Original Article

High-Risk Genotypes Associated with Poor Response to Controlled Ovarian Stimulation in Indian Women

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Abstract

Background: Infertility is a global burden and has become exceedingly common in the preceding years; controlled ovarian stimulation (COS) is a pre-requisite for couples opting to conceive via *in vitro* fertilisation (IVF). Based on the number of oocytes retrieved upon COS, a patient may be classified as a good responder or poor responder. The genetic aspect of response to COS has not been elucidated in the Indian population. **Aims:** This study aimed to establish a genomic basis for COS in IVF in the Indian population and to understand its predictive value. **Settings and Design:** The patient samples were collected at both Hegde Fertility Centre and GeneTech laboratory. The test was carried out at GeneTech, a diagnostic research laboratory based in Hyderabad, India. Patients with infertility without any history of polycystic ovary syndrome and hypogonadotropic hypogonadism were included in the study. Detailed clinical, medical and family history was obtained from patients. The controls had no history of secondary infertility or pregnancy losses. **Materials and Methods:** A total of 312 females were included in the study comprising 212 women with infertility and 100 controls. Next-generation sequencing technology was employed to sequence multiple genes associated with response to COS. **Statistical Analysis Used:** Statistical analysis using odds ratio was carried out to understand the significance of the results obtained. **Results:** Strong association of c.146G>T of *AMH*, c.622-6C>T of *AMHR2*, c.453-397T>C and c.975G>C of *ESR1*, c.2039G>A of *FSHR* and c.161+4491T>C of *LHCGR* with infertility and response to COS was established. Further, combined risk analysis was carried out to establish a predictive risk factor for patients with a combination of the genotypes of interest and biochemical parameters commonly considered during IVF procedures. **Conclusion:** This study has enabled the identification of potential markers pertaining to response to COS in the Indian population.

Keywords: *Controlled ovarian stimulation, genotypes, infertility*

Introduction

Controlled ovarian stimulation (COS) is a crucial step of infertility treatment and a pre-requisite step for *in vitro* fertilisation (IVF) or intra-cytoplasmic sperm injection (ICSI). Retrieving good number of oocytes during COS is an important pre-requisite for the success of IVF treatment. Further, the number of oocytes retrieved might be used as an independent predictor of the live birth rate of the treatment cycle.[1] A great range of variability can be observed in response amongst women undergoing COS and poor response is a frequent challenge faced by infertility specialists. Poor ovarian response-related dropout is one of the most important contributing factors to such high failure

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rate. Although certain clinical parameters such as low anti-Mullerian hormone (AMH), low antral follicle count (AFC), high follicle-stimulating hormone (FSH), high oestradiol (E2) and maternal age add value as indicators of poor ovarian response, none are absolute measures and they only serve as broad indicators.[2,3] This limited accuracy of current tests available makes it difficult to distinguish Poor responder (PRs) from Good responder (GRs) leading to inadequate counselling strategy and treatment adaptations at IVF centres.

Early researchers in the field of reproductive health have predicted that although hormonal and functional biomarkers are more commonly used tools to predict ovarian response, genetic biomarkers may well become the best predictive tool to guide individualised treatment.[4]

It is often seen that women who are young, with normal hormonal profile and without any clinical signs also can have poor response to COS. This could be due to genetic markers associated with response which predispose them to poor response. The genetic pathology of poor response to COS was demonstrated by multiple studies for individual genes. In addition to patient and biochemical factors, common poor response genetic markers that are commonly known are reported in genes *FSHR*, *LHB* and *ESR1*.[5-7] With the availability of affordable sequencing technologies, genetic phenotyping of relevant gene variants in different population groups is gaining momentum. In addition, research has unravelled the role of many other genes that contribute to COS response such as *AMH*, *AMHR2*, *CYP19A1*, *BMP15*, *ESR2*, *KISS1*, *KISS1R*, *LHCGR*, *MTHFR*, *PAI*, *VEGFA*, *COMT* and *TNF*. The current cohort study was carried out to identify risk genotypes of those genes associated with poor response to COS in Indian women.

