Original Paper

Associations of Polymorphisms in Anti-Müllerian Hormone (*AMH* Ile49Ser) and its Type II Receptor (*AMHRII* -482 A>G) on Reproductive Outcomes and Polycystic Ovary Syndrome: a Systematic Review and Meta-Analysis

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Key Words

Anti-Müllerian hormone • Anti-Müllerian hormone type II receptor • *AMH* • *AMHRII* polymorphisms • Reproductive outcomes • Polycystic ovary syndrome • Meta-analysis

Abstract

Background/Aims: Reported associations of reproductive outcomes (RO) and polycystic ovary syndrome (PCOS) with genotypes of the Ile49Ser and -482A>G polymorphisms in the Anti-Müllerian hormone (*AMH*) gene and its type II receptor (*AMHRII*), respectively, have conflicting results. **Methods:** PubMed, Google Scholar and Science Direct databases were searched for studies that investigated Ile49Ser and -482A>G in RO and PCOS. Using the meta-analytic approach, we estimated risk (odds ratio [OR] with 95% confidence intervals) using standard genetic models. **Results:** All calculated summary effects were non-significant. Overall associations of Ile49Ser and -482A>G with RO were absent (OR 0.95-0.99, *P* = 0.76-0.96) but implied increased risk in PCOS (OR 1.07-1.17, *P* = 0.49-0.55). Where heterogeneity of the pooled ORs were present, its sources were explored using the Galbraith plot. Detection and omission of the outlying studies in both polymorphisms not only erased heterogeneity of the recalculated pooled outcomes but also changed direction of association, where null effects turned to increased risk (Ile49Ser in RO) and increased risk became reduced risk (-482A>G in PCOS). Implications of the Ile49Ser and -482A>G, effects pointed to protection for Caucasians

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(OR 0.64–0.89, $P = 0.36$ – 0.73) in RO and increased risk in PCOS (OR 1.19–1.45, $P = 0.28$ – 0.65). Asian effects in RO and PCOS were variable (OR 0.97–1.24, $P = 0.58$ – 0.91). **Conclusions:** In summary, we found no evidence of significant associations of Ile49Ser and -482A>G with RO and PCOS, although contrasting Ile49Ser effects were implied among Caucasians between RO (up to 0.36% reduced risk) and PCOS (up to 1.5-fold increased risk).

Introduction

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Reproductive outcomes (RO) in women encompass a range of gynecological issues which include ovarian insufficiency and failure as well as infertility. Infertility which could be attributed to precursor conditions such as polycystic ovary syndrome (PCOS) involves failure to achieve pregnancy after a year of unprotected intercourse [1]. RO has become a global health issue warranting couples to seek assisted reproductive technology methods [2]. Success of these methods depends on variability of patient characteristics, much of which is attributed to genetic factors that regulate female reproductive function. Single nucleotide polymorphisms (SNPs) in coding regions in the signaling pathways of the ovary could play an important role in RO, as they are responsible for synthesis of hormone receptors, metabolic enzymes or transport molecules.

The Anti-Müllerian hormone (AMH) aka Müllerian inhibiting substance is homodimeric and a member of the transforming growth factor-beta (TGF- β) superfamily [3]. AMH in the female is produced exclusively by ovarian granulosa cells of pre-antral and small antral follicles [4]. Its concentration declines with age and become undetectable after menopause [5]. The number of antral follicles are strongly correlated with the size of primordial follicle pool [6] which indicate status of ovarian function and reserve as shown by serum AMH levels [7]. AMH is involved in the regulation of follicular growth [8] and continues to be expressed in the growing follicles until they have reached the appropriate size and differentiation state [9]. AMH with its serum has been shown to be a good indicator of a patient's follicular response to controlled ovarian stimulation with gonadotropins [7]. A meta-analysis has reported AMH to be a good predictor of ovarian response [10].

