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Genetic variants in anti-Müllerian hormone-related genes and breast cancer risk: Results from the AMBER consortium

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Abstract

Purpose—Circulating anti-Müllerian hormone (AMH) levels are positively associated with time to menopause and breast cancer risk. We examined breast cancer associations with single nucleotide polymorphisms (SNPs) in the *AMH* gene or its receptor genes, *ACVR1* and *AMHR2*, among African American women.

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Compliance with Ethical Standards
Conflict of interest: The authors have no conflicts to disclose.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Methods—In the AMBER consortium, we tested 65 candidate SNPs, and 1,130 total variants, in or near *AMH*, *ACVR1*, and *AMHR2* and breast cancer risk. Overall, 3,649 cases and 4,230 controls contributed to analyses. Odds ratios (OR) and 95% confidence intervals (CI) for breast cancer were calculated using multivariable logistic regression.

Results—After correction for multiple comparisons (false discovery rate of 5%), there were no statistically significant associations with breast cancer risk. Without correction for multiple testing, four candidate SNPs in *ACVR1* and one near *AMH* were associated with breast cancer risk. In *ACVR1*, rs13395576[C] was associated with lower breast cancer risk overall (OR=0.84; 95% CI: 0.72, 0.97) and for ER+ disease (OR=0.75; CI: 0.62, 0.89)($p<0.05$). Rs1220110[A] and rs1220134[T] each had ORs of 0.89–0.90 for postmenopausal and ER+ breast cancer (*p* 0.03). Conversely, rs1682130[T] was associated with higher risk of ER+ breast cancer (OR=1.17; 95% CI: 1.04, 1.32). Near *AMH*, rs6510652[T] had ORs of 0.85–0.90 for breast cancer overall and after menopause (*p* 0.02).

Conclusions—The present results, from a large study of African American women, provide limited support for an association between AMH-related polymorphisms and breast cancer risk and require replication in other studies.

Keywords

genetic polymorphisms; breast cancer; anti-Müllerian hormone; case-control

Introduction

Anti-Müllerian hormone (AMH), also called Müllerian inhibiting substance, is a peptide hormone produced by the granulosa cells of pre- and small antral ovarian follicles and is a member of the transforming growth factor β family (1). AMH is used clinically in adult women as a measure of ovarian reserve. AMH levels peak in the mid-20s and decline thereafter, becoming non-detectable prior to menopause (2). Polymorphisms in the *AMH* gene and its two receptor genes, AMH type 1 receptor (*ACVR1)* and AMH type 2 receptor (*AMHR2)* have previously been associated with later age at menopause (3, 4), an established risk factor for invasive breast cancer (5).

Polymorphisms in the AMH receptor gene *ACVR1* have also been associated with circulating anti-Müllerian hormone levels (22). A recent pooled analysis of 10 prospective studies reported a positive association between circulating AMH concentrations and future breast cancer risk (6), possibly mediated by later ages at menopause. Polymorphisms in *AMH*, *ACVR1*, and *AMHR2* were examined in relation to breast cancer risk in the Breast Cancer Health Disparities (7) and Women's Insights and Shared Experiences (WISE)(8) case-control studies. The Breast Cancer Health Disparities Study combined information from Hispanic and non-Hispanic White women in three studies across the United States and Mexico, but was not designed to evaluate risk among African American women. In the WISE study, breast cancer associations with AMH-related polymorphisms in *ACVR1* and *AMHR2* appeared to vary by race; however, the study was limited by the relatively small number of African American women with breast cancer (N=149)(8).

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Previous findings suggest variation in the distribution of AMH-related polymorphisms between racial and ethnic groups, with potential implications for breast cancer risk. To examine AMH-related polymorphisms associated with breast cancer among African American women, we used data from the African American Breast Cancer Epidemiology and Risk (AMBER) consortium (9).

Materials and methods

Study Population

The AMBER Consortium, an etiologic study of breast cancer subtypes in African American women, has been described previously (9), and combines data and specimens from four of the largest studies of breast cancer in African American women. These include two case-control studies: the Carolina Breast Cancer Study (CBCS) (10) and the Women's Circle of Health Study (WCHS) (11, 12); and two cohort studies: the Black Women's Health Study (BWHS) (13) and the Multiethnic Cohort (MEC)(14). Each study has published its own design and methods as referenced. All study participants provided written informed consent and all studies obtained Institutional Review Board approval.