Subjects

Informed consent was obtained from all the participants of the study following ethical clearance from the institution's internal review board (ECA2019D01). The patient group subjects were all from a single centre (Hegde Fertility Centre, Hyderabad) undergoing treatment for infertility and samples were collected during 3 years (2019–2022). The patients were further classified as (a) poor responders (PR) if the average oocytes retrieved in previous IVF cycles are <5 or with total FSH consumption >3000 units or those with prolonged stimulation of more than 12-day duration and (b) good responders (GR) if the average oocytes retrieved were between 5 and 15.[3] Recorded clinical and scan parameters of patients included age, body mass index (BMI) and AFC. A detailed clinical, medical and family history was obtained from patients along with informed consent to use the results of data analysis for scientific publication assuring non-disclosure of patient identity. Patients with polycystic ovary syndrome (PCOS) and hypogonadotropic hypogonadism were excluded from the study. All female participants were non-smokers and did not have any health conditions that are reportable. No sample size calculation was performed.

Methodology and Data Analysis

Patients with infertility with at least one previously failed IVF or ICSI and controls with minimum of two live children and with no history of secondary infertility or pregnancy losses were included in the study [Table 1]. Levels of day 2 FSH, day 2 luteinising hormone (LH), day 2 E2 and AMH were tested at a single in-house laboratory of the fertility centre. For the *in vitro* quantitative determination of AMH, FSH, LH and E2, 'electrochemiluminescence immunoassay' was done with Elecsys AMH Plus, Elecsys FSH, Elecsys LH and Oestradiol III, respectively, on the cobas e 801 immunoassay analyser [Table 2].

A multi-gene, next-generation sequencing (NGS)-based, research panel designed for diagnosing large number

Table 1: Number of subjects with age range (years) in groups and subgroups included in the study

	Control	Infertility	
		Patients with GR to COS	Patients with PR to COS
Number	100	85	127
Age range (years)	23–48	25–42	22–43

COS=Controlled ovarian stimulation, PR=Poor responder, GR=Good responder

Table 2: Patient clinical and biochemical parameters

Parameter	Mean	SD
Patients with GR		
BMI	27.71	4.84
AFC	8.76	4.28
FSH	6.45	2.46
AMH	3.16	2.41
LH	8.51	10.84
Oestradiol	44.60	29.42
Patients with PR		
BMI	26.54	4.56
AFC	5.38	3.36
FSH	7.60	3.86
AMH	1.67	1.38
LH	6.26	3.55
Oestradiol	74.45	225.85

SD=Standard deviation, BMI=Body mass index, AFC=Antral follicle count, FSH=Follicle-stimulating hormone, AMH=Anti-Mullerian hormone, LH=Luteinising hormone, PR=Poor responder, GR=Good responder

of conditions associated with infertility or reproductive failure was used in the laboratory, including multiple genes reported to be associated with response to COS. The test was carried out at GeneTech Pvt. Ltd., a diagnostic research laboratory in Hyderabad, India. Table 3 provides the list of 15 genes and 53 genotypes analysed in the study. The loci are labelled in terms of cDNA location and the corresponding mRNA along with rsID wherever available is also mentioned in Table 3. 3 mL of blood sample was obtained from each of the subjects in EDTA vacutainer and DNA was isolated using the Gentra Puregene Blood Kit (Qiagen, Germany) as per manufacturer guidelines. 100 ng of genomic DNA was obtained from blood and was used to process the custom-designed multigene panel (Ion AmpliSeq™) using ion semiconductor technology (Ion GeneStudio S5 System; Thermo Fisher Scientific, USA). DNA corresponding to targeted genomic regions is amplified using Ion AmpliSeq™ library 2.0 and custom-designed primers. Purified library is quantified, sequenced on a NGS platform, and 500MB raw sequencing data obtained were aligned to hg38. Uniform coverage is ~99% at mean read depth of 150X. Gene variants are called using torrent variant caller using default parameters and annotated using ion reporter Software. Sequencing data of the 53 selected genotypes were selected for data analysis to derive clinical correlations reported in this study. SNPSTAT software was adopted for the calculation of the odds ratios, as the estimates of relative risk of disease, with 95% confidence intervals and 0.05 probability to determine codominant inheritance model for the polymorphisms studied (www.snpstats.net). Linkage disequilibrium test was carried out by SHEsis software (http://shesisplus.bio-x.cn/SHEsis.html) to determine any possible association with the disease phenotype.[8] Combined genotype analysis was adopted to determine the risk of combination of alleles with the poor response phenotype.