The gene for *AMH* is located on the small arm of chromosome 19 and consists of 5 exons [11, 12]. *AMH* is produced as a pro-hormone, which after secretion undergoes cleavage to generate a transforming growth factor-C-terminal fragment [13]. *AMH* exerts its biological effects through the *AMH* type II receptor (*AMHRII*), which in turn activates *AMH* type I receptor (*AMHRI*) and creates a downstream signaling pathway. The human gene for *AMHRII* is located on chromosome 12, consists of 11 exons and is expressed in granulosa and theca cells [14]. Recent studies in normo-ovulatory women have demonstrated an association of the polymorphisms of *AMH* and its receptor (*AMHRII*) with estradiol levels during the early follicular phase of the menstrual cycle [15]. Therefore, genetic variants in the *AMH* and *AMHRII* genes may influence hormone function in folliculogenesis causing infertility. Two polymorphisms in the *AMH* gene and *AMHRII* are found at the Ile49Ser (rs10407022) and -482A>G (rs2002555) restriction sites, respectively [3]. Genetic studies have shown that these variants are associated with reproductive status of women which include infertility [16] and in the *AMH* signaling pathway, play a role in usage of the primordial follicle pool, and also affect follicle counts as well as folliculogenesis [17]. Carriers of the *AMH* 49Ser and -482G alleles have higher follicular phase estradiol levels compared to non-carriers [15, 18]. Also, carriers of the *AMH* 49Ser allele in PCOS, women were less likely to exhibit polycystic ovaries and low androgen levels (hyperandrogenism) [19–21], suggesting a contribution of *AMH* polymorphism to the severity of PCOS. Considering these roles of *AMH* in regard to regulating ovarian function, it has been postulated that the genetic variants in the *AMH* signaling pathway could lead to an impaired reproductive function of women. Given the variable outcomes in the human reproduction investigations of the *AMH* and *AMHRII* variants, we performed a meta-analysis to evaluate the role of Ile49Ser and -482A>G in RO and PCOS.

Materials and Methods

Selection of studies

Using the terms “*AMH*”, “*Anti-Müllerian hormone*”, “*polymorphism*”, “*reproductive outcomes*” and “*polycystic ovary syndrome*”, we searched MEDLINE using PubMed, Science Direct and Google Scholar for association studies as of May 31, 2016 without language restrictions. References cited in the retrieved articles were also screened manually to identify additional eligible studies. Inclusion criteria included: (1) case-control study design evaluating the association between *AMH* and *AMHRII* polymorphisms and RO, as well as PCOS, (2) sufficient genotype frequency data presented to calculate the odds ratios (ORs) and 95% confidence intervals (CI).

Data extraction

Two investigators independently extracted data and reached consensus on all the items. The following information was obtained from each publication: first author's name, published year, country of origin, dominant ancestry of the study populations, study design, context of the study, type of ovarian response, use of the Hardy-Weinberg Equilibrium (HWE), *AMH* and *AMHRII* polymorphism studied, sample source, genotyping approach, matching information and genotype data. We extracted data that pertained to our investigation of RO and PCOS. We also calculated frequencies of the variant allele, deviations of the controls from HWE. Where studies reported the mean and 95% CI, SD was derived as follows: $SD = \sqrt{N \times (\text{upper limit} - \text{lower limit}) / 3.92}$.

Quality assessment of the studies

We used the Clark-Baudouin Score (CBS) to evaluate methodological quality of the included studies [22]. This scale emphasizes statistics (i.e. P values, power and corrections for multiplicity) and includes genotyping methods as well as HWE. These among others, were addressed in several of the included papers. Thus we felt this to be most appropriate in assessing the methodological quality of the included studies. For case-control studies, CBS scores range from 0 (worst) to 10 (best) where scoring is based on quality (low for <5 and high for ≥5).

Meta-analysis

We investigated polymorphisms in *AMH* (Ile49Ser) and *AMHRII* (-482A>G) whose influence was estimated using the standard genetic models (homozygous, recessive, dominant and codominant). These associations were expressed as OR, 95% CI. Raw data for genotype frequencies, without adjustment, were used for calculating study-specific estimates of the OR. Significance of the pooled estimates was determined by the Z-test. Pooled estimates were obtained using either the fixed [23] (in the absence of heterogeneity) or random [24] (in its presence) effects models. Heterogeneity between studies was estimated using the χ^2 -based Q test [25]. Recognizing the low power of this test [26], significance threshold was set at P = 0.10. We also quantified heterogeneity with the I^2 statistic which measures the degree of inconsistency among studies [27]. Significance was set at a P-value of ≤0.05 throughout except in heterogeneity estimation. Pooled estimates were subjected to sensitivity analysis which involved omitting one study at a time and recalculating the pooled estimates, to test for robustness of the summary effect. Subgroup analysis based on ethnicity was performed on those comprising of three or more studies. Data were analyzed using Review Manager 5.3 (Copenhagen: Nordic Cochrane Centre, Cochrane Collaboration, 2014) and SigmaStat 2.3 (Systat Software, San Jose, CA). All comparisons in our meta-analysis had <10 studies, and because of the low sensitivity of the qualitative and quantitative tests [28], we did not investigate publication bias.