Overall, 3,663 cases and 4,687 controls in the AMBER consortium provided either blood or saliva for DNA analysis. For the case-control studies, controls were identified either through Division of Motor Vehicles lists (age <65 years) and Health Care Financing Administration lists (age 65) (CBCS), or random digit dialing (WCHS). The prospective cohort studies (BWHS and MEC) were sampled as nested-case control studies with breast cancer cases frequency-matched to controls based on 5-year age groups, geographic location, and most recent questionnaire completed (15). Eligible cases were African American women with incident invasive breast cancer or *ductal carcinoma in situ* (DCIS) diagnosis. Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) oncogene overexpression was determined using pathology data from hospital or cancer registry records.

Genotyping and Quality Control

Genotyping of DNA from participants in the BWHS, CBCS, and WCHS was performed by the Center for Inherited Disease Research (CIDR) using the Illumina Human Exome Beadchip v1.1. This array includes >200,000 coding variants, as well as tagged single nucleotide polymorphisms (SNPs) for loci identified in previous genome-wide association studies (GWAS), a grid of common variants, and ancestry informative markers. A description of the exome chip design is available from http://genome.sph.umich.edu/wiki/Exome_Chip_Design. In addition to the standard Beadchip, approximately 159,000 SNPs of custom content focused on 433 unique genes in pathways potentially related to breast cancer (e.g., FGFR2, steroid hormone metabolism, and vitamin D) were added. Tag SNPs were selected with minor allele frequency at 10% (at r2 0.8) based on the haplotype structure of the Yoruban (YRI) population in 1000 Genomes Phase 1 reference panel (http://www.1000genomes.org).

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A total of 246,519 SNPs were genotyped, and 231,705 SNPs remained after excluding variants that failed technical or QC filters. Briefly, genotypes with a GenCall score <0.15 were classified as missing, and SNPs were removed if they had poor cluster properties (e.g. cluster separation <0.2 or <0.3 depending on allele frequency), call rates <0.98, Hardy-Weinberg Equilibrium $p<1\times10^{-4}$, >1 Mendelian error in trios from HapMap, or >2 discordant calls in duplicate samples. Mitochondrial and Y chromosome SNPs were also excluded. Genotyping was attempted for 6,936 participants from the BWHS, CBCS, and WCHS, and 6,828 participants had genotypes with a call rate >98%, including 3,130 cases (963 ER−, 1,674 ER+, 493 ER unknown) and 3,698 controls. Imputation was performed by the University of Washington using the IMPUTE2 software (16) and the 1000 Genomes Phase I reference panel (5/21/2011 1000 Genomes data, December 2013 haplotype release). Genetic data from 533 cases (135 ER−, 309 ER+, and 89 ER unknown) and 989 controls in the MEC were available from a previous GWAS on the Illumina Human 1 M-Duochip (17). SNPs from MEC were imputed to the same release of 1000 Genomes and combined with the genotype data from the Illumina Human Exome BeadChip v.1.1. Additional exclusion criteria applied to the four-study merged dataset were: variants with mismatching alleles or allele frequencies that differed by >0.15 in MEC when compared with the other three studies, variants with allele frequencies <0.5%, and variants with imputation score INFO <0.5 in any of the four studies. For this analysis, measured and imputed genotypes from MEC and the three other AMBER studies were combined into a final data set containing 10,684,077 SNPs for 8,350 women; 3,663 cases and 4,687 controls.

Principal components were calculated using the smartpca program in the EIGENSOFT package (18) based on ~42,000 common SNPs, most of which were custom content additions to the exome chip for use in other AMBER projects. Genotype principal components were tested for association with case status after controlling for the study covariates: study, DNA source (blood, saliva [Oragene], saliva [mouthwash]), and the matching variables. While no principal components were strongly associated in the multivariable model, we included terms for principal components with p <0.1 in our analyses (20).

Analysis sample

Among the genotyped and imputed SNPs, we selected variants from introns, exons, and within 20 kb upstream of transcription and 20 kb downstream for *AMH* and its receptor genes, *ACVR1* and *AMHR2,* resulting in 1,130 variants after QC exclusions. Among these variants were 65 SNPs across 36 loci (linkage disequilibrium r2<0.3) that were selected *a priori* as candidate SNPs. These 65 SNPs included 62 SNPs that were evaluated among African American women in the WISE study (4/62 SNPs were associated with breast cancer in either Causasian or African American women—rs1146031, rs12694937, rs2883605, and rs2002555 (8)) and 3 additional SNPs (rs1146035, rs2033962, rs920522) that were associated with breast cancer in the Breast Cancer Health Disparities Study (7) but were not evaluated in WISE. Among the 8,350 women with genetic data, we excluded 471 women (5.6%) who were missing questionnaire covariate data on menopausal status. Ultimately, 7,879 women (3,649 cases and 4,230 controls) contributed to our analyses.

