Int J Mol Epidemiol Genet 2014;5(3):145-151
www.ijmeg.org /ISSN:1948-1756/IJMEG0001522

Original Article

Genetic variants in anti-Mullerian hormone and anti-Mullerian hormone receptor genes and breast cancer risk in Caucasians and African Americans

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Received July 23, 2014; Accepted September 10, 2014; Epub October 22, 2014; Published October 30, 2014

Abstract: Anti-Mullerian hormone (AMH) regulates ovarian folliculogenesis by signaling via its receptors, and elevated serum AMH levels are associated with an increased risk of breast cancer. No previous studies have examined the effects of genetic variants in AMH-related genes on breast cancer risk. We evaluated the associations of 62 single nucleotide polymorphisms (SNPs) in *AMH* and its receptor genes, including AMH type 1 receptor (*ACVR1*) and AMH type 2 receptor (*AMHR2*), with the risk of breast cancer in the Women's Insights and Shared Experiences (WISE) Study of Caucasians (346 cases and 442 controls), as well as African Americans (149 cases and 246 controls). Of the 62 SNPs evaluated, two showed a nominal significant association (*P* for trend < 0.05) with breast cancer risk among Caucasians, and another two among African Americans. The age-adjusted additive odds ratios (ORs) (95% confidence interval (95% CI)) of those two SNPs (*ACVR1* rs12694937[C] and *ACVR1* rs2883605[T]) for the risk of breast cancer among Caucasian women were 2.33 (1.20-4.52) and 0.68 (0.47-0.98), respectively. The age-adjusted additive ORs (95% CI) of those two SNPs (*ACVR1* rs1146031[G] and *AMHR2* functional SNP rs2002555[G]) for the risk of breast cancer among African American women were 0.63 (0.44-0.92) and 1.67 (1.10-2.53), respectively. However, these SNPs did not show significant associations after correction for multiple testing. Our findings do not provide strong supportive evidence for the contribution of genetic variants in AMH-related genes to the risk of developing breast cancer in either Caucasians or African Americans.

Keywords: Single nucleotide polymorphism, anti-Mullerian hormone, anti-Mullerian hormone receptors, breast cancer

Introduction

Anti-Mullerian hormone (AMH) is a member of the transforming growth factor-β (TGFβ) family of growth and differentiation factors known primarily for its role in regulating the in-utero sexual differentiation of males [1]. In women, AMH is secreted by the premenopausal ovary [2-4] and regulates ovarian folliculogenesis by signaling via its receptors, including non-specific activin receptor-like kinase 2 receptor (ALK2) encoded by AMH type 1 receptor (*ACVR1*) gene and specific AMH type II receptor (AMHR2) encoded by *AMHR2* gene. Upon binding to AMHR2, AMH promotes heterodimerization with ALK2, resulting in downstream signaling via Smads [5]. Moreover, binding of AMH to AMHR2 suppresses follicle maturation by inhibiting recruitment of primordial follicles into the pool of growing follicles and by decreasing responsiveness of growing follicles to follicle stimulating hormone (FSH) [6]. Our previous study showed that women with elevated serum levels of AMH are at a 10-fold excess risk of developing breast cancer [7].

Given that ovarian follicle development is intimately related to steroidogenesis and breast

cancer risk has clearly been shown to be related to the ovarian steroid hormones, genetic variants in folliculogenesis-related genes, such as *AMH* and its receptors, could be related to breast cancer risk via effects on hormone production or other as yet unidentified mechanisms. However, only a few previous studies have focused on genes involved in regulation of folliculogenesis [8-11] and none have evaluated associations of genetic variants in *AMH* and its receptors with breast cancer risk.

In this study, we evaluated the associations of 62 single nucleotide polymorphisms (SNPs) in *AMH* and its receptor genes (*ACVR1* and *AMHR2*) with the risk of breast cancer in the Women's Insights and Shared Experiences (WISE) study of Caucasians (346 cases and 442 controls), as well as African Americans (149 cases and 246 controls).

Materials and methods

Study population and data collection

The WISE study is a population-based retrospective case-control study. Incident primary breast cancer cases were identified through hospitals and the Pennsylvania State Cancer Registry, and frequency-matched controls were identified from the community using random digit dialing. The source population for this study was from the three counties of Philadelphia (Pennsylvania), Delaware (Pennsylvania), and Camden (New Jersey). Details of the study have been reported previously [12-14].