Results

Included studies

Figure 1 outlines our study selection process in a flowchart following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [29]. We identified a total of 116 citations during the initial search, from which 17 were omitted following a review of titles and abstracts. We retrieved the remaining 99 abstracts which after review, 73

articles were excluded for not conforming to our inclusion criteria. Full-texts of the remaining 26 were obtained and read including the reference lists. From the 26, 10 were excluded for being duplicates or with absence of genotypic data. The remaining 16 articles were included in the meta-analysis. The epidemiological features and clinical characteristics these studies are outlined in Table 1 and 2. Nine and six examined RO [16, 30–37] and PCOS [19, 38–42], respectively, and one study examined both [43]. Publication years of the included studies ranged from 2008 to 2016. Eight studies each were Asian [33, 35–37, 40–43] and Caucasian [16, 19, 30–32, 34, 38, 39]. In terms of study context for *Ile49Ser*, five focused on infertility or ovarian insufficiency [16, 33, 36, 37, 43] and three addressed stimulation issues [30, 32, 35]. One was about assisted reproductive technology [31] and another addressed follicle recruitment [34]. All 16 articles addressed HWE and used blood as tissue source, but none of them explicitly mention about controls being matched to cases. For *-482A>G*, the studies addressed relationships between genes [39, 42], hormone levels [19, 38, 41] and PCOS. One PCOS study engaged in haplotype analysis [40]. Based on the CBS scores, methodological quality of the included papers was high (mean and standard deviation of 6.40 ± 0.84 and 5.57 ± 1.40 for RO and PCOS, respectively). Tables 3 and 4 summarize the sample sizes, genotype and minor allele frequencies as well as HWE values for both polymorphisms in RO and PCOS. Controls in two studies for PCOS deviated from HWE [41, 42]. Where genotype frequency values are zero, we applied the Laplace correction by adding a pseudo-count of one to all values of the data set [44] prior to generating the forest plots.

Meta-analysis

Tables 5 and 6 show no evidence of significant associations in all comparisons. The *Ile49Ser* overall effects in RO were essentially null (OR 0.95–0.99, $P = 0.76$ –0.98) but implied increased risk in PCOS (OR 1.07–1.16, $P = 0.51$ –0.55). Outlier analysis changed these overall effects to increased risk for RO (OR 1.15–1.18, $P = 0.17$ –0.47) and reduced risk for PCOS (OR 0.90–0.93, $P = 0.30$ –0.69). Figures 2 to 4 show effects of outlier treatment in the dominant model of association. Figure 2 shows a pooled OR of <1.00 (OR 0.95). Figure 3 identifies two outlier studies below the -2 confidence limit [16, 43]. Figure 4 shows the forest plot without the outliers that resulted in a changed pooled OR of >1 (OR 1.17). The *-482A>G* overall effects yielded both an implied increased risk in RO (OR 1.02–1.24, $P = 0.28$ –0.82) and in PCOS (OR 1.05–1.31, $P = 0.54$ –0.80). Asian *Ile49Ser* effects were variable for RO but implied increased risk in *-482A>G* in PCOS (OR 1.14–1.16, $P = 0.50$ –0.76). By contrast, Caucasian effects implied an *Ile49Ser* protection in RO (OR 0.64–0.89, $P = 0.36$ –0.73) but increased risk in PCOS (OR 1.19–1.45, $P = 0.28$ –0.51). Like-

Fig. 1. Flowchart of literature search and study selection.

Table 1. Characteristics of the included articles that examined the association of *AMH* and *AMHR11* polymorphisms with RO in women. RO: Reproductive Outcomes; [R]: Reference; HWE: Hardy-Weinberg Equilibrium; RT-PCR: Reverse Transcriptase Polymerase Chain Reaction; CBS: Clark-Baudouin Score: assessment of methodological quality of the included studies