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Statistical analysis

Single variant association analyses were conducted assuming an additive genetic model using logistic regression as implemented in PLINK version 1.07 (19). We ran case-control models with the 1,130 variants among 1) all women (3,649 cases and 4,230 controls), 2) premenopausal women (N=1,276 cases and 1,518 controls), 3) postmenopausal women (N=2,095 cases and 2,422 controls), 4) all controls and cases with ER positive status (N=2,076 cases), 5) all controls and cases with ER negative status (N=1,133 cases), and 6) all controls and cases with triple negative status (ER negative, PR negative and HER2 negative; 629 cases). All models were adjusted for age group (by ~10 year intervals), study site, geographic region, DNA source, and 3 principal components. To account for multiple comparisons among the 65 candidate SNPs, we used a false-discovery rate (FDR) of 5% and calculated a p-value threshold required to meet statistical significance with 65 tests based on the method proposed by Benjamini and Hochberg (21). In addition, we did a separate FDR correction for the 1,130 total variants and calculated the p-value threshold required for statistical significance with 1,130 tests (21).

Results

Participant characteristics according to case-control status are shown in Table 1. The average age at breast cancer diagnosis was 54.9 years (SD=11.7, range: 22–87). Over half of the breast cancers were estrogen receptor positive (ER+) (N=2,076, 56.9% of cases); 31% were ER− (N=1,133). Among ER− tumors, 629 were also progesterone receptor (PR) negative and did not overexpress the HER2 oncogene (triple negative).

Controlling for an overall FDR of 5%, none of the 65 candidate SNPs selected *a priori* were associated with breast cancer. We further evaluated the 65 candidate SNPs without correction for multiple comparisons for comparison to the previous literature. Of these 65 SNPs, 16 SNPs were associated with breast cancer risk in one or more prior studies (rs1146031, rs1146035, rs1220134, rs2033962, rs12694937, rs10497191, rs4380178, rs10497192, rs4233672, rs10933443, rs2883605, rs920522, rs10497193, rs4664901, rs2002555, rs17182166)(7, 8), the remaining 49 SNPs were previously examined, but not associated, among African American women in the WISE Study (8). The 16 SNPs that were associated with breast cancer in previous studies, and 5 additional SNPs that were nominally significant in the current analysis (rs13395576, rs1220110, rs7561419, rs16842130, rs6510652), are shown in Tables 2–4. One polymorphism in *ACVR1* (rs13395576) and one in *AMH* (rs6510652) were associated with breast cancer at p<0.05 (Table 2). In *ACVR1*, rs13395576[C] was associated with lower risk of breast cancer overall (OR=0.84; 95% CI: 0.72, 0.97; Table 2) and ER+ disease (OR=0.75; 95% CI: 0.62, 0.89; Table 4). Rs6510652[T] in *AMH* was associated with 10–15% lower odds of breast cancer overall (OR=0.90; CI: 0.83, 0.98; Table 2) and after menopause (OR=0.85; CI: 0.76, 0.95; Table 3).

Two additional SNPs in *ACVR1* (rs1220110 and rs1220134) were associated with postmenopausal or ER+ breast cancer, and were in almost complete linkage disequilibrium with each other (LD, r2=0.977). Rs1220110[A] and rs1220134[T] were associated with 10–11% lower risk of postmenopausal breast cancer (OR=0.89; 95% CI: 0.81, 0.99 and OR=0.90; 95% CI: 0.81, 0.99, respectively; Table 2) and 11% lower risk ER+ breast cancer

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(OR=0.89; 95% CI: 0.82, 0.98 and OR=0.89; 95% CI: 0.82, 0.97, respectively; Table 4). Conversely, rs1682130[T] in *ACVR1* was associated with ER+ breast cancer with an OR of 1.17 (95% CI: 1.04, 1.32).

Controlling for an overall FDR of 5%, none of the 1,130 genotyped and imputed SNPs within 20 kb upstream of transcription and 20 kb downstream of *AMH* and its receptor genes, *ACVR1* and *AMHR2,* were associated with breast cancer (Supplementary Tables).

Discussion

Our analysis expands the evidence base for evaluating potential associations between genetic variation in or near *AMH* and *ACVR1* and breast cancer risk among African American women. Identified associations have some biologic plausibility, but were not robust to correction for multiple comparisons calling for caution in interpretation. In the AMBER consortium, rs1220134[T] in *ACVR1* was nominally associated with 11% lower odds of ER + breast cancer (OR=0.89, p=0.01) and 10% lower odds of postmenopausal breast cancer (OR=0.90, p=0.03). The alternate allele, rs1220134[A] has been associated with higher circulating AMH concentrations in women with polycystic ovary syndrome (22). Corresponding lower circulating levels of AMH associated with the rs1220134[T] allele would be in agreement with studies reporting a positive association between circulating AMH and breast cancer risk (6, 23–25).