Potentially eligible cases were women residing in these counties at the time of diagnosis who were aged 50-79 years and newly diagnosed with breast cancer between July 1, 1999 and June 30, 2002. The cases were identified through active surveillance at hospitals in these counties. Pennsylvania Cancer Registry lists were reviewed quarterly to validate completeness of case ascertainment. Breast cancer diagnoses were validated by review of pathology reports and medical records. Breast cancer was confirmed if a pathology report was compatible with a first primary, invasive breast cancer. Controls were selected from the same geographic region as the cases and were frequency matched to the cases on race, age (in 5-year age groups) and calendar date of interview (within 3 months). Eligible controls had no history of breast cancer. Both cases and controls were required to live in a non-institutional setting, to have a household telephone, to have the ability to speak English, and to have no severe cognitive, language, or speech impairment.

Telephone interviews were used to collect data on demographic characteristics, anthropometry, family history of breast cancer, menstrual and menopausal history, reproductive history, medical history, oral contraceptive (OC) and hormone replacement therapy (HRT) use, smoking and alcohol ingestion. Participants collected buccal swabs according to standard directions and mailed them to the University of Pennsylvania. A total of 346 cases and 442 controls for Caucasians, as well as 149 cases and 246 controls for African Americans were included in this study.

Participants provided verbal informed consent for the interview and written informed consent for the buccal samples. The University of Pennsylvania Committee on Studies Involving Human Beings, the institutional review boards at University of Maryland School of Medicine, and the institutional review boards of all the participating hospitals approved this study.

Laboratory assays

Using the International HapMap project, we identified single nucleotide polymorphisms (SNPs) that effectively cover 3 candidate genes of interest. Some of these SNPs are in linkage disequilibrium; therefore, a more efficient set of tagging SNPs can be used to capture the same genetic variation [15]. Using Haploview program and a minimum r^2 threshold of 0.8, we identified a set of 62 parsimonious tagging SNPs with minor allele frequency greater than 5% to capture genetic variation in each locus (introns and exons, as well as 20 kb upstream of the start of transcription and 10 kb downstream of the end of transcription) of three genes including *AMH* (13), *ACVR1* (42), and *AMHR2* (7), in a race specific manner for Caucasians and African Americans separately. Information on these 62 SNPs is presented in Supplementary Table 1.

We genotyped these SNPs using the Sequenom platform with 10ng of all DNA samples in 384-

Table 1. Basic characteristics of breast cancer cases and controls in WISE case-control study

Characteristic	Caucasians		African Americans	
	Cases (n = 346)	Controls (n = 442)	Cases (n = 149)	Controls (n = 246)
Age (yrs, mean)	63.4	62.5	62.2	61.0
Body-mass index (kg/m^2, mean)	24.0	24.0	25.7	26.0
Age at menarche (yrs, mean)	12.5	12.7	12.8	12.7
Age at menopause (yrs, mean)	48.2	48.4	48.2	47.5
Age at first full term pregnancy among parous women (yrs, mean)	24.3	24.6	21.2	20.9
Number of full term pregnancies (%)				
0	19.9	11.5	16.8	6.9
1~2	32.7	39.6	43.0	41.5
≥3	47.4	48.9	40.3	51.6
Duration of breast feeding (%)				
Never	69.1	56.1	66.9	67.1
< 12 months	22.3	28.1	19.6	19.8
≥ 12 months	8.7	15.8	13.5	13.2
Menopausal status (%)				
Premenopausal	7.2	7.7	7.4	8.1
Postmenopausal	76.0	80.1	80.5	72.8
Induced (e.g., surgical)/unknown	16.8	12.2	12.1	19.1
Family history of breast cancer in 1st degree relative (%)				
Yes	18.8	19.0	18.1	11.4
No	81.2	81.0	81.9	88.6
Duration of combined estrogen and progestin (CHRT) use (%)				
Never/other HRT use	75.1	70.6	92.6	87.8
< 3 years	8.4	13.6	1.3	6.9
≥ 3 years	16.5	15.8	6.0	5.3
Duration of oral contraceptive (OC) use (%)				
Never	53.8	47.7	55.7	46.8
< 3 years	22.0	26.6	19.5	22.4
≥ 3 years	24.3	25.7	24.8	30.9

well format. Laboratory personnel were blinded to case-control status, and 3% blinded quality control samples were inserted to validate genotyping procedures; concordance for the blinded quality control samples was 100%.