	First Author (Year)	[R]	Country	Ethnic group	Polymorphism	Context of study	Study design	Used HWE	Tissue Source	Genotyping platform	Age of subjects: mean $\pm$ SD (range)	CBS
1	Hanevik 2010	[30]	Norway	Caucasian	<i>AMH</i> : Ile49Ser <i>AMHR11</i> : -482A>G	Response to ovarian stimulation	Retrospective case-control	Yes	blood	Taqman	cases: 32.6 $\pm$ 5.33y; controls: 32.4 $\pm$ 2y	7
2	Karagiorga 2015	[31]	Greece	Caucasian	<i>AMH</i> : Ile49Ser <i>AMHR11</i> : -482A>G	Markers in assisted reproduction technology	Prospective cross-sectional	Yes	blood	RT-PCR	36 $\pm$ 5y	6
3	Lazaros 2016	[32]	Greece	Caucasian	<i>AMHR11</i> : -482A>G	Ovarian response to gonadotropin stimulation	Prospective case-control	Yes	blood	PCR	33 $\pm$ 4y for the study population (28-38y) for both groups	7
4	Rigon 2010	[16]	Italy	Caucasian	<i>AMH</i> : Ile49Ser <i>AMHR11</i> : -482A>G	Unexplained infertility	Observational case-control	Yes	blood	PCR	63 $\pm$ 3.4y for both groups	6
5	Voorhuis 2011	[34]	Netherlands	Caucasian	<i>AMHR11</i> : -482A>G	Follicle recruitment	Prospect-Epic cohort	Yes	blood	PCR-SNP	Cases: 27 $\pm$ 6y; controls: 28 $\pm$ 5y	5
6	Chunhua 2012	[43]	China	Asian	<i>AMH</i> : Ile49Ser <i>AMHR11</i> : -482A>G	Premature ovarian failure	Association study	Yes	blood	PCR	cases: 32 $\pm$ 6y controls: 49 $\pm$ 5y	6
7	Qin 2014	[33]	China	Asian	<i>AMH</i> : Ile49Ser	Primary ovarian insufficiency at menopause	Retrospective case-control	Yes	blood	PCR	(20-35y) for both groups	7
8	Wang 2015	[35]	China	Asian	<i>AMH</i> : Ile49Ser	Pathogenesis of early ovarian hyperstimulation syndrome	Association study	Yes	blood	PCR	>45y post-menopausal	8
9	Yoon 2013	[36]	Korea	Asian	<i>AMH</i> : Ile49Ser <i>AMHR11</i> : -482A>G	Idiopathic primary ovarian insufficiency	Association case-control	Yes	blood	Taqman	Homozygous: 38.7 $\pm$ 4.2y Wild: 38 $\pm$ 4.7y	6
10	Yoshida 2014	[37]	Japan	Asian	<i>AMH</i> : Ile49Ser <i>AMHR11</i> : -482A>G	Infertility	Association case-control	Yes	blood	Taqman		

Table 2. Characteristics of the included articles that examined the association *AMH* and *AMHR11* polymorphisms with PCOS. [R]: Reference; PCOS: Polycystic Ovary Syndrome; HWE: Hardy-Weinberg Equilibrium; NM: No Mention; MALDI-TOF: Matrix-Assisted Laser Desorption/ Ionization Time-of-Flight; CBS: PCR: Polymerase Chain Reaction; Clark-Baudouin Score: assessment of methodological quality of the included studies

	First Author (Year)	[R]	Country	Ethnic group	Polymorphism	Context of study	Study design	Used HWE	Tissue Source	Genotyping platform	Age of subjects: mean $\pm$ SD (range)	Cases	Controls	CBS
1	Kevenaar 2008	[19]	Netherlands	Caucasian	<i>AMH</i> : Ile49Ser <i>AMHR11</i> : -482A>G	Follicle number and androgen levels in PCOS	Association study in cohort	Yes	blood	Taqman	28.7 $\pm$ 4.7y	28.7 $\pm$ 4.7y	30 $\pm$ 4y	6
2	Sprout 2010	[39]	USA	Caucasian	<i>AMH</i> : Ile49Ser	Folliculogenesis genes in PCOS	Association study	Yes	blood	NM	27 $\pm$ 11y	27 $\pm$ 11y	32.5 $\pm$ 17y	6
3	Georgopoulos 2013	[38]	Greece	Caucasian	<i>AMHR11</i> : -482A>G	Relationship of luteinizing hormone in women with PCOS	Case-control in cohort	Yes	blood	MALDI-TOF	24 $\pm$ 5.8y	24 $\pm$ 5.8y	30.8 $\pm$ 5.8y	7
4	Xu 2013	[40]	China	Asian	<i>AMH</i> : Ile49Ser	Haplotype analysis	Association study	Yes	blood	Taqman	25.9 $\pm$ 4.2y	25.9 $\pm$ 4.2y	29.9 $\pm$ 3.9y	5
5	Zheng 2016	[41]	China	Asian	<i>AMH</i> : Ile49Ser	Androgen levels in PCOS	Case-control association study	Yes	blood	PCR	27.8 $\pm$ 4.1y	27.8 $\pm$ 4.1y	27.4 $\pm$ 4.7y	7
6	Jian 2008	[42]	China	Asian	<i>AMH</i> : Ile49Ser <i>AMHR11</i> : -482A>G	Relationship of <i>AMH</i> and <i>AMHR11</i> with PCOS	Case-control association study	Yes	blood	PCR	(21 - 37y)	(21 - 37y)	23 - 38y	5
7	Chunhua 2012	[43]	China	Asian	<i>AMH</i> : Ile49Ser <i>AMHR11</i> : -482A>G	Relationship of <i>AMH</i> and <i>AMHR11</i> with PCOS	Case-control association study	Yes	blood	PCR	(28-31y)	(28-31y)	21 - 38y	7

wise, the -482A>G Caucasian effects in RO implied increased risk (OR 1.08-1.20, $P = 0.42$ -0.65).