To our knowledge, two prior studies have evaluated the association between polymorphisms in AMH-related genes and breast cancer risk, the Women's Insights and Shared Experiences (WISE)(8) and the Breast Cancer Health Disparities case-control studies (7). In WISE, two SNPs (rs1146031in *ACVR1* and rs200255 in *AMHR2*) were related to breast cancer risk among African American women ages 50–79 (N=149 cases, 246 controls). The OR for associations with total breast cancer was 0.63 (CI: 0.43–0.93) for rs1146031[G] and 1.62 (CI: 1.04–2.52) for rs200255[G]; however, few African American women (N=7 cases, 1 control) were homozygous for the rs200255[G] allele (8). In our analysis, rs1146031 and rs200255 were not associated with breast cancer in a much larger sample of African American women (N= 3,649 cases, 4,230 controls), among whom 63 cases (1.7%) and 46 controls (1.1%) were homozygous for the rs200255[G] allele.

In the Breast Cancer Health Disparities Study, a consortium formed by the 4-Corner's Breast Cancer Study, the Mexico Breast Cancer Study, and the San Francisco Bay Area Breast Cancer Study, 13 SNPs in *ACVR1* were associated with breast cancer risk (7). Of these, eight (rs2033962, rs4380178, rs920522, rs1146035, rs10497191, rs10497192, rs4233672, rs10933443) were associated with ER+/PR+ breast cancer, four (rs920522, rs2883605, rs10497193, rs4664901) were associated with ER+/PR− breast cancer, and two (rs17182166, rs11169953) were associated with ER−/PR− breast cancer after correction for multiple comparisons (p=0.005=0.05/10 independent SNPs in *ACVR1)* (7). Rs2883605[T] was also nominally associated with increased breast cancer risk in the WISE study (8), but only among European American women (8). In WISE, the T allele was extremely rare (<1% of Causcasian women, and no African American women, were homozygous for the T allele; 83% of Caucasian women and 98% of African American women were homozygous for the

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G allele). Among African American women in the AMBER consortium, 0.2% were homozygous for the T allele and 96.3% were homozygous for the G allele; rs2883605 was not associated with breast cancer risk.

Among the 13 SNPs that emerged in the Breast Cancer Health Disparities Study, one (rs11169953) was not available in our analysis and 11 were not associated with breast cancer among AMBER participants. The remaining SNP, *ACRV1* rs1220134[T] was nominally associated with ORs of 0.89–0.90 for ER+ or postmenopausal breast cancer breast cancer in AMBER, in contrast to the lower premenopausal breast cancer risk associated with the alternate allele (rs1220134[A]) among women with low Native American Ancestry in the Breast Cancer Health Disparities study (7). No association was observed between rs1220134 and breast cancer among African American or Caucasian women in WISE (8). Rs1220134 is found in the intronic region of *ACRV1* and shows evidence of falling in enhancer regions with expression in mesodermal stem cells, fat tissues, and breast tissue (26).

The functional roles of other SNPs associated with breast cancer in our analysis are not well understood (*AMH* rs6510652[G]; *ACVR1* rs13395576[C], rs1220110[A], rs7561419[T], and rs16842130[T]). These SNPs were not associated with breast cancer risk in the WISE study among African American or European American women (8). Rs7561419 was assessed as a candidate SNP in a study of pre-eclampsia risk, but was monomorphic in the Norwegian study population (27).

The few studies of AMH-related genes and breast cancer risk that have been conducted to date include heterogeneous study populations that emphasize potential variation according to ancestral groups. Our study provided the opportunity to examine these associations in one of the largest studies of African American women, an understudied group that is more likely to be diagnosed with breast cancer at younger ages and with more aggressive features than their European American peers. Additionally, because linkage disequilibrium structures vary by ancestry with African-Americans exhibiting weaker between-SNP correlations than Europeans or Asians, SNPs associated with breast cancer in African-Americans correspond to smaller genomic regions and may help to locate specific causal loci (28).

We identified five SNPs in *ACVR1* and one in *AMH* that were nominally associated with breast cancer risk among African American women in our study. Of these, rs13395576[C] on *ACVR1* is expressed in breast tissue, although its function is unclear. A second SNP, rs1220134[T] (in high LD with rs1220110), has biologic plausibility based on evidence for variation in circulating anti-Müllerian hormone levels in one study (22) but did not replicate results among African American women (8) or those with low Native American ancestry (7) in other studies. These suggestive findings require replication; in addition, future studies to address whether these SNPs are associated with differences in gene expression would aid interpretation.

Supplementary Material

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Table 1.