Results

The study comprised 212 women with infertility and 100 controls.

Patient and clinical parameters

Amongst the biochemical parameters analysed [Table 2], AMH and E2 have shown a significant difference between the PR and GR groups while FSH and LH levels did not. Ultrasound parameter AFC was significantly lower in the PR group. Age and BMI correlations could not be established with poor response in our study.

Table 3: List of genomic and coding loci (hg38) analysed in the study

Gene	Transcript	Coding region	rsIDs
AMH	NM_000479.5	c.*511A>G	rs10417628
	NM_000479.5	c. 546G>A	
	NM_000479.5	c. 555+50G>A	rs8112524
	NM_000479.5	c. 252G>A	rs61736572
	NM_000479.5	c. 303G>A	rs61736575
	NM_000479.5	c. 146G>T	rs10407022
AMHR2	NM_020547.3	c. 1288+29C>T	rs201217654
	NM_020547.3	c. 1425+77A>G	rs11170555
	NM_020547.3	c. 622-6C>T	rs2071558
	NM_020547.3	c. 853-10G>A	rs3741664
	NM_020547.3	c. 425-29C>T	
BMP15	NM_005448.2	c. 308A>G	rs41308602
	NM_005448.2	c. 319C>A	
	NM_005448.2	c. 329-74A>C	rs73488037
	NM_005448.2	c. 852C>T	rs17003221
	NM_005448.2	c.-9C>G	
COMT	NM_001135161.2	c. 472G>A	rs4680
CYP19A1	NM_001347255.2	c. 240A>G	rs700518
ESR 1	NM_001122742.1	c. 1782G>A	rs2228480
	NM_001122742.1	c. 229G>A	rs9340773
	NM_001122742.1	c. 261G>C	rs746432
	NM_001122742.1	c. 30T>C	rs2077647
	NM_001122742.1	c. 453-397T>C	
	NM_001122742.1	c. 729T>C	rs4986934
	NM_001122742.1	c. 975G>C	
FSHR	NM_000145.3	c. 152+152A>G	rs7589810
	NM_000145.3	c. 2039G>A	rs6166
	NM_000145.3	c. 919G>A	rs6165
KISS1	NM_002256.4	c. 107A>G	rs35431622
	NM_002256.4	c. 242C>G	rs4889
	NM_002256.4	c. 58G>A	rs12998
KISS1R	NM_032551.5	c. 244+128C>T	
	NM_032551.5	c. 369+25A>G	
	NM_032551.5	c. 369+34T>G	
	NM_032551.5	c. 369+8C>G	
LHB	NM_000894.2	c.-34T>A	rs3752210
	NM_000894.2	c. 285T>C	rs1056917
LHCGR	NM_000233.4	c. 161+4386G>A	rs13431355
	NM_000233.4	c. 161+4491T>C	rs13405728
MTHFR	NM_005957.4	c. 665C>T	rs1801133
SERPINE1	NM_000602.4	c. 1087+162C>T	rs2227692
	NM_000602.4	c. 43G>A	rs6092
	NM_000602.4	c. 555G>C	rs201293550
	NM_000602.4	c. 701-45G>A	rs2227684
TNF	NM_000594.4	c.-418G>A	rs361525
VEGFA	NM_001025366.3	c. 1085+52T>C	
	NM_001025366.3	c. 534C>T	rs25648
	NM_001025366.3	c. 659-111C>A	rs2146323
	NM_001025366.3	c. 659-99G>A	rs3024997
	NM_001025366.3	c. 856-28C>T	rs3025000
	NM_001025366.3	c. 963-112G>A	rs3025017
	NM_001025366.3	c. 963-119G>A	

Contd...