Heterogeneity analysis

Effects of outlier treatment on the overall pooled ORs in both polymorphisms was generally erasure of heterogeneity (Tables 5 and 6). Figures 2 to 4 show effects of outlier treatment in the dominant model of Ile49Ser on heterogeneity. Figure 2 shows the pre-outlier pooled OR with moderate heterogeneity ($P = 0.02$, $I^2 = 58\%$) and Figure 4 shows the post-outlier effect resulting in zero heterogeneity ($P = 0.74$, $I^2 = 0\%$). Zero heterogeneity ($I^2 = 0\%$) was also observed in the following comparisons: (i) Ile49Ser post-outlier pooled ORs in the codominant models of RO and homozygous/recessive models of PCOS. (ii) Caucasian

Table 3. Genotype frequencies of *AMH* and *AMHRII* polymorphisms with RO in women. RO: Reproductive Outcome; maf: minor allele frequency; HWE: Hardy-Weinberg Equilibrium

First Author Year		Sample sizes			Cases			Controls			maf	HWE
		Case	Control	Total	wt-wt	wt-var	var-var	wt-wt	wt-var	var-var		
AMH Ile49Ser (rs10407022)												
1	Chunhua 2012 [43]	32	94	126	15	13	4	20	48	26	0.53	0.81
2	Hanevik 2010 [30]	91	100	191	60	28	3	66	28	6	0.20	0.21
3	Karagiorgia 2015 [31]	151	100	251	97	50	4	72	24	4	0.16	0.28
4	Qin 2014 [33]	120	120	240	77	36	7	89	26	5	0.15	0.10
5	Rigon 2010 [16]	76	100	176	64	12	0	70	30	0	0.15	0.08
6	Wang 2015 [35]	62	60	122	26	24	12	26	30	4	0.32	0.23
7	Yoon 2013 [36]	211	233	444	100	88	23	111	95	27	0.32	0.34
8	Yoshida 2014 [37]	15	21	36	4	8	3	9	11	1	0.31	0.30
AMHRII -482A>G (rs2002555)												
1	Chunhua 2012 [43]	32	94	126	20	11	1	79	14	1	0.09	0.67
2	Hanevik 2010 [30]	100	91	191	64	34	2	59	29	3	0.19	0.81
3	Karagiorgia 2015 [31]	148	100	248	103	43	2	68	30	2	0.17	0.53
4	Lazaros 2016 [32]	300	300	600	224	69	7	233	63	4	0.12	0.91
5	Rigon 2010[16]	76	100	176	56	18	2	63	31	6	0.22	0.41
6	Voorhuis 2010 [34]	2,892	524	3,416	1,879	905	108	343	167	14	0.19	0.23
7	Yoon 2013 [36]	211	233	444	131	73	7	142	85	6	0.21	0.10
8	Yoshida 2014 [37]	17	23	40	12	2	3	11	9	3	0.33	0.60

Table 4. Genotype frequencies of *AMH* and *AMHRII* polymorphisms with PCOS. PCOS: Polycystic Ovary Syndrome; maf: minor allele frequency; HWE: Hardy-Weinberg Equilibrium

First Author Year		Sample sizes			Cases			Controls			mafHWE		
		Case	Control	Total	wt-wt	wt-var	var-var	wt-wt	wt-var	var-var			
AMH Ile49Ser (rs10407022)													
1	Chunhua 2012 [43]	94	94	188	34	29	31	20	48	26	0.53	0.81	
2	Jian 2008 [42]	103	79	182	49	38	16	29	39	1	0.30	0.003	
3	Kevenaar 2008 [18]	331	32	363	208	110	13	24	8	0	0.13	0.42	
4	Sproul 2010 [39]	335	198	533	238	89	8	129	62	7	0.19	0.89	
5	Xu 2013 [40]	475	512	987	191	228	56	191	255	66	0.38	0.18	
6	Zheng 2016 [41]	94	94	188	20	48	26	34	29	31	0.48	0.0002	
AMHRII -482A>G (rs2002555)													
1	Chunhua 2012 [43]	94	94	188	80	13	1	79	14	1	0.09	0.67	
2	Georgopoulos 2013 [38]	858	309	1,167	585	248	25	234	71	4	0.13	0.59	
3	Jian 2008 [42]	103	79	182	55	41	7	52	22	5	0.20	0.22	
4	Kevenaar 2008 [18]	331	32	363	224	94	13	20	12	0	0.19	0.19	

Ile49Ser effects in RO; and (iii) all homozygous and recessive effects (overall and subgroups) in -482A>G of both polymorphisms (Tables 5 and 6).