Participant characteristics according to case-control status, AMBER Consortium.

	Breast cancer cases N=3,649		Controls N=4,230	
Age at diagnosis / reference age (mean, SD)	54.9	(11.7)	54.5	(11.6)
Study	N	%	N	%
Black Women's Health Study	896	24.6	2,229	52.7%
Carolina Breast Cancer Study	1,408	38.6	615	14.5%
Women's Circle of Health Study	821	22.5	834	19.7%
Multiethnic Cohort Study	524	14.4	552	13.0%
Menopausal status				
Pre	1,276	35.0	1,518	35.9
Post	2,095	57.4	2,422	57.3
Unknown	278	7.6	290	6.9
Tumor subtype (cases only)				
Estrogen receptor (ER)+	2,076	56.9		
ER-	1,133	31.0		
Triple negative (ER-/PR-/HER2-)	629	17.2		

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Table 2.

Odds ratios for breast cancer and 21 selected variants in the Anti-Mullerian hormone (AMH) gene or its receptors, AMH type 1 receptor (ACVR1) and AMH type 2 receptor (AMHR2) in all African American women.[a]

Variant ID	Chr	Position (hg19)	Gene of interest	Distance from and region based on gene of interest	Coded/ Noncoded Alleles [b]	All women (3649 cases, 4230 controls)					
						Frequency Coded Allele	OR	95% lower CI	95% upper CI	P-value	P-value threshold required at FDR<5% [c]
rs13395576	2	158585549	ACVR1	7.4kb_3prime_of_ACVR1	C/T	0.06	0.84	0.72	0.97	0.019	0.002
rs1220110	2	158588460	ACVR1	4.5kb_3prime_of_ACVR1	A/T	0.32	0.94	0.87	1.01	0.097	0.005
rs17182166	2	158596197	ACVR1	intronic_ACVR1	T/G	0.21	1.07	0.98	1.16	0.118	0.005
rs1220134	2	158603556	ACVR1	intronic_ACVR1	T/A	0.32	0.94	0.87	1.01	0.093	0.004
rs1146031	2	158626980	ACVR1	synonymous_ACVR1	C/T	0.23	0.97	0.89	1.05	0.464	0.019
rs1146035	2	158633411	ACVR1	intronic_ACVR1	A/C	0.22	0.98	0.90	1.07	0.672	0.032
rs2033962	2	158638441	ACVR1	intronic_ACVR1	A/C	0.27	1.00	0.93	1.09	0.906	0.047
rs12694937	2	158657433	ACVR1	intronic_ACVR1	C/T	0.17	1.02	0.93	1.12	0.673	0.033
rs7561419	2	158661789	ACVR1	intronic_ACVR1	T/C	0.26	1.04	0.96	1.13	0.318	0.012
rs10497191	2	158667217	ACVR1	intronic_ACVR1	C/T	0.21	0.96	0.88	1.05	0.365	0.014
rs4380178	2	158668445	ACVR1	intronic_ACVR1	A/G	0.20	0.99	0.91	1.08	0.858	0.040
rs10497192	2	158671700	ACVR1	intronic_ACVR1	T/C	0.16	0.97	0.88	1.07	0.486	0.024
rs16842130	2	158687087	ACVR1	intronic_ACVR1	T/C	0.13	1.10	0.99	1.22	0.077	0.003
rs4233672	2	158691926	ACVR1	intronic_ACVR1	G/A	0.31	1.01	0.94	1.09	0.829	0.039
rs10933443	2	158694121	ACVR1	intronic_ACVR1	T/C	0.37	0.98	0.91	1.05	0.574	0.029
rs2883605	2	158696529	ACVR1	intronic_ACVR1	T/G	0.02	0.91	0.71	1.17	0.476	0.022
rs920522	2	158705824	ACVR1	intronic_ACVR1	C/T	0.14	1.00	0.90	1.10	0.967	0.049
rs10497193	2	158709678	ACVR1	intronic_ACVR1	G/A	0.43	1.06	0.98	1.13	0.131	0.006
rs4664901	2	158712538	ACVR1	intronic_ACVR1	T/C	0.24	0.97	0.90	1.06	0.526	0.026
rs2002555	12	53817237	AMHR2	400bp_5prime_of_AMHR2	G/A	0.12	1.02	0.92	1.14	0.645	0.030
rs6510652	19	2244903	AMH	4.2kb_5prime_of_AMH	T/G	0.24	0.90	0.83	0.98	0.015	0.001

Abbreviations: Chr - chromosome; OR - odds ratio; 95% lowerCI - 95% lower confidence interval; 95% upperCI - 95% upper confidence interval

[a] Models were all adjusted for the standard covariates including: 10 year age groups (ages 50–59years, 60–69years, 70–79 years, 80+years; referent ages 50–59 years), geographic region (i.e. New Jersey, Northeast US except New Jersey, South US, Midwest US, West US; referent Northeast US except New Jersey), principle components 5,6 and 8, DNA source (i.e. blood, mouthwash, saliva; referent blood), and study (i.e. MEC, CBCS, BWHS, WCHS; referent BWHS).