Table 3: Contd...

Gene	Transcript	Coding region	rsIDs
	NM_001025366.3	c. 1034+103T>C	

*Denotes that the position of the nucleotide mentioned is in the upstream region of AMH gene. This nomenclature is according to standard guidelines of nucleotide notation. AMH=Anti-Mullerian hormone, ESR1=Oestrogen receptor 1, FSHR=Follicle-stimulating hormone receptor

Phenotype–genotype correlations (control vs. infertility)

Relative risk was calculated for all the genotypes mentioned in Table 3 using odds ratio, and the significance was established on the basis of confidence intervals and $P < 0.05$ [Table 4]. Amongst the 15 genes and 53 loci analysed, results of 6 loci corresponding to c.146G>T of *AMH*, c.622-6C>T of *AMHR2*, c. 453-397T>C and c. 975G>C of *ESR1*, c. 2039G>A of *FSHR* and c. 161+4491T>C of *LHCGR* were of particular interest. The TT genotype of c.146G>T of *AMH* and AA of c.161+4491T>C of *LHCGR* were found to be significantly associated with the infertility group and this association held strong across codominant, dominant and recessive models of inheritance as well. The genotypes TT of c.453-397T>C, CC of c.975G>C of *ESR1* and CC of c.2039G>A of *FSHR* were found to be significantly associated with the infertility group in both codominant and recessive models of inheritance. TT genotype of c.622-6C>T of *AMHR2* was also considered for further analysis because this genotype was completely absent in the control population and thus could indicate an association with infertility.

Phenotype–genotype correlations (poor responders vs. good responders)

The six genotypes identified in the infertility group were subjected to relative risk analysis which showed that five genotypes of *AMH* (GT and TT of c.146G>T) ($P = 0.032$), *AMHR2* (TT of c.453-397T>C) ($P = 0.04$), *ESR1* (TT of c.453-397T>C) ($P = 0.04$), *ESR1* (CC of c.975G>C) ($P = 0.03$) and *FSHR* (CC of c.2039G>A) ($P = 0.03$) were significantly associated with poor response to COS [Table 5]. Although the presence of A allele of *LHCGR* genotype (AA) was significantly higher in the infertility group than the control group, there was no significant difference of prevalence between the PR and GR groups.

Haplotype analysis

Haplotype analysis was carried out using combination of alleles proven to have a significant association with poor response in this study to identify if any of the alleles is inherited as a unit. Upon analysis, G-C-C-G-C (<0.0001), T-C-C-G-T ($P < 0.0001$), T-C-C-C-C ($P < 0.0001$) and T-C-C-C-T ($P < 0.0001$) were identified as high-risk haplotypes corresponding to *AMH* c.146G>T, *AMHR2* c.622-6C>T, *ESR1* c.453-397T>C and c.975G>C and *FSHR* c.2039G>A [Table 6].

Cumulative effect

Combined risk analysis was carried out to identify the effect of a combination of risk genotypes derived from relative risk analysis and patient parameter analysis. In addition to the genotypes mentioned earlier, AFC count below 5, AMH levels below 1.2 ng/ml and E2 levels above 70 pg/ml on day 2 were considered risk factors in this study [Table 7]. The analysis showed that poor response risk to COS was enhanced as the number of risk genotypes increased in the patient group. Women with two of the risk genotypes had a 2-fold increased risk towards poor response, while a carrier of three risk genotypes had a nearly 5-fold increased risk. Cases with four and five risk alleles were excluded from the analysis, as the number is too low and none were identified in patients with good response. We also demonstrate in our study that there is an additional one-fold increase in risk of poor response in carriers of one-risk genotype coupled with any one of the high-risk clinical parameters (low AMH, low AFC and high E2), with a categorical one-fold increase with the addition of more high-risk clinical parameters. Further analysis including 2 high-risk genotypes and clinical parameters could not be carried out as none of the cases with good response were found to be carriers of such a combination.