Sensitivity analysis

Table 7 summarizes the changes in association resulting from sensitivity treatment. These changes affected all comparisons in every genetic model. Of the four comparisons, Ile49Ser in RO was least robust as the pooled ORs were altered with omission of a total of five studies [16, 30, 31, 36, 43] in every genetic model. By contrast, -482A>G was non-robust in the dominant model only, attributed to two studies [32, 43]. In terms of genetic model, pooled ORs in the homozygous and recessive models were deemed robust in -482A>G comparisons.

Discussion

With a sample size of 9,210 for both polymorphisms in RO and PCOS, our meta-analysis has shown that genotypes in the *AMH* and *AMHRII* genes are not significantly associated with RO and PCOS. Despite the non-significance, subgroup analysis delineated pooled effects between the ethnic groups and outlier treatment erased heterogeneity, which thus rendered greater confidence in the post-outlier results. However, another consequence of outlier treatment was change in direction of association such that the original close to null pooled ORs became 1.2-fold increased risk in Ile49Ser and 1.2-fold increased risk became

Fig. 2. Forest plot showing the dominant influence of the Ile49Ser polymorphism on RO outcomes in women. Black diamond denotes the pooled OR. Blue squares indicate the OR in each study, with square sizes directly proportional to the weight contribution (%) of the study. Horizontal lines represent 95% confidence intervals. RO: Reproductive Outcomes; OR: Odds Ratio; M-H: Mantel-Haenszel; CI: confidence interval; df: degree of freedom

Fig. 3. Galbraith plot analysis to detect sources of heterogeneity in the dominant model. OR: Odds Ratio; SE: Standard Error.

Fig. 4. Forest plot showing the dominant post-outlier influence of the Ile49Ser polymorphism on RO in women. Black diamond denotes the pooled OR. Blue squares indicate the OR in each study, with square sizes directly proportional to the weight contribution (%) of the study. Horizontal lines represent 95% confidence intervals. RO: Reproductive Outcomes; OR: Odds Ratio; M-H: Mantel-Haenszel; CI: confidence interval; df: degree of freedom

protective for up to 10% in -482A>G. Despite the clinical heterogeneity of RO, post-outlier values were statistically non-heterogeneous, zero in the dominant and codominant models.

Table 5. Summary associations of the *AMH* Ile49Ser and *AMHRII* -482A>G polymorphisms with RO outcomes in women. RO: Reproductive Outcomes; N: number of studies; OR: Odds Ratio; CI: Confidence Interval; P^a : P value for association with significance set at <0.05; P^b : P value for heterogeneity with significance set at <0.10; I^2 values as measure of heterogeneity are considered low (<44%), moderate (45-74%) or high (>75%); R: Random-effects; F: Fixed-effects

<i>AMH</i> Ile49Ser	N	Test of association			Test of heterogeneity		
		OR	95% CI	P^a	P^b	I^2 (%)	Analysis Model
Overall							
Homozygous	8	0.98	0.52-1.85	0.96	0.08	45	R
Recessive	8	0.99	0.68-1.45	0.98	0.18	31	F
Dominant	8	0.95	0.66-1.35	0.76	0.02	58	R
Codominant	8	0.99	0.73-1.33	0.94	0.01	62	R
Post-outlier							
Homozygous	7	1.18	0.77-1.80	0.45	0.40	3	F
Recessive	7	1.16	0.77-1.75	0.47	0.33	14	F
Dominant	6	1.17	0.93-1.48	0.17	0.74	0	F
Codominant	6	1.15	0.96-1.38	0.13	0.46	0	F
Asian							
Homozygous	5	1.18	0.47-2.97	0.73	0.02	66	R
Recessive	5	1.24	0.58-2.63	0.58	0.07	55	R
Dominant	5	0.97	0.60-1.59	0.91	0.03	61	R
Codominant	5	1.07	0.70-1.63	0.75	0.009	70	R
Caucasian							
Homozygous	3	0.68	0.26-1.73	0.42	0.90	0	F
Recessive	3	0.64	0.25-1.64	0.36	0.85	0	F
Dominant	3	0.89	0.47-1.70	0.73	0.04	68	R
Codominant	3	0.87	0.53-1.43	0.58	0.09	58	R
<i>AMHRII</i> -482A>G							
Overall							
Homozygous	8	1.21	0.81-1.80	0.35	0.74	0	F
Recessive	8	1.24	0.84-1.85	0.28	0.82	0	F
Dominant	8	1.02	0.88-1.17	0.82	0.12	38	F
Codominant	8	1.03	0.92-1.17	0.59	0.14	36	F
Asian							
Homozygous	3	1.29	0.52-3.16	0.58	0.69	0	F
Recessive	3	1.44	0.59-3.48	0.42	0.86	0	F
Dominant	3	0.88	0.44-1.77	0.72	0.01	77	R
Codominant	3	0.88	0.44-1.77	0.72	0.01	77	R
Caucasian							
Homozygous	5	1.19	0.76-1.86	0.44	0.47	0	F
Recessive	5	1.20	0.77-1.87	0.42	0.53	0	F
Dominant	5	1.08	0.77-1.51	0.65	0.09	51	R
Codominant	5	1.08	0.77-1.51	0.65	0.09	51	R

