Pharmacogenetics of ovarian response

Effective controlled ovarian stimulation (COS) is crucial for IVF outcome. Ovarian response to follicle-stimulating hormone, however, varies widely among women undergoing ovarian stimulation. Advance identification of patients who will elicit a poor or high response to standard treatment would be of great clinical benefit for such patients. Application of pharmacogenetics to ovarian response may predict stimulation success but also help in the adjustment and design of doses prior to treatment. Different studies have examined the impact of variations in follicle-stimulating hormone receptor, biochemical pathways involved in estrogen production and action, folliculogenesis and other aspects. Recently, gene-association studies have tried to identify a number of genetic variations affecting interindividual variability in COS.

KEYWORDS: controlled ovarian stimulation ■ COS ■ genotype ■ ovarian response ■ pharmacogenetics ■ single nucleotide polymorphism ■ SNP

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Infertility is defined by the failure to conceive after 12 months of regular unprotected sexual intercourse [1]. Approximately 10% of couples have difficulty conceiving a child naturally [2] and more than 80 million couples worldwide are infertile and may be treated using assisted reproduction techniques. The most used and successful assisted reproduction technique is *in vitro* fertilization (IVF). IVF is a complex and multistep process. Oocytes are collected after controlled ovarian stimulation (COS) with gonadotropins. After fertilization and embryo cleavage, the embryos will be transferred to the uterus for implantation, whereas other may be cryopreserved for future implantation attempts. All these steps are critical for successful IVF. The aim of COS is to safely obtain a high number of mature oocytes that allows us to select the most viable embryo for transfer [3]. Approximately 8–10 oocytes are regarded as an optimal prerequisite for successful outcome in conventional COS protocols and it has been recently published that the number of eggs to maximize the success rates is approximately 15 [4]. However, ovarian response varies widely among women undergoing ovarian stimulation [5]. Approximately 9–24% of women undergoing IVF respond more poorly than expected to the ovarian stimulation protocol selected according to their clinical characteristics [5]. On the other hand high responses can cause a serious medical condition, ovarian hyperstimulation syndrome (OHSS). Identification in advance of patients who will carry out a poor or high response to standard treatment would be of great clinical interest for such patients. Various predictive markers of COS outcome have been proposed such as age [6], ovarian reserve [7], cigarette smoking [8] and hormonal status [9]. Besides these parameters, genetic variability also seems to be an important factor. It is well recognized that individual variability in response to drugs exists [10]. Application of pharmacogenetics to ovarian response may predict stimulation success [11] but also contribute to adjustment and design of doses in order to tailor treatment.

More than 19 million SNPs have been identified in the human genome [12]. Some of these SNPs have already been associated with changes in the effects of drugs. The aim for pharmacogenetics is to establish the relationship between genetic variants and medication response, to develop diagnostic tests that can predict drug action and adjust therapy accordingly [13].

The influence of polymorphisms in genes on the outcome of ovarian stimulation in IVF has been analyzed by several groups, but the pharmacogenetic approach in regards to follicle-stimulating hormone (FSH) dosing is still emerging. Most studies have been focused on polymorphisms in the FSH receptor gene (*FSHR*) [14]. The effect of variations in different biochemical pathways involved in estrogen production and action (aromatase and estrogen receptor genes), folliculogenesis (*BMP15*, *GDF9* and *AMH*) and other aspects have also been examined by a few groups (Table 1) [14]. Recently, gene-association

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studies have identified a number of no genomic SNPs affecting interindividual variability in the COS [15].

FSHR

FSH is a key hormone in human reproduction. FSH and its receptor (FSHR) play a major role in follicular development and regulation of steroidogenesis in the ovary [16]. The *FSHR* gene is localized on chromosome 2p21 and spans a region of 54 kb [17]. It consists of ten exons [18].

Almost 1000 SNPs have been located in the *FSHR* gene, but only eight are in the exons. Six of them are nonsymptomatic and the other two at codon 307 (rs6165) and 680 (rs6166) are located in exon 10 and are related to ovarian response. Codon 307 is found in the extracellular domain and codon 680 is in the intracellular domain. Both SNPs affect gene function by changing the properties of the protein and then modifying the response to FSH [19]. Threonine (T) can be substituted by alanine (A) at position 307 and

Table 1. Genetic variations studied in relation to controlled ovarian hyperstimulation.