Linkage disequilibrium for the poor responder group

Linkage disequilibrium (LD) was estimated using ShEsisPlus software, and LD plot analysis with r^2 values was done to identify any non-Mendelian association between the 5 sites proven to have an association with poor response to COS (*AMH* c.146G>T, *AMHR2* c.622-6C>T, *ESR1* c.453-397T>C, *ESR1* c.975G>C and *FSHR* c. 2039G>A) [Figure 1]. The analysis failed to show any significant linkage disequilibrium between the sites. This could indicate that despite having a strong role individually, these may not influence each other at the gene/allele level and the interaction may be at a downstream level.

Discussion

Ovarian stimulation is an integral part of IVF treatment and is a crucial factor that determines the outcome of treatment, as the number and quality of oocytes retrieved are related to the chance of achieving a pregnancy and thereby a live birth. Patient (age and

Table 4: Risk estimation for infertility group with significant associations

Gene	Coding	Model	Genotype	Controls	Infertility	OR (95% CI)	*P*
AMH	c. 146G>T	Codominant	G/G	44	67	1.00	0.003*
			T/T	28	100	2.35 (1.33–4.13)	
		Dominant	G/G	44	67	1.00	0.03*
			G/T-T/T	56	145	1.70 (1.04–2.77)	
		Recessive	G/G-G/T	72	112	1.00	0.001*
			T/T	28	100	2.30 (1.37–3.83)	
AMHR2	c. 622-6C>T	Codominant	C/C	84	167	1.00	0.93
			C/T	16	31	0.97 (0.50–1.88)	
			T/T	0	14	NA (0.00–NA)	
ESR1	c. 453-397T>C	Codominant	C/C	21	31	1.00	0.0034*
			T/T	23	99	2.91 (1.42–5.97)	
		Recessive	C/C-C/T	77	113	1.00	0.0001*
			T/T	23	99	2.93 (1.71–5.02)	
ESR1	c. 975G>C	Codominant	G/G	56	105	1.00	0.007*
			C/C	7	43	3.28 (1.38–7.76)	
		Recessive	G/G-G/C	93	169	1.00	0.0044*
			C/C	7	43	3.38 (1.46–7.81)	
FSHR	c. 2039G>A	Codominant	T/T	32	54	1.00	0.0031*
			C/C	22	97	2.61 (1.38–4.94)	
		Recessive	T/T-T/C	78	115	1.00	0.0001*
			C/C	22	97	2.99 (1.73–5.16)	
LHCGR	c. 161+4491T>C	Codominant	G/G	7	4	1.00	0.014*
			A/A	63	175	4.86 (1.37–17.16)	
		Dominant	G/G	7	4	1.00	0.03*
			G/A-A/A	93	208	3.91 (1.19–13.70)	
		Recessive	G/G-G/A	37	37	1.00	0.0002*
			A/A	63	175	2.78 (1.62–4.76)	

OR=Odds ratio, CI=Confidence interval, NA=Not available, AMH=Anti-Mullerian hormone, ESR1=Oestrogen receptor 1, FSHR=Follicle-stimulating hormone receptor