To our knowledge, this is the first meta-analysis to address *AMH* associations in both RO and PCOS. Study specific findings of RO and PCOS were variable, with contradictions among the results. Absence of associations between the *AMH* and *AMHRII* polymorphisms and ovarian stimulation have been reported [30, 35] and contradicted in another study [31]. Regarding ovarian insufficiency, findings of no association [36] were countered by other studies that found associations [33, 43]. Associations of Ile49Ser and -482A>G with PCOS have been reported [38, 41, 43] but found absent in other studies [19, 39, 40, 42]. These findings indicate lack of consistency in the outcome of these association studies. Here, then enters the utility of our meta-analysis.

AMH levels are currently used in clinical practice to predict response to stimulation [6], its value is recognized in a range of clinical settings, from PCOS to ovarian assessment following cancer treatment [45]. PCOS has been found to cause significantly increased risk of ovarian hyperstimulation syndrome [46]. In addition, impact of AMH receptor polymorphism have been studied in other diseases such as breast cancer [47] and cryptorchidism [48].

Hence, reproductive studies in women have focused on the role of serum AMH as marker for ovarian response and reserve [31]. AMH has the advantage of having relatively stable levels throughout the menstrual cycle [49, 50] and is more accurate in providing information on the expected ovarian response to controlled stimulation [50]. However, AMH is limited in extreme responses as it is not reliably predictive in this situation, and also its value has been questioned [51]. Nevertheless, considering the important role of the AMH signaling pathway in regulating sensitivity in the ovary and follicular recruitment, it is appropriate to consider that variation in the genes encoding key proteins in the pathway may influence ovarian response. Genetic variation in *AMH* studies demonstrated that Ile49Ser and -482A>G were associated with an increase in estradiol (E2) and AMH levels during the follicular phase of

Table 6. Summary associations of the *AMH* Ile49Ser and *AMHRII* -482A>G polymorphisms with PCOS. PCOS: Polycystic Ovary Syndrome; HWE: Hardy-Weinberg Equilibrium; N: number of studies; OR: Odds Ratio; CI: Confidence Interval; P^a : p value for association with significance set at <0.05; P^b : p value for heterogeneity with significance set at <0.10; I^2 values as measure of heterogeneity are considered low (<44%), moderate (45-74%) or high (>75%); R: Random-effects; F: Fixed-effects

<i>AMH</i> Ile49Ser	N	Test of association			Test of heterogeneity		
		OR	95% CI	P^a	P^b	I^2 (%)	Analysis Model
Overall							
Homozygous	8	1.16	0.71-1.89	0.55	0.08	47	R
Recessive	8	1.15	0.74-1.78	0.53	0.08	47	R
Dominant	8	1.12	0.80-1.56	0.51	0.00009	74	R
Codominant	8	1.07	0.87-1.31	0.54	0.02	59	R
Post-outlier							
Homozygous	5	0.90	0.66-1.22	0.48	0.53	0	F
Recessive	5	0.93	0.71-1.22	0.60	0.57	0	F
Dominant	5	0.93	0.65-1.32	0.69	0.03	62	R
Codominant	5	0.93	0.82-1.07	0.30	0.36	8	F
HWE only							
Homozygous	5	0.93	0.68-1.27	0.66	0.25	25	F
Recessive	5	1.06	0.80-1.42	0.68	0.37	6	F
Dominant	5	0.95	0.66-1.38	0.80	0.00	75	R
Codominant	5	1.01	0.89-1.15	0.88	0.01	70	R
Asian							
Homozygous	4	1.10	0.61-1.98	0.76	0.08	56	R
Recessive	4	1.15	0.65-2.02	0.64	0.04	64	R
Dominant	4	1.08	0.63-1.85	0.79	0.004	77	R
Codominant	4	0.97	0.83-1.12	0.64	0.39	1	F
Caucasian							
Homozygous	3	1.45	0.74-2.86	0.28	0.14	48	F
Recessive	3	1.40	0.71-2.75	0.33	0.22	34	F
Dominant	3	1.19	0.71-1.99	0.51	0.02	75	R
Codominant	3	1.20	0.73-1.96	0.47	0.009	79	R
<i>AMHRII</i> -482A>G							
Overall							
Homozygous	4	1.28	0.53-3.09	0.58	0.95	0	F
Recessive	4	1.31	0.55-3.10	0.54	0.94	0	F
Dominant	4	1.05	0.72-1.54	0.80	0.16	42	F
Codominant	4	1.09	0.79-1.51	0.60	0.46	0	F
Asian							
Homozygous	3	1.16	0.45-2.97	0.76	0.94	0	F
Recessive	3	1.16	0.46-2.92	0.76	0.96	0	F
Dominant	3	1.16	0.74-1.80	0.52	0.11	55	F
Codominant	3	1.14	0.78-1.66	0.50	0.30	17	F