Gene	Chromosome localization	Genetic variation		Major findings	Ref.
		rs number	*Protein or coding sequence variation*		
FSHR	2p21	rs6166	N680S	Patients with the S680 allele need more FSH during the stimulation phase	[20–36]
		rs1394205	-29G/A	Women with AA genotype at position -29 require higher doses of FSH, had lower E_2 levels, produced fewer follicles and showed a lower number of retrieved oocytes	[37,38]
LHB	11p13	rs1800447 rs3439826	W8R I15T	Women that carry the variants W8R (rs1800447) and I15T (rs3439826) require higher recombinant FSH consumption while having fewer oocytes retrieved	[39,40]
LHCGR	2p21	rs4073366	+28G>C	The C variant carrier status was associated with a threefold increase risk of developing OHSS	[41–43]
ESR1	6q25	rs2234693	-397T>C	Patients carrying the CC genotype had higher number of follicles, mature oocytes, fertilization rate and good quality embryos	[44,45]
		rs9340799	-351A>G	Patients carrying the GG genotype had higher number of mature oocytes and fertilization rate	
		rs3138774	$(TA)_n$	Longer TA repeats were associated with better COH	
AMH	19p13	rs10407022	I49S	Women carrying both *AMH* Ser and *AMHR2* -482G alleles had increased follicular sensitivity to FSH	[46–48]
AMHR2	12q13	rs2002555	-482A>G		
SHBG	17p13	rs6761	$(TAAAA)_n$	An increase in the follicle and oocyte number was observed in women carrying long *SHBG* $(TAAAA)_n$	[49–50]
CYP19A1	15q21	rs60271534	$(TTTA)_n$	Short *CYP19A1* allele carriers need higher gonadotropin administration during COH	[51,52]
MTHFR	1p36	rs1801133	677C>T	Heterozygote individuals seems to have more favorable outcomes compared to homozygotes	[53–55]
BMP15	Xp11	rs58995369 rs3810682 rs3897937 rs6165	- 673C>T -9C/G IVSI+905 N103S	The haplotype TGGA was was higher in patients with OHSS	[56–58]
GDF-9	5q31	rs10491279	546G>A	The A allele was correlated with poor COH and IVF outcomes	[58]
SOD2	6q25	rs4880	A16V	The AA genotype was associated with a higher number of total oocytes following COH	[59]
p53	17p13	rs1042522	P72R	Women who were homozygous for A72 had a greater number of oocytes than women who were PP homozygous and heterozygous	[3]

COH: Controlled ovarian hyperstimulation; E_2: Estradiol; FSH: Follicle-stimulating hormone; IVF: In vitro *fertilization; OHSS: Ovarian hyperstimulation syndrome.*

serine (S) can be substituted by asparagine (N) at position 680. These polymorphisms are in linkage disequilibrium resulting in the most frequent allelic combinations of T307–N680 and A307–S680. In order to simplify, most studies focus almost exclusively on polymorphisms at codon 680.

These polymorphisms are the most extensively studied to assess the response of the receptor to the FSH simulation. Although there is some discordance [20,21], there is sufficient evidence to state that N680S polymorphism determines ovarian response to FSH stimulation in patients undergoing IVF treatment [22–24]. To achieve similar peak estradiol levels the amount of FSH needed for COS was lower in women with the genotype N/N at position 680, suggesting a lower sensitivity to FSH for the S680 allele and a poor response to gonadotropins [25]. Patients with the S680 allele need more FSH during the follicular phase [26–29]. Estradiol levels per oocyte retrieved in the S/S group were significantly lower as compared with the levels in the N/S and N/N groups at the time of hCG administration. To evaluate stimulation and embryo implantation potential oocyte donation is the best model. Donors are young women of similar age with normal ovarian function. Interestingly a study performed on fertile egg donors agree with previous results, showing that in the S/S group the gonadotropin dosage is higher and the oocytes retrieved are less than other genotype groups in COS [30]. The higher gonadotropin consumption in the S/S group could be explained by the fact that patients with the S/S genotype have increased basal FSH levels and require higher FSH doses as reported in the Yao *et al.* meta-analysis [23]. Several investigations have confirmed these original findings in different populations [26,31–34]. These findings implied that women with the S/S variant were more resistant to FSH action than women carrying the other variants [27,29]. However Yao did not find an association between the number of oocytes and genotype. One explanation could be that there is a bias on the IVF treatment: the FSH dose of poor responders is increased to achieve an adequate number of eggs and the dose of good responders is lowered to avoid hyperstimulation. The only clinical trial examining gene variants and COS outcome conducted so far has confirmed the previous finding of N680S polymorphism effect, indicating that lower FSH sensitivity of S/S carriers may be overcome by higher FSH doses during COS protocols [35]. Furthermore, a meta-analysis where patients were divided into poor or good responders confirmed the role of the N680S variant in poor responders during COS [36].