Table 5: Risk estimation for poor responder group with significant associations

Gene	Coding	Model	Genotype	Controls	Infertility	OR (95% CI)	*P*
AMH	c. 146G>T	Dominant	G/G	34	33	1.00	0.032*
			G/T-T/T	51	94	1.90 (1.05–3.42)	
AMHR2	c. 622-6C>T	Codominant	C/C	68	99	1.00	0.04*
			T/T	2	12	5.62 (1.08–29.42)	
ESR1	c. 453-397T>C	Codominant	C/C	12	19	1.00	0.04*
			T/T	20	79	2.49 (1.04–5.97)	
		Recessive	C/C-C/T	65	48	1	0.0001*
			T/T	20	79	5.35 (2.89–9.91)	
ESR1	c. 975G>C	Codominant	G/G	45	60	1.00	0.03*
			C/C	10	33	2.47 (1.11–5.54)	
		Recessive	G/G-G/C	75	94	1.00	0.0095*
			C/C	10	33	2.63 (1.22–5.69)	
FSHR	c. 2039G>A	Codominant	T/T	18	36	1.00	0.04*
			C/C	18	79	2.19 (1.02–4.71)	
		Recessive	T/T-T/C	67	48	1.00	<0.0001*
			C/C	18	79	6.12 (3.25–11.52)	

OR=Odds ratio, CI=Confidence interval, AMH=Anti-Mullerian hormone, ESR1=Oestrogen receptor 1, FSHR=Follicle-stimulating hormone receptor

BMI), biochemical (AMH, FSH, LH, E2 and inhibin) and ultrasound (AFC) factors are usually considered in clinics to identify the poor response group. In addition, genetic markers that are traditionally used by some clinics include *FSHR*, *LHB* and *ESR1* genotypes. To our knowledge, this is the first study which looked for 53

Table 6: Haplotype association of risk genotypes with poor response group

AMH- c. 146G>T	*AMHR2-* c. 622-6C>T	*ESR1-* c. 453-397T>C	*ESR1 -* c. 975G>C	*FSHR-* c. 2039G>A	Frequency	OR (95% CI)	*P*
G	C	T	G	C	0.1826	1.00	-
T	C	T	G	T	0.1102	0.44 (0.21–0.93)	0.032
T	C	T	G	C	0.1016	0.44 (0.20–0.96)	0.039
T	C	T	C	T	0.1002	0.61 (0.26–1.40)	0.24
T	C	T	C	C	0.0623	2.06 (0.56–7.57)	0.28
G	C	C	G	C	0.0618	>1 (>1–>1)	<0.0001*
G	C	T	G	T	0.0499	1.76 (0.40–7.66)	0.45
T	C	C	G	T	0.048	>1 (>1–>1)	<0.0001*
T	C	C	C	C	0.0474	>1 (>1–>1)	<0.0001*
G	C	T	C	T	0.0419	0.54 (0.15–1.97)	0.35
T	C	C	C	T	0.0354	>1 (>1–>1)	<0.0001*

OR=Odds ratio, CI=Confidence interval, AMH=Anti-Mullerian hormone, ESR1=Oestrogen receptor 1, FSHR=Follicle-stimulating hormone receptor

Table 7: Combined risk analysis of risk genotypes and clinical parameters

Number of risk genotypes	Number of clinical risk factors	Patients with good response	Patients with poor response	OR (95% CI)	*P*
1	0	53	77	-	-
2	0	11	36	2.25 (1.05–4.82)	0.04*
3	0	2	4	4.82 (1.05–22.08)	0.04*
1	1	23	61	1.83 (1.01–3.30)	0.05*
1	2	9	30	2.29 (1.01–5.22)	0.05*
1	3	4	19	3.27 (1.05–10.16)	0.04*

OR=Odds ratio, CI=Confidence interval

genotypes and demonstrated a cumulative risk for poor response based on genetic results combined with patient and biochemical parameters in Indian women.

Age and body mass index

Even though advancing age is considered a marker for declined response to ovarian stimulation, it does not affect all women equally.[9] In the present study, we did not find any significant difference between the PR and GR groups for patient parameters – age and BMI. The mean age of the GR and PR groups is 31.4 and 31.7 years, respectively. The mean BMI in the GR group is 27.71 and 26.54 in the PR group.