Table 7. Summary of sensitivity analysis findings. Entries with references imply non-robustness of the summary effects; * least robust based on absence of robust comparisons; ** most robust as it had the most number of robust comparisons; RO: Reproductive outcomes; *AMH*: Anti-Müllerian Hormone; *AMHRII*: Anti-Müllerian Hormone Receptor II; PCOS: Polycystic Ovary Syndrome

Genetic Model	Outcome Polymorphism			
	RO Ile49Ser	RO -482A>G	PCOS Ile49Ser	PCOS -482A>G
Homozygous	[30, 31, 36, 43]	Robust	[42]	Robust
Recessive	[30, 31, 36, 43]	Robust	[42]	Robust
Dominant	[16, 43]	[32, 43]	Robust	[42]
Codominant	[16, 30, 43]	Robust	[38]	[42]
Number of references that contributed to instability	5	2	2	1
Number of robust comparisons	0 *	3 **	1	2

the menstrual cycle in healthy normo-ovulatory women [15] as well as with the number of embryos produced [52]. Significant differences between certain subgroups were found following controlled ovarian stimulation amongst women with wild type for the *AMH* polymorphism [31]. In addition, women with wild-type for the *AMHRII* polymorphism had a higher number of follicles, and those with higher peak serum E2 levels required a lower gonadotropin dose [31]. These polymorphisms have been demonstrated to be of predictive utility in ovarian stimulation procedures (reviewed in [53]).

Interpreting the results of this meta-analysis warrant awareness of its strengths and limitations. Strengths include the following: (i) all tissue sources were blood; (ii) all of the studies addressed the HWE issues; (iii) efficiency of outlier treatment in erasing heterogeneity and altering direction of association enabling the findings to be without the baggage of variability; (iii) zero heterogeneity in most comparisons of the -482A>G polymorphism. (iv) Pooled ORs of -482A>G in RO and PCOS were robust in the homozygous and recessive models. On the other hand, there are a number of limitations in our study: (i)

low statistical power of most included studies. Of the 16 studies, only five (31%) had sample sizes of >500 [32, 34, 38-40]. (ii) The -482A>G comparisons had a small number of studies thus with limited statistical power; (iii) since we included studies whose controls deviated from the HWE, we risked methodological weakness, such as biased selection of subjects, genotyping errors and population stratification [54]. However, non-significance remained with omission of these studies followed by recalculation. (iv) Genotyping approaches were not uniform although most of the studies used PCR (Polymerase Chain Reaction). (v) Pooled ORs in Ile49Ser were generally not robust.

Conclusion

It is possible that Ile49Ser and -482A>G do not play any direct functional role in RO and PCOS, but are in linkage disequilibrium (LD) with other polymorphisms [19]. It is also likely that variants in other than *AMH* or *AMHRII* are relevant to RO and PCOS [15, 31, 32, 40]. It is possible that the Ile49Ser and -482A>G polymorphisms are merely markers for the truly functional polymorphism elsewhere in these genes [15]. Lazaros et al [32] suggested a concerted effect between -482A>G and 1749C4T, since both polymorphisms in *AMHRII* were found to be in strong LD. Genome wide association studies may be promising as did a recent one that found significant correlation with ovarian response [55]. It is conceivable that RO and PCOS related to any one locus will be small because gene-gene as well as gene-environment interactions are likely to operate. Genotyping the *AMH* and *AMHRII* polymorphisms together with some additional markers may provide a means of identifying RO. Meanwhile, further studies regarding other SNPs (or haplotypes) in the *AMH* and *AMHRII* genes may help better understand their roles in RO/PCOS. Since potential biases and confounders could not be ruled out completely in this meta-analysis, additional large case-control studies or later update meta-analysis may be warranted to validate or modify our findings. It would help that well-designed studies based on sample sizes commensurate with detection of small genotypic risks should allow more definitive conclusions about the association of *AMH* and *AMHRII* polymorphisms and RO/PCOS.

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Disclosure Statement

The authors have nothing to disclose and no conflicts of interest to declare.

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