[b] Coded alleles refer to the variant allele; non-coded alleles are the referent alleles.

[c] To evaluate the impact of p-value correction for multiple comparisons, we used an false-discovery rate of 5% and calculated an adjusted p-value necessary to meet significance based on that proposed by Benjamini and Hochberg 1995. We ranked all the p-values (max number of tests = 65) from most to least significant and then calculated the adjusted p-value, as (i/m) * Q, where i = the raw p-value rank and m= the total number of tests, and Q= 5% FDR.

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Table 3.

Odds ratios for breast cancer and 21 selected variants in the Anti-Mullerian hormone (AMH) gene or its receptors, AMH type 1 receptor (ACVR1) and AMH type 2 receptor (AMHR2) in African American women by menopausal status.[a]

Variant ID	Chr	Gene of interest	Coded/ Noncoded Alleles [b]	Premenopausal women (1276 cases, 1518 controls)						Postmenopausal women (2095 cases, 2422 controls)					
				Frequency Coded Allele	OR	95% lower CI	95% upper CI	P-value	P-value threshold required at FDR<5% [c]	Frequency Coded Allele	OR	95% lower CI	95% upper CI	P-value	P-value threshold required at FDR<5% [c]
rs13395576	2	ACVR1	C/T	0.06	0.81	0.63	1.04	0.102	0.001	0.06	0.87	0.71	1.06	0.158	0.015
rs1220110	2	ACVR1	A/T	0.32	0.99	0.87	1.13	0.919	0.044	0.32	0.90	0.81	0.99	0.026	0.002
rs17182166	2	ACVR1	T/G	0.20	1.058	0.918	1.220	0.436	0.015	0.21	1.102	0.987	1.230	0.085	0.006
rs1220134	2	ACVR1	T/A	0.32	0.99	0.87	1.13	0.898	0.043	0.32	0.90	0.81	0.99	0.026	0.002
rs1146031	2	ACVR1	C/T	0.22	0.99	0.86	1.14	0.871	0.042	0.23	0.93	0.84	1.04	0.220	0.017
rs1146035	2	ACVR1	A/C	0.22	1.00	0.86	1.15	0.973	0.050	0.22	0.99	0.88	1.10	0.794	0.042
rs2033962	2	ACVR1	A/C	0.28	0.98	0.86	1.11	0.741	0.032	0.27	1.03	0.93	1.14	0.596	0.032
rs12694937	2	ACVR1	C/T	0.17	1.01	0.86	1.18	0.944	0.047	0.16	1.02	0.91	1.15	0.733	0.039
rs7561419	2	ACVR1	T/C	0.25	1.09	0.95	1.26	0.197	0.005	0.26	1.01	0.91	1.12	0.864	0.045
rs10497191	2	ACVR1	C/T	0.20	1.06	0.91	1.23	0.452	0.015	0.21	0.91	0.81	1.02	0.108	0.009
rs4380178	2	ACVR1	A/G	0.21	0.91	0.79	1.04	0.171	0.003	0.20	1.03	0.92	1.15	0.624	0.035
rs10497192	2	ACVR1	T/C	0.16	1.04	0.88	1.23	0.676	0.029	0.16	0.91	0.80	1.03	0.152	0.012
rs16842130	2	ACVR1	T/C	0.13	1.05	0.88	1.25	0.584	0.023	0.13	1.13	0.98	1.29	0.090	0.007
rs4233672	2	ACVR1	G/A	0.31	1.08	0.95	1.23	0.251	0.010	0.31	0.94	0.85	1.04	0.205	0.015
rs10933443	2	ACVR1	T/C	0.36	1.05	0.93	1.19	0.401	0.013	0.38	0.93	0.84	1.02	0.106	0.009
rs2883605	2	ACVR1	T/G	0.02	1.33	0.85	2.07	0.210	0.007	0.02	0.75	0.55	1.03	0.077	0.005
rs920522	2	ACVR1	C/T	0.14	1.05	0.88	1.25	0.574	0.022	0.15	0.99	0.87	1.13	0.900	0.045
rs10497193	2	ACVR1	G/A	0.42	1.08	0.96	1.21	0.217	0.008	0.44	1.07	0.97	1.17	0.157	0.014
rs4664901	2	ACVR1	T/C	0.23	1.03	0.89	1.19	0.679	0.030	0.24	0.93	0.84	1.04	0.215	0.016
rs2002555	12	AMHR2	G/A	0.13	1.05	0.88	1.25	0.605	0.025	0.12	0.98	0.85	1.13	0.810	0.043
rs6510652	19	AMH	T/G	0.24	0.96	0.83	1.10	0.536	0.019	0.24	0.85	0.76	0.95	0.005	0.001