Clinical parameters

As far as biochemical and ultrasound parameters, our study shows that AMH, AFC and E2 are probably better clinical predictors of poor response to COS than day 3 FSH and LH. Serum AMH levels are known to reflect the primordial follicle pool indirectly, along with AFC to determine response to COS.[10] Suboptimal AMH (<1.2 ng/ml) is a fairly good predictor of low ovarian response and is used in a tailored approach to COS based on AMH although no consensus is reached on the variability of serum AMH assay methodologies, cut-off levels to predict ovarian response.[11-13] AFC, which is the number of follicles detected by ovarian ultrasound, is significantly associated with AMH levels when the count is <5 and is also accepted as a predictor of poor ovarian response but with limitations of lack of a consistent methodology of AFC assessment.[14] Our study results are consistent with the claim that elevated levels of serum E2 on cycle days 2 or 3 can be used to predict poor response to COS.[15] Our study did not reveal any significant difference between the FSH and LH levels of the PR and GR groups.

Risk genotypes for poor response to controlled ovarian stimulation

Multiple genes and genotypes were studied to understand their role on response to ovarian stimulation in different population groups. In our study, we report a significantly higher proportion of *AMH*, *AMHR2*, *ESR1* and *FSHR* risk genotypes in the poor response group of Indian women.

The potential influence of genes encoding the AMH signalling pathway such as *AMH* and *AMHR2* on the outcome of ovarian stimulation was proposed by several researchers. We reported that T allele of c.146G>T (rs10407022) is significantly higher in the poor responder group. It replaces serine with isoleucine in position 49 of AMH protein and is known to result in reduced bioactivity of AMH and poor response to COS with less number of oocytes retrieved and fewer

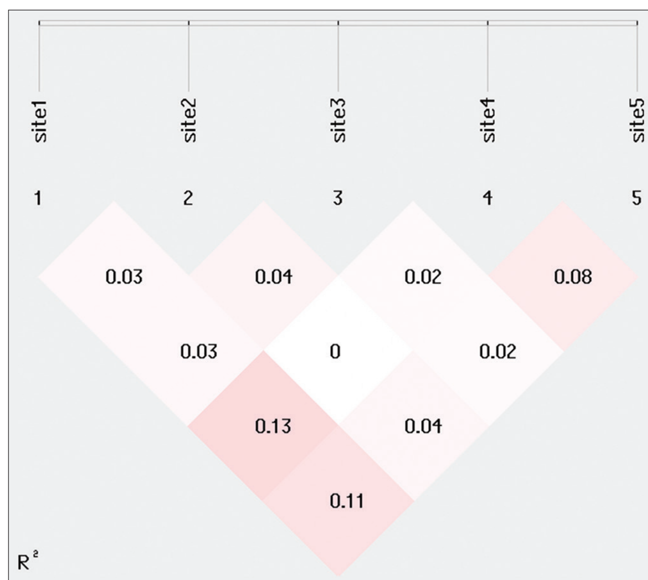

Figure 1: LD plot analysis with r^2 values between the 5 sites failed to show non-Mendelian inheritance with poor response to COS. COS = Controlled ovarian stimulation, LD = Linkage disequilibrium

MII oocytes.[16] We reported a strong association of TT, c.622-6C>T (rs2071558) of AMHR2 gene. Lazaros *et al*. studied *AMHR2* 6C>T (rs2071558) and 482A>G (rs2002555) polymorphisms and reported that women with the 6C/T genotype had an increased number of total and small follicles compared to women with the 6C/C genotype.[17] They hypothesised that these women needed a higher gonadotropin dose to achieve better response.

Another set of candidate genes involved in the ovarian response to gonadotropins is oestrogen receptor gene (ESR). They mediate oestrogen effects on follicle growth, maturation, oocyte release and implantation. PvuII and Xbal of ESR1 and *Rsal* and *Alul* of ESR2 are associated with female infertility, ovarian reserve, oocyte maturation, release and premature ovarian insufficiency.[18] We show that TT (PvuII) genotype is significantly higher in the PR group. The genotype was also reported with poor response association and requirement for longer stimulation period and higher doses of gonadotropins in Brazilian population.[7] We also identified that genotype CC of c.975G>C (a silent mutation) on ESR1 gene is present in significantly higher proportion in the PR group. This variant was reported in male breast cancer patients, and no reports are available on infertility associations.[19] Further studies with larger number of patients may be required to confirm or rule out its association with poor response to ovarian stimulation.