Statistical methods

We used the X^2 test to assess whether the genotypes for all 62 SNPs were in Hardy-Weinberg equilibrium (HWE) among the controls. We evaluated the association between each SNP and breast cancer risk using unconditional logistic regression. An additive model was used to calculate the *p*-value for trend on breast cancer risk according to an ordinal coding for genotype (0, 1 or 2 copies of SNP minor allele). All statistical analyses were two-sided and carried out using SAS V9.2 (SAS Institute, Cary, NC).

Results and discussion

Descriptive characteristics of cases and controls in this study are summarized in **Table 1**. The mean age at diagnosis of breast cancer cases was 63.4 years for Caucasians and 62.2 years for African Americans. Compared with controls, breast cancer cases in both Caucasians and African Americans had lower number of full term pregnancies. Among Caucasians, cases were less likely to breast-feed, more likely to have used long-term (≥ 3 years) combined estrogen and progestin hormone replacement therapy (CHRT). Among

African Americans, cases were more likely to have a family history of breast cancer in a first degree relative and less likely to have used long-term (≥ 3 years) oral contraceptives (OCs).

The distributions of genotypes for the four SNPs (rs1220135, rs13395576, rs16842009, and rs16842143 in ACVR1) among Caucasians and two SNPs (rs10933441 in ACVR1 and rs17695156 in AMHR2) among African Americans were not in Hardy-Weinberg equilibrium among controls (*P* for HWE = 0.000); thus were excluded from the analyses (Supplementary **Table 1**). We evaluated the association of each SNP with breast cancer risk among Caucasians and African Americans separately. Of the 62 SNPs evaluated, two showed a nominal significant association with breast cancer risk among Caucasians (*P* for rs12694937, 0.01; *P* for rs2883605, 0.04), and another two among African Americans (*P* for rs1146031, 0.01; *P* for rs2002555, 0.02). The age-adjusted additive odds ratios (ORs) (95% confidence interval (95% CI)) of those two SNPs (ACVR1 rs12694937[C] and ACVR1 rs2883605[T]) for the risk of breast cancer among Caucasian women were 2.33 (1.20-4.52) and 0.68 (0.47-0.98), respectively. The age-adjusted additive ORs (95% CI) of those two SNPs (ACVR1 rs1146031[G] and AMHR2 functional SNP rs2002555[G]) for the risk of breast cancer among African American women were 0.63 (0.44-0.92) and 1.67 (1.10-2.53), respectively (**Table 2**). These findings remained consistent after adjusting for breast cancer-related factors (**Table 2**). After correction for multiple testing (Bonferroni correction), these SNPs did not show significant associations with breast cancer risk (all *p*-values > 0.05/62 = 0.001).

Considering that the relationship of genetic variants in folliculogenesis-related genes with breast cancer risk could be affected by HRT or OC use, for those four SNPs that showed nominal associations in the main effect analyses, we conducted additional analyses in which users of HRT or OC were excluded. The results did not materially change for each of the four SNPs (data not shown).

A few previous studies have examined genetic variants in genes involved in regulation of folliculogenesis. A previous study of Dutch and German cohorts reported that women who carry the variant allele of the common polymorphism Ile49Ser (rs10407022) in the *AMH* gene have significantly higher serum estradiol levels in the follicular phase of the menstrual cycle compared to those who are homozygous wild-type [11]. This variant was also associated with altered hormonal profiles in polycystic ovary syndrome (PCOS) patients [16], and its interaction with *AMHR2* variant may modify age at natural menopause [17]. Similar to women who carry the Ile49Ser variant allele in *AMH*, women who carry the variant allele of the common A-482G promoter polymorphism in *AMHR2* gene (rs2002555) have significantly higher follicular phase serum estradiol levels compared to women who are homozygous wild-type in the cohorts of Dutch and German [11]. Furthermore, women who carry variant alleles for both Ile49Ser in *AMH* and A-482G in *AMHR2* have the highest follicular phase estradiol levels. It has been shown that the A-482G polymorphisms in *AMHR2* is associated with infertility in Italy [18] and Japanese women [19], and interacts with parity in relation to age at menopause [10]. In this study, -482G allele in *AMHR2* (rs2002555) was associated with an increased risk of breast cancer among African American women. Considering the effect of A-482G polymorphisms on serum estradiol level and well-known relationship between ovarian steroid hormone and breast cancer risk, it is plausible that this SNP is related to breast cancer risk partially through alteration of hormone production. For *ACVR1*, several tagging SNPs (rs1220134, rs10497189, and rs2033962/rs13021202) and their corresponding haplotypes were associated with AMH levels and follicle number in PCOS patients [20]. However, little is known about variants in *ACVR1* in relationship to AMH and follicle number in healthy women. In this study, we did not find an association of these SNPs with breast cancer risk.

