.61 (1.30, 1.98)	<.0001

[1] Defined using consortium-wide 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 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).
[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.

Appendix Table 4. Odds ratios[1] (ORs) and 95% confidence intervals (95% CIs) for breast cancer associated with AMH concentration by age at blood draw and age at diagnosis

		AMH quartiles[2]				P_{trend}[3]	$P_{interaction}$[4]
		Q1	Q2	Q3	Q4		
Age at blood draw, years							0.16
≤40	Cases/Controls	172/194	166/194	213/210	237/228		
	Adjusted OR (95% CI)	1.00 (Referent)	1.02 (0.74, 1.39)	1.15 (0.84, 1.58)	1.26 (0.93, 1.71)	0.10	
41-44	Cases/Controls	179/211	182/214	189/206	201/190		
	Adjusted OR (95% CI)	1.00 (Referent)	1.01 (0.74, 1.37)	1.12 (0.82, 1.51)	1.22 (0.90, 1.66)	0.15	
45-49	Cases/Controls	212/301	226/267	222/273	306/267		
	Adjusted OR (95% CI)	1.00 (Referent)	1.38 (1.06, 1.82)	1.25 (0.95, 1.65)	1.83 (1.38, 2.42)	<0.001	
≥50	Cases/Controls[5]	125/155	59/73	76/76	70/63		
	Adjusted OR (95% CI)	1.00 (Referent)	1.01 (0.64, 1.60)	1.18 (0.72, 1.92)	1.65 (1.03, 2.65)	0.05	
Age at diagnosis, years							0.73
≤45	Cases/Controls	112/133	109/144	146/164	160/157		
	Adjusted OR (95% CI)	1.00 (Referent)	0.96 (0.64, 1.43)	1.06 (0.73, 1.55)	1.23 (0.84, 1.79)	0.20	
46-50	Cases/Controls	181/263	209/234	211/219	220/238		
	Adjusted OR (95% CI)	1.00 (Referent)	1.40 (1.06, 1.86)	1.47 (1.10, 1.97)	1.51 (1.13, 2.02)	0.01	
51-55	Cases/Controls	149/195	150/188	162/187	230/185		
	Adjusted OR (95% CI)	1.00 (Referent)	1.05 (0.76, 1.45)	1.08 (0.77, 1.52)	1.70 (1.23, 2.35)	0.001	
≥56	Cases/Controls[5]	246/270	165/182	181/195	204/168		
	Adjusted OR (95% CI)	1.00 (Referent)	1.03 (0.76, 1.39)	0.97 (0.72, 1.32)	1.32 (0.97, 1.81)	0.12	

[1] 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 (<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- and age-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 tumor characteristic.

[5] Because a high proportion of values were below the LDV for women ≥50, the sample size is largest for the lowest quartile because it includes all values <LDV. For cohorts with >25% of values below the LDV among women ≥50 (NYU and Sister), values below the LDV were assigned to the lowest quartile, while values above LDV were divided into tertiles (i.e., the top three quartiles).

Appendix Table 5. Odds ratios[1] (ORs) and 95% confidence intervals (95% CIs) for breast cancer associated with AMH concentration by subject baseline characteristics