Abbreviations: Chr - chromosome; OR - odds ratio; 95% lowerCI - 95% lower confidence interval; 95% upperCI - 95% upper confidence interval

[a] Models were all adjusted for the standard covariates including: 10 year age groups (ages 50–59years, 60–69years, 70–79 years, 80+years; referent ages 50–59 years), geographic region (i.e. New Jersey, Northeast US except New Jersey, South US, Midwest US, West US; referent Northeast US except New Jersey), principle components 5,6 and 8, DNA source (i.e. blood, mouthwash, saliva; referent blood), and study (i.e. MEC, CBCS, BWHS, WCHS; referent BWHS).

[b] Coded alleles refer to the variant allele; non-coded alleles are the referent alleles.

[c] To evaluate the impact of p-value correction for multiple comparisons, we used an false-discovery rate of 5% and calculated an adjusted p-value necessary to meet significance based on that proposed by Benjamini and Hochberg 1995. We ranked all the p-values (max number of tests = 65) from most to least significant and then calculated the adjusted p-value, as (i/m) * Q, where i = the raw p-value rank and m= the total number of tests, and Q= 5% FDR.

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Table 4.

Odds ratios by breast cancer subtype and 21 selected variants in the Anti-Mullerian hormone (AMH) gene or its receptors, AMH type 1 receptor (ACVR1) and AMH type 2 receptor (AMHR2) in African American women. [a]

Variant ID	Chr	Gene of interest	Coded / Noncoded Alleles [b]	ER+ breast cancer (2076 cases, 4230 controls)						ER- breast cancer (1133 cases, 4230 controls)					
				Freq Coded Allele	OR	95% lower CI	95% upper CI	P-value	P-value threshold required at FDR<5% [c]	Freq Coded Allele	OR	95% lower CI	95% upper CI	P-value	P-value threshold required at FDR<5% [c]
rs13395576	2	ACVR1	C/T	0.06	0.75	0.62	0.89	0.001	0.001	0.06	0.88	0.71	1.09	0.246	0.007
rs1220110	2	ACVR1	A/T	0.32	0.89	0.82	0.98	0.013	0.004	0.33	1.01	0.90	1.12	0.881	0.041
rs17182166	2	ACVR1	T/G	0.21	1.06	0.960	1.171	0.246	0.015	0.20	1.03	0.912	1.171	0.605	0.022
rs1220134	2	ACVR1	T/A	0.32	0.89	0.82	0.97	0.011	0.002	0.33	1.01	0.91	1.13	0.846	0.037
rs1146031	2	ACVR1	C/T	0.23	0.97	0.88	1.07	0.595	0.031	0.23	1.01	0.89	1.14	0.878	0.040
rs1146035	2	ACVR1	A/C	0.22	0.94	0.85	1.04	0.230	0.013	0.22	1.00	0.89	1.14	0.942	0.046
rs2033962	2	ACVR1	A/C	0.27	0.97	0.89	1.07	0.545	0.028	0.27	1.00	0.89	1.12	0.966	0.049
rs12694937	2	ACVR1	C/T	0.16	0.95	0.86	1.06	0.399	0.021	0.17	1.10	0.96	1.25	0.166	0.005
rs7561419	2	ACVR1	T/C	0.26	1.11	1.01	1.22	0.025	0.005	0.25	0.96	0.85	1.08	0.486	0.018
rs10497191	2	ACVR1	C/T	0.21	0.94	0.85	1.05	0.284	0.016	0.21	1.02	0.89	1.16	0.799	0.034
rs4380178	2	ACVR1	A/G	0.20	1.00	0.90	1.10	0.945	0.049	0.20	0.97	0.86	1.10	0.660	0.026
rs10497192	2	ACVR1	T/C	0.16	0.97	0.87	1.09	0.662	0.035	0.16	0.99	0.85	1.14	0.847	0.038
rs16842130	2	ACVR1	T/C	0.13	1.17	1.04	1.32	0.010	0.002	0.12	1.03	0.88	1.20	0.715	0.029
rs4233672	2	ACVR1	G/A	0.30	0.96	0.88	1.05	0.421	0.023	0.31	1.08	0.97	1.21	0.168	0.005
rs10933443	2	ACVR1	T/C	0.37	1.00	0.92	1.09	0.943	0.049	0.37	0.93	0.84	1.03	0.159	0.004
rs2883605	2	ACVR1	T/G	0.02	0.83	0.61	1.12	0.223	0.012	0.02	1.10	0.78	1.56	0.591	0.021
rs920522	2	ACVR1	C/T	0.14	0.95	0.85	1.07	0.421	0.023	0.14	0.96	0.83	1.12	0.610	0.022
rs10497193	2	ACVR1	G/A	0.43	1.06	0.98	1.15	0.146	0.007	0.43	1.01	0.92	1.12	0.778	0.032
rs4664901	2	ACVR1	T/C	0.23	0.94	0.85	1.04	0.236	0.014	0.24	1.03	0.92	1.16	0.611	0.023
rs2002555	12	AMHR2	G/A	0.12	1.05	0.93	1.19	0.394	0.020	0.12	0.97	0.83	1.13	0.687	0.028
rs6510652	19	AMH	T/G	0.24	0.92	0.84	1.02	0.124	0.006	0.24	0.89	0.78	1.01	0.061	0.001