One limitation of this study is potential recall bias from the retrospective case-control study design. However, deviations due to the major factors related to AMH, such as use of HRT and OC, could be minimized, because OC use is only modestly related to breast cancer risk and risk is limited in duration after discontinuation [21], and WISE was completed before the Women's Health Initiative heightened public awareness of the association of HRT use with breast cancer risk.

In summary, we evaluated the associations between 62 SNPs in three *AMH*-related genes

Table 2. SNPs significantly associated with breast cancer risk in WISE case-control study

SNP (gene)			Caucasians				African Americans	
rs12694937 (*ACVR1*)	Cases (%)	Controls (%)	Age-adjusted OR	Multivariate-adjusted OR[a]	Cases (%)	Controls (%)	Age-adjusted OR	Multivariate-adjusted OR[a]
TT	292 (92.4)	417 (96.5)	1.00	1.00	102 (71.8)	163 (71.5)	1.00	1.00
TC	24 (7.6)	15 (3.5)	2.33 (1.20-4.52)	2.35 (1.20-4.63)	38 (26.8)	59 (25.9)	1.02 (0.64-1.65)	0.89 (0.53-1.48)
CC	0 (0.0)	0 (0.0)	-	-	2 (1.4)	6 (2.6)	0.51 (0.10-2.61)	0.47 (0.09-2.49)
Additive OR			2.33 (1.20-4.52)	2.35 (1.20-4.63)			0.93 (0.61-1.42)	0.83 (0.53-1.29)
p for trend			0.01	0.01			0.74	0.40
rs2883605 (*ACVR1*)	Cases (%)	Controls (%)	Age-adjusted OR	Multivariate-adjusted OR[a]	Cases (%)	Controls (%)	Age-adjusted OR	Multivariate-adjusted OR[a]
GG	286 (86.1)	347 (80.5)	1.00	1.00	138 (97.9)	232 (97.9)	1.00	1.00
GT	44 (13.3)	80 (18.6)	0.66 (0.44-0.99)	0.67 (0.44-1.01)	3 (2.1)	5 (2.1)	1.09 (0.26-4.67)	1.34 (0.30-5.94)
TT	2 (0.6)	4 (0.9)	0.60 (0.11-3.31)	0.67 (0.12-3.74)	0 (0.0)	0 (0.0)	-	
Additive OR			0.68 (0.47-0.98)	0.69 (0.47-1.01)			1.09 (0.26-4.67)	1.34 (0.30-5.94)
p for trend			0.04	0.06			0.91	0.70
rs1146031 (*ACVR1*)	Cases (%)	Controls (%)	Age-adjusted OR	Multivariate-adjusted OR[a]	Cases (%)	Controls (%)	Age-adjusted OR	Multivariate-adjusted OR[a]
AA	323 (97.6)	430 (99.1)	1.00	1.00	95 (66.0)	133 (55.6)	1.00	1.00
AG	7 (2.1)	4 (0.9)	2.30 (0.67-7.92)	2.55 (0.71-9.10)	46 (31.9)	89 (37.2)	0.73 (0.47-1.14)	0.70 (0.43-1.12)
GG	1 (0.3)	0 (0.0)	-	-	3 (2.1)	17 (7.1)	0.24 (0.07-0.84)	0.28 (0.08-1.03)
Additive OR			2.60 (0.83-8.17)	2.82 (0.86-9.27)			0.63 (0.44-0.92)	0.63 (0.43-0.93)
p for trend			0.10	0.09			0.01	0.02
rs2002555 (*AMHR2*)	Cases (%)	Controls (%)	Age-adjusted OR	Multivariate-adjusted OR[a]	Cases (%)	Controls (%)	Age-adjusted OR	Multivariate-adjusted OR[a]
AA	216 (69.9)	304 (71.2)	1.00	1.00	98 (68.5)	176 (76.5)	1.00	1.00
AG	77 (24.9)	109 (25.5)	0.99 (0.71-1.40)	0.99 (0.70-1.41)	38 (26.6)	53 (23.0)	1.32 (0.81-2.15)	1.23 (0.73-2.08)
GG	16 (5.2)	14 (3.3)	1.67 (0.79-3.50)	1.84 (0.86-3.94)	7 (4.9)	1 (0.4)	12.4 (1.51-103)	13.3 (1.53-115)
Additive OR			1.12 (0.86-1.46)	1.14 (0.87-1.50)			1.67 (1.10-2.53)	1.62 (1.04-2.52)
p for trend			0.41	0.34			0.02	0.03