FSHR polymorphism has also been associated with OHSS. Among OHSS patients, the N/N allele has been found to be a risk factor predictor of severity of symptoms [60]. Nevertheless, researches have not replicated these findings [61,62]. The disagreed results could, in part, be explained by women's age. It has been suggested that in younger women, S carriers have a 'follicle-burning phenotype' recruiting a higher number of follicles during every natural cycle and therefore being at a higher risk of OHSS [63]. Meanwhile, in the same individuals, ovarian reserve depletion could occur earlier, leading to an age-dependent poor responder phenotype in later life [63]. Further research is needed to clarify the role of *FHSR* polymorphism in OHSS.

The level of FSHR expression also has an impact on FSH action. The G/A polymorphism at position -29 (rs1394205) of the *FSHR* gene can also modulate ovarian response to gonadotropin. Women with the AA genotype at position -29 require higher doses of FSH, had lower estradiol levels, produced fewer follicles and showed a lower number of retrieved oocytes [37]. This effect could be caused by reduced expression of FSHR on granulosa cells [38]. However, further larger studies are necessary to confirm this finding.

FSHR genotype is an important factor for determining the prognosis of COS cycles. Genotyping *FSHR* N680S together with some additional markers may therefore provide a means of identifying a group of poor responders before infertility treatment is initiated [19].

Luteinizing hormone β subunit

FSH and luteinizing hormone (LH) share a common α subunit, while the β subunit is hormone specific and includes the receptor binding domain. LH acts with FSH in promoting follicular development, ovulation and lutenization of the leading follicle [64]. The LH β subunit is coded by the *LHB* gene, localized on chromosome 11p13 and it has three exons. Polymorphisms in the gene have been identified and three in the coding sequence have been found to decrease LH activity [39]. Women that carry the variants W8R (rs1800447) and I15T (rs3439826) require higher FSH doses while fewer oocytes are retrieved [40]. Further studies with larger study groups are needed to clarify whether this variant could predict COS. Women carrying this variant could benefit from exogenous LH supplementation during COS.

LH receptor

The LH receptor (LHR) is located in the cell surface and binding with its ligand allows for the maintenance of the theca, maturation of follicles and ovulation. LHR is encoded by the *LHCGR* gene located on chromosome 2p21, consisting of 11 exons. *LHCGR* harbors at least 300 polymorphisms some of which have a significant impact on sexual development and fertility [41]. Recently, a new polymorphism was detected (rs4073366 +28G>C), which could potentially impact on LHCGR mRNA processing [42]. The C variant carrier status was associated with a threefold increased risk of developing OHSS [43]. This interesting finding requires further investigations in other COS populations.

Estrogen receptors

In COS, endogenously produced estrogens extend the action of FSH in stimulating folliculogenesis. In addition to folliculogenesis, estrogens play an important role in endometrial preparation. Estrogen signaling is mediated by estrogen receptors. Estrogen receptors are encoded by *ESR1* (located on 6q25) and *ER2* (located on 14q22). The first pharmacogenetic approach applied to IVF focused on *ESR1* gene polymorphisms [65]. The most studied polymorphisms in *ESR1* are T>C at position -397 (rs2234693) and A>G at position -351 (rs9340799) both in intron 1, and a $(TA)_n$ repeat polymorphism (rs3138774) in the promoter region. In reference to SNPs rs2234693 in intron 1, patients carrying the CC genotype had a higher number of follicles, mature oocytes, fertilization rate and good quality embryos [44,45]. In reference to SNPs rs9340799 in intron 1, patients carrying the GG genotype had a higher number of mature oocytes and fertilization rate [45]. Finally, longer TA repeats were associated with better COS [44].

Studying the G>A polymorphism at position +1730 (rs49866938) on *ER2* alone did not reveal any effect [44]. However, previous studies [3,28,66] support the hypothesis that a multigenic model, including *ESR2* and different genes, is involved in the controlled ovarian stimulation outcome.