FSH receptor gene plays a central role in oogenesis, follicle proliferation and recruitment and is the most studied gene in relation to response to ovarian stimulation. Its gene variants are extensively studied in association with COS. In our study, we report a strong correlation of CC genotype (G allele) at c.2039G>A (rs6166) of FSHR gene with the PR group. Several studies have been reported in past on the same lines demonstrating the association of the genotype with less oocytes retrieved, lower follicle-to-oocyte index, more resistant to FSH action and requiring stronger stimulus.[20-22]

Even though AA genotype of *LHCGR* (c.161+4491T>G) was significantly higher in combined infertility patient group, there was no difference between poor and good responders. Literature demonstrates the correlation of *LHCGR* genotype with PCOS, slow response to ovarian stimulation along with relation to FSH consumption and dose of LH required.[23-25] Interestingly, 82% of the patient group had AA genotype which indicates that it could be a strong biomarker for female infertility.

The study could not demonstrate previously reported risk genotypes associated with poor response in genes *CYP19A1, BMP15, ESR2, KISS1, KISS1R, LHB, MTHFR, PAI, VEGFA, COMT* and *TNF*. It is worthwhile to carry out further studies with larger sample size to establish or rule out the role of these genes on response to COS.

Combined analysis has shown that the risk of poor response increases in carriers with the increase in number of risk alleles. Additional risk is conferred due to the presence of low AMH, low AFC and high E2. This can further be proven as none of the good responders carried more than 1 risk allele in combination with the above patient biochemical parameters, indicating that only poor responders would carry such a combination. We strongly recommend that all reported risk genotypes be included in diagnostic plan for identifying poor responders to COS in clinics which will help immensely in their management.

Sunkara *et al*. showed that the number of eggs to maximise live birth rate is approximately 15.[1] Although we have considered patients with >5 oocytes retrieved as good responders and <5 as poor responders, there is another unique group of patients with suboptimal response group with oocyte numbers 5–10 which may need special attention. If not handled properly, the suboptimal response can easily turn into a poor response and can even result in cycle cancellation.[26] The authors intend to take up larger studies including this suboptimal response group in future to (a) identify associated genomic risk pattern

in the subgroup, (b) demonstrate the possibility of pushing suboptimal response patients to the good response group by modulating stimulation protocols based on genomic risk.

Good response to COS is desired in every single IVF cycle. Identification of risk genotypes using multi-genomic sequencing panels will help in identification of the poor responder group with better accuracy than purely depending on clinical parameters. With fast accumulating genomic data in different population groups, pharmacogenomic approach may become a solution to the challenge of heterogeneous response of women to ovarian stimulation. Based on genomic risk and its implications, infertility clinics can prevent multiple stimulations by individualising treatment suitable for the patients and recommending embryo cryopreservation.

Limitations

The study is observational and genetic correlations in polycystic ovarian syndrome, and ovarian hyperstimulation syndrome patients were not included in the study. The study is relatively small and limited to patients of a single centre. Larger multi-centre studies including women fulfilling Poseidon or Bologna criteria of poor responders are required to strengthen our correlations in the population and also to identify the role of wider range of genes contributing to ovarian development function and insufficiency.

Conclusion

The study demonstrates that multi-gene cumulative risk analysis may offer an additional tool for forecasting a poor responder to COS. Precise identification of the poor response group will facilitate evidence-based, customised COS plan for patients with choice of suitable drugs, doses and adjunct therapies, which in turn may lead to improved success rates of treating infertility patients using assisted reproductive technology.

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Conflicts of interest

Anuradha Udumudi (Director, GeneTech), K. Chaitra Lava (Medical Geneticist, GeneTech) and Vandana Hegde (IVF Specialist, Hegde Fertility) declare no conflict of interest.

Data availability statement

The information will be accessible through the corresponding author and can be provided upon request.

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