[a]Multivariate-adjusted ORs are adjusted for age, age at menarche (< 12 yr, 12 yr, or > 12 yr), number of full term pregnancies (0, 1 to 2, or ≥ 3), menopausal status (premenopausal, postmenopausal, or induced/unknown), family history of breast cancer in 1st degree relative (yes or no), body-mass index (< 25, 25 to 30, or ≥ 30), duration of CHRT use (never/other HRT use, < 3 yrs, or ≥ 3 yrs), and duration of OC use (never, < 3 yrs, or ≥ 3 yrs).

and breast cancer risk in both Caucasians and African Americans. We did not find strong supportive evidence for the contribution of genetic variants in AMH-related genes to the risk of developing breast cancer, although four SNPs showed suggestive association. The sample size of this study was modest, and additional larger studies are warranted to confirm the suggestive associations observed in the present study.

Acknowledgements

We thank our collaborators Brian L. Strom, Angela DeMichele, Greta Bunin, and Sandra Norman who were involved in the design and execution of the WISE study. We are also in debt to the database manager Dr. Anita L. Weber; the project manager for the Hospital Network Core, Elene Turzo; and the project manager for the Field Core, Desiree Burgh, for their incredible efforts in coordinating the logistic aspects of obtaining institutional review board approvals in participating hospitals and for ascertaining and recruiting the large number of participants in this study. We also thank Karen Venuto who managed the tracking database and the vast correspondence involved in this study, Shawn Fernandes for performing extensive quality control checks and helping with the development of the questionnaire database and Stephen Gallagher for data management. We are grateful for the cooperation of the hospitals in the Greater Delaware Valley and for the support of the physicians who sponsored their study in these institutions, as without this help we could not have performed this study. We are indebted to the participants in the WISE Study for their dedication and commitment. National Cancer Institute, National Institutes of Health (R03 CA150072).

Disclosure of conflict of interest

None.

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References

[1] Teixeira J, Maheswaran S and Donahoe PK. Mullerian inhibiting substance: an instructive developmental hormone with diagnostic and possible therapeutic applications. Endocr Rev 2001; 22: 657-674.

[2] Lee MM, Donahoe PK, Hasegawa T, Silverman B, Crist GB, Best S, Hasegawa Y, Noto RA, Schoenfeld D and MacLaughlin DT. Mullerian inhibiting substance in humans: normal levels from infancy to adulthood. J Clin Endocrinol Metab 1996; 81: 571-576.

[3] de Vet A, Laven JS, de Jong FH, Themmen AP and Fauser BC. Antimullerian hormone serum levels: a putative marker for ovarian aging. Fertil Steril 2002; 77: 357-362.

[4] van Rooij IA, Broekmans FJ, Scheffer GJ, Looman CW, Habbema JD, de Jong FH, Fauser BJ, Themmen AP and te Velde ER. Serum antimullerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study. Fertil Steril 2005; 83: 979-987.

[5] Clemente N, Josso N, Gouedard L and Belville C. Components of the anti-Mullerian hormone signaling pathway in gonads. Mol Cell Endocrinol 2003; 211: 9-14.

[6] Durlinger AL, Visser JA and Themmen AP. Regulation of ovarian function: the role of anti-Mullerian hormone. Reproduction 2002; 124: 601-609.

[7] Dorgan JF, Stanczyk FZ, Egleston BL, Kahle LL, Shaw CM, Spittle CS, Godwin AK and Brinton LA. Prospective case-control study of serum mullerian inhibiting substance and breast cancer risk. J Natl Cancer Inst 2009; 101: 1501-1509.

[8] Kevenaar ME, Themmen AP, van Kerkwijk AJ, Valkenburg O, Uitterlinden AG, de Jong FH, Laven JS and Visser JA. Variants in the ACVR1 gene are associated with AMH levels in women with polycystic ovary syndrome. Hum Reprod 2009; 24: 241-249.