Variant ID	Chr	Gene of interest	Coded/ Non-coded Allele [b]	Triple negative breast cancer (629 cases, 4230 controls)					
				Freq Coded Allele	OR	95% lower CI	95 upper CI	P-value	P-value threshold required at FDR<5% [c]
rs13395576	2	ACVR1	C/T	0.06	0.81	0.61	1.09	0.162	0.005
rs1220110	2	ACVR1	A/T	0.33	1.02	0.89	1.18	0.762	0.035
rs17182166	2	ACVR1	T/G	0.20	1.064	0.906	1.249	0.448	0.021
rs1220134	2	ACVR1	T/A	0.33	1.01	0.88	1.17	0.850	0.041
rs1146031	2	ACVR1	C/T	0.23	0.97	0.82	1.13	0.664	0.030
rs1146035	2	ACVR1	A/C	0.22	1.00	0.85	1.18	0.990	0.049

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rs2033962	2	ACVR1	A/C	0.27	0.99	0.85	1.14	0.856	0.042
rs12694937	2	ACVR1	C/T	0.17	1.15	0.97	1.36	0.103	0.002
rs7561419	2	ACVR1	T/C	0.25	0.90	0.77	1.05	0.180	0.005
rs10497191	2	ACVR1	C/T	0.21	1.04	0.88	1.22	0.667	0.031
rs4380178	2	ACVR1	A/G	0.20	0.93	0.79	1.10	0.415	0.017
rs10497192	2	ACVR1	T/C	0.16	0.99	0.82	1.20	0.935	0.047
rs16842130	2	ACVR1	T/C	0.12	0.90	0.73	1.11	0.311	0.015
rs4233672	2	ACVR1	G/A	0.31	1.14	0.99	1.32	0.065	0.001
rs10933443	2	ACVR1	T/C	0.37	0.93	0.81	1.06	0.264	0.010
rs2883605	2	ACVR1	T/G	0.02	0.85	0.51	1.40	0.518	0.025
rs920522	2	ACVR1	C/T	0.14	1.00	0.82	1.20	0.972	0.048
rs10497193	2	ACVR1	G/A	0.43	0.99	0.87	1.13	0.895	0.045
rs4664901	2	ACVR1	T/C	0.24	1.04	0.89	1.21	0.605	0.029
rs2002555	12	AMHR2	G/A	0.12	0.89	0.73	1.10	0.276	0.011
rs6510652	19	AMH	T/G	0.24	0.90	0.77	1.06	0.214	0.008

Abbreviations: Chr - chromosome; OR - odds ratio; 95% lowerCI - 95% lower confidence interval; 95% upperCI - 95% upper confidence interval

[a]Models were all adjusted for the standard covariates including: 10 year age groups (ages 50–59years, 60–69years, 70–79 years, 80+years; referent ages 50–59 years), geographic region (i.e. New Jersey, Northeast US except New Jersey, South US, Midwest US, West US; referent Northeast US except New Jersey), principle components 5,6 and 8, DNA source (i.e. blood, mouthwash, saliva; referent blood), and study (i.e. MEC, CBCS, BWHS, WCHS; referent BWHS).

[b]Coded alleles refer to the variant allele; non-coded alleles are the referent alleles.

[c]To evaluate the impact of p-value correction for multiple comparisons, we used an false-discovery rate of 5% and calculated an adjusted p-value necessary to meet significance based on that proposed by Benjamini and Hochberg 1995. We ranked all the p-values (max number of tests = 65) from most to least significant and then calculated the adjusted p-value, as (i/m) * Q, where i = the raw p-value rank and m= the total number of tests, and Q= 5% FDR.