		AMH quartiles[2]				P_{trend}[3]	$P_{interaction}$[4]
		Q1	Q2	Q3	Q4		
White race/ethnicity	Cases/Controls	583/713	619/700	636/700	724/687		
	OR (95% CI)	1.00 (Referent)	1.18 (1.00, 1.38)	1.30 (1.09, 1.55)	1.65 (1.36, 2.01)	<.0001	
Education							0.69
High school or less	Cases/Controls	196/246	203/227	175/212	185/188		
	OR (95% CI)	1.00 (Referent)	1.19 (0.90, 1.58)	1.14 (0.83, 1.57)	1.53 (1.07, 2.21)	0.04	
Some college or higher	Cases/Controls	367/473	407/477	456/500	528/513		
	OR (95% CI)	1.00 (Referent)	1.16 (0.95, 1.42)	1.30 (1.05, 1.61)	1.59 (1.26, 2.02)	<.0001	
BMI, kg/m^2							0.29
18.5-25	Cases/Controls	327/409	383/430	453/433	539/507		
	OR (95% CI)	1.00 (Referent)	1.19 (0.96, 1.47)	1.46 (1.16, 1.82)	1.61 (1.26, 2.06)	<.0001	
25-30	Cases/Controls	194/198	182/200	159/216	175/163		
	OR (95% CI)	1.00 (Referent)	0.98 (0.73, 1.32)	0.87 (0.63, 1.21)	1.42 (0.98, 2.06)	0.15	
>=30	Cases/Controls	97/161	101/126	82/115	73/87		
	OR (95% CI)	1.00 (Referent)	1.79 (1.19, 2.70)	1.62 (1.02, 2.58)	2.32 (1.33, 4.05)	0.01	
Oral contraceptive use							0.52
Never user	Cases/Controls	202/242	183/204	168/159	183/167		
	OR (95% CI)	1.00 (Referent)	1.21 (0.90, 1.62)	1.63 (1.16, 2.30)	1.94 (1.31, 2.85)	<0.001	
Former user	Cases/Controls	377/485	446/505	468/554	539/539		
	OR (95% CI)	1.00 (Referent)	1.19 (0.98, 1.45)	1.17 (0.95, 1.44)	1.48 (1.18, 1.86)	0.02	
No history of partial oophorectomy	Cases/Controls	597/750	661/738	697/751	792/750		
	OR (95% CI)	1.00 (Referent)	1.20 (1.02, 1.40)	1.33 (1.12, 1.57)	1.64 (1.35, 1.98)	<.0001	
Smoking status							0.57
Never smoker	Cases/Controls	339/435	369/466	391/464	477/482		
	OR (95% CI)	1.00 (Referent)	1.06 (0.86, 1.30)	1.24 (0.99, 1.55)	1.56 (1.22, 2.00)	<0.001	
Former smoker	Cases/Controls	159/211	193/177	196/196	204/167		
	OR (95% CI)	1.00 (Referent)	1.60 (1.17, 2.18)	1.57 (1.12, 2.19)	2.12 (1.45, 3.11)	<0.001	
Current smoker	Cases/Controls	93/98	81/89	89/82	89/90		
	OR (95% CI)	1.00 (Referent)	1.10 (0.70, 1.73)	1.18 (0.71, 1.95)	1.14 (0.65, 2.02)	0.62	

[1] Estimated using unconditional logistic regression adjusting for cohort, age, and the following variables (with the exception of the variable under consideration): 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).

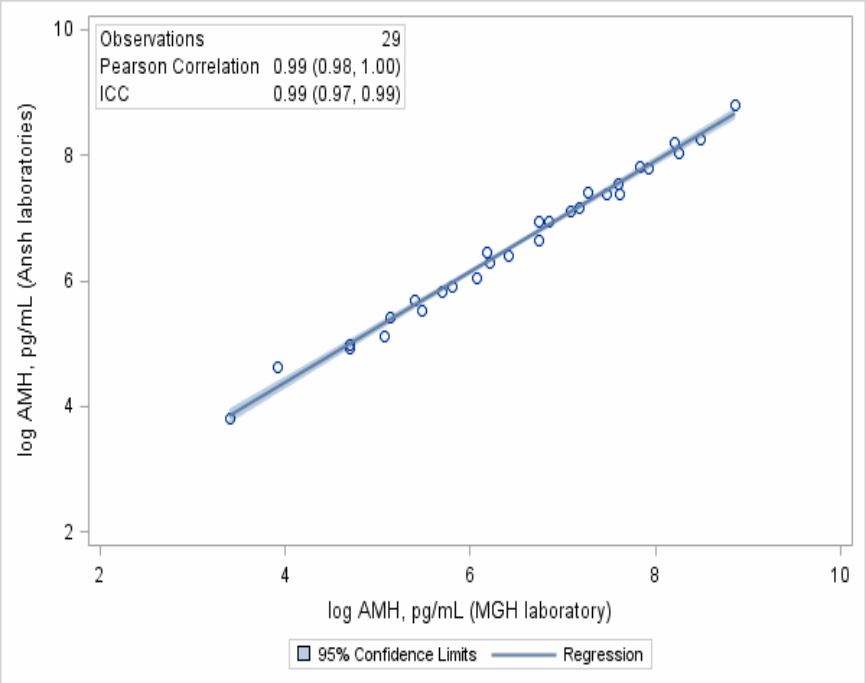
[2] Defined using cohort-specific cutpoints.

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

[4] $P_{interaction}$ was calculated by including an interaction term between AMH quartiles (ordered categorical) and variable under consideration.

Appendix Figure 1. AMH measurement at Ansh Lab vs. laboratory used for previous AMH measurements (Core Laboratory, Massachusetts General Hospital Pathology Service for the NYUWHS and Reproductive Endocrinology Laboratory, University of Southern California for the Sister Study) for samples with AMH concentrations above the lowest detectable value.

(a) NYUWHS



(b) Sister study