[9] Kevenaar ME, Laven JS, Fong SL, Uitterlinden AG, de Jong FH, Themmen AP and Visser JA. A functional anti-mullerian hormone gene polymorphism is associated with follicle number and androgen levels in polycystic ovary syndrome patients. J Clin Endocrinol Metab 2008; 93: 1310-1316.

[10] Kevenaar ME, Themmen AP, Rivadeneira F, Uitterlinden AG, Laven JS, van Schoor NM, Lips P, Pols HA and Visser JA. A polymorphism in the AMH type II receptor gene is associated with age at menopause in interaction with parity. Hum Reprod 2007; 22: 2382-2388.

[11] Kevenaar ME, Themmen AP, Laven JS, Sonntag B, Fong SL, Uitterlinden AG, de Jong FH, Pols HA, Simoni M and Visser JA. Anti-Mullerian hormone and anti-Mullerian hormone type II receptor polymorphisms are associated with follicular phase estradiol levels in normo-ovulatory women. Hum Reprod 2007; 22: 1547-1554.

[12] Rebbeck TR, Troxel AB, Walker AH, Panossian S, Gallagher S, Shatalova EG, Blanchard R, Norman S, Bunin G, DeMichele A, Berlin M, Schinnar R, Berlin JA and Strom BL. Pairwise combinations of estrogen metabolism genotypes in postmenopausal breast cancer etiology. Cancer Epidemiol Biomarkers Prev 2007; 16: 444-450.

[13] Rebbeck TR, Troxel AB, Norman S, Bunin GR, DeMichele A, Baumgarten M, Berlin M, Schinnar R and Strom BL. A retrospective case-control study of the use of hormone-related supplements and association with breast cancer. Int J Cancer 2007; 120: 1523-1528.

[14] Rebbeck TR, Troxel AB, Wang Y, Walker AH, Panossian S, Gallagher S, Shatalova EG, Blanchard R, Bunin G, DeMichele A, Rubin SC, Baumgarten M, Berlin M, Schinnar R, Berlin JA and Strom BL. Estrogen sulfation genes, hormone replacement therapy, and endometrial cancer risk. J Natl Cancer Inst 2006; 98: 1311-1320.

[15] Haiman CA and Stram DO. Utilizing HapMap and tagging SNPs. Methods Mol Med 2008; 141: 37-54.

[16] Kevenaar ME, Laven JSE, Fong SL, Uitterlinden AG, de Jong FH, Themmen APN and Visser JA. A functional anti-mullerian hormone gene polymorphism is associated with follicle number and androgen levels in polycystic ovary syndrome patients. J Clin Endocrinol Metab 2008; 93: 1310-1316.

[17] Braem MG, Voorhuis M, van der Schouw YT, Peeters PH, Schouten LJ, Eijkemans MJ, Broekmans FJ and Onland-Moret NC. Interactions between genetic variants in AMH and AMHR2 may modify age at natural menopause. PLoS One 2013; 8: e59819.

[18] Rigon C, Andrisani A, Forzan M, D'Antona D, Bruson A, Cosmi E, Ambrosini G, Tiboni GM and Clementi M. Association study of AMH and AMHRII polymorphisms with unexplained infertility. Fertil Steril 2010; 94: 1244-1248.

[19] Yoshida Y, Yamashita Y, Saito N, Ono Y, Yamamoto H, Nakamura Y, Hayashi A, Terai Y and Ohmichi M. Analyzing the possible involvement of anti-Mullerian hormone and anti-Mullerian hormone receptor II single nucleotide polymorphism in infertility. J Assist Reprod Genet 2014; 31: 163-8.

[20] Kevenaar ME, Themmen AP, van Kerkwijk AJ, Valkenburg O, Uitterlinden AG, de Jong FH, Laven JS, Visser JA. Variants in the ACVR1 gene are associated with AMH levels in women with polycystic ovary syndrome. Hum Reprod 2009; 24: 241-9.

[21] Henderson BE, Pike MC, Bernstein L and Ross RK. Breast cancer. In: Schottenfeld D, Fraumeni JF, editors. Cancer Epidemiology and Prevention. Second. New York: Oxford University Press; 1996. pp. 1022-1039.

Supplementary Table 1. 62 SNPs in *AMH*, *ACVR1*, and *AMHR2* genes

		Caucasians			African Americans		
Gene	rs#	Major/minor allele	MAF (%)[a]	*P* for HWE	Major/minor allele	MAF (%)[a]	*P* for HWE
Anti-mullerian hormone (AMH)	rs733846	T/G	16	0.340	G/T	43	0.016
	rs2074860	A/G	18	0.379	G/A	42	0.224
	rs3746158	A/G	23	0.528	A/G	42	0.203
	rs3761021	T/C	2	0.654	T/C	15	0.537
	rs4806834	C/T	3	0.456	C/T	29	0.281
	rs6510652	G/T	9	0.098	G/T	26	0.976
	rs6510653	A/G	10	0.427	G/A	45	0.068
	rs7249235	C/A	9	0.052	C/A	29	0.170
	rs7250822	G/C	16	0.228	G/C	18	0.366
	rs7253181	T/G	22	0.398	G/T	48	0.859
	rs8112524	A/G	38	0.229	G/A	37	0.038
	rs10407022	T/G	13	0.012	T/G	39	0.723
	rs10415913	G/A	9	0.816	A/G	49	0.073
AMH type 1 receptor (ACVR1)	rs12936	G/T	0	0.961	G/T	5	0.605
	rs1146031	A/G	0	0.923	A/G	26	0.691
	rs1146033	A/G	1	0.904	A/G	26	0.024
	rs1146037	T/C	22	0.569	T/C	19	0.042
	rs1220110	T/A	27	0.739	A/T	36	0.090
	rs1220133	G/A	28	0.477	A/G	33	0.583
	rs1220134	T/A	28	0.761	A/T	35	0.234
	rs1220135	T/C	0	0.000	T/C	8	0.778
	rs2883605	G/T	10	0.796	G/T	1	0.870
	rs3738927	T/C	0	0.981	T/C	5	0.581
	rs4233672	G/A	19	0.023	A/G	32	0.363
	rs4380178	G/A	16	0.124	G/A	20	0.369
	rs4664898	A/G	19	0.661	A/G	26	0.522
	rs4664901	T/C	23	0.140	C/T	23	0.560
	rs7561419	C/T	0	0.962	C/T	23	0.415
	rs7563276	G/A	6	0.886	G/A	9	0.932
	rs7565550	C/A	20	0.329	C/A	29	0.688
	rs7603425	T/C	0	0.981	T/C	9	0.151
	rs9288697	G/T	5	0.924	G/T	29	0.312
	rs10168000	C/T	18	0.137	T/C	23	0.068
	rs10497189	T/C	12	0.542	T/C	1	0.842
	rs10497190	C/T	19	0.215	C/T	27	0.776
	rs10497191	C/T	13	0.312	T/C	21	0.006
	rs10497192	T/C	27	0.494	C/T	16	0.002
	rs10497193	A/G	18	0.728	A/G	42	0.413
	rs10933441	C/T	7	0.913	C/T	5	0.000
	rs10933443	T/C	25	0.885	C/T	38	0.581
	rs12694937	T/C	2	0.713	T/C	16	0.812
	rs12987698	T/G	17	0.071	G/T	30	0.020
	rs13021202	C/T	19	0.060	C/T	27	0.474
	rs13395576	T/C	0	0.000	T/C	6	0.884
	rs13398650	G/A	8	0.340	A/G	44	0.026
	rs13426299	C/G	0	0.981	C/G	20	0.435
	rs16842009	C/T	0	0.000	C/T	5	0.703
	rs16842018	G/T	1	0.902	G/T	32	0.106
	rs16842023	C/T	1	0.904	C/T	15	0.710
	rs16842091	C/T	0	0.962	C/T	5	0.407
	rs16842126	A/T	1	0.828	A/T	11	0.445
	rs16842128	G/T	0	0.981	G/T	6	0.888
	rs16842130	C/T	0	0.961	C/T	12	0.033

	rs16842143	G/A	0	0.000	G/A	6	0.351
	rs17182166	G/T	18	0.232	G/T	17	0.394
AMH type 2 receptor (AMHR2)	rs784888	C/G	0	0.922	C/G	33	0.623
	rs784892	C/T	0	0.962	C/T	27	0.715
	rs784893	T/C	1	0.904	T/C	47	0.825
	rs2002555	T/C	16	0.279	T/C	12	0.152
	rs11170550	G/T	16	0.396	G/T	16	0.625
	rs17695156	C/T	7	0.169	C/T	1	0.000
	rs36120387	C/T	10	0.254	C/T	2	0.768

[a]Minor allele frequency (MAF) was calculated among controls in this study.