Anti-Mullerian hormone & receptor

The serum concentration of anti-Mullerian hormone (AMH) is increasingly used as a predictor of ovarian reserve and ovarian response to stimulation [67]. AMH exerts its influence by way of the AMHR2 receptor. The genes encoding *AMH* an *AMHR2* are located on chromosomes 19p13 and 12q13, respectively. Genetic variation in AMH function is of interest and has already been described by Kevenaar *et al.* [46]. In their study on normogonadotrophic women, the I49S (rs10407022) SNP in the *AMH* gene and the -482 A>G (rs2002555) polymorphism in the *AMHR2* gene were associated with an increase in estradiol concentrations during the menstrual cycle. This may represent an indirect measure of increased follicular sensitivity to FSH. There is some discordance in the association between any of these SNPs and high or low response to ovarian stimulation [47,48]. Further investigations are needed in order to clarify these results.

SHBG

SHBG constitutes the main plasma transport glycoprotein of the sex steroid hormones to target tissues. The gene encoding SHBG is located on chromosone 17p13. A pentanucleotide $(TAAAA)_n$ repeat polymorphism (rs6761) at the 5-boundary of the gene promoter has been described, which has been shown to influence its transcriptional activity [68]. Previous studies have shown significant association between the length in TAAAA repeats on SHBG and ovarian response [49,50]. An increase in the follicle and oocyte number was observed in women carrying long SHBG $(TAAAA)_n$ allele homozygotes compared with short SHBG $(TAAAA)_n$ allele homozygotes using eight repeats as the cutoff allele. Larger population studies are needed to validate this finding.

CYP19

Intrafollicular steroids and their metabolism are crucial for follicular growth. Intrafollicular estrogen production takes place in follicular granulosa cells using androgens, which are synthesized in theca cells under the catalytic action of aromatase, as substrates. The gene encoding aromatase is *CYP19A1*, located at chromosome region 15q21. A tetranucleotide $(TTTA)_n$ repeat polymorphism (rs60271534) in intron 4 has been involved in steroid hormone regulation [69]. Women carrying shorter TTTA repeats, using nine as the cutoff allele, exhibit lower estrogen concentrations [51] and reduced aromatase activity. Short *CYP19A1* allele carriers need higher gonadotropin administration during COS in order to achieve follicle numbers as high as those observed in long *CYP19A1* allele carriers [50,52]. Further studies are needed to clarify the effect of aromatase gene variants on COS outcome.

MTHFR

MTHFR (1p36.3) plays a central role in folate and homocysteine metabolism by catalyzing the

conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulatory form of folate, which is utilized in homocysteine remethylation to methionine. Folate is important during periods of rapid cell growth, which occurs during early follicle development, affecting immature follicles [70]. The common 677C>T *MTHFR* polymorphism (rs1801133) gives rise to an unstable enzyme with reduced activity. Heterozygous individuals seems to have a more favorable outcome compared with homozygotes. However, there is some discordance in the association between C677T polymorphism on *MTHFR* and COS outcome [53–55]. The majority of women who are trying to get pregnant take folate supplements, which could be one explanation for the contradictory results. Given the involvement of folate in folliculogenesis, *MTHFR* polymorphisms should be further studied in COS response.

BMP15

BMP15 is a member of the TGF-β superfamily of proteins that controls many aspects of development. The gene that encodes BMP15 is located on Xp11.2. According to animal models, reduced levels of BMP15 might result in higher levels of FSHR in the granulose cells, high levels of estrogens and increased follicle production, with the possible presentation of a side effect related to OHSS [71]. A SNP in the gene for BMP15, which renders the protein less bioactive or inhibits its secretion, would theoretically increase the follicles' sensitivity to FSH. An activating SNP could have the opposite effect. Four SNPs (-673C>T, rs58995369; -9C/G, rs3810682; IVSI +905, rs3897937; N103S, rs6165) that could predict over-response to recombinant FSH and OHSS have been identified in the *BMP15* gene [56]. In addition, in another study, an association between the -9 G allele and a high response to gonadotropin stimulation was reported [57]. These results support the existence of a new pathway to activate estrogen and follicle production in humans. This result has to be confirmed by independent research teams.

GDF9

GDF9 also belongs to the TGF-β superfamily and is preferentially expressed in the oocytes of humans. GDF9 stimulates granulosa cell proliferation and cumulus cell expansion, inhibits follicular apoptosis and enhances oocyte and embryo development. The gene for *GDF9* is located at chromosome region 5q31.1. In the *GDF9* gene 546G>A polymorphism (rs10491279), the A allele was correlated with poor COS and IVF outcomes in women with diminished ovarian reserve [58]. Polymorphisms in the TFG-β superfamily genes are promising candidates in COS outcomes owing to the important role of these TFG-β superfamily members in folliculogenesis. The involvement of *GDF9* polymorphisms in different responses to FSH should be investigated further.

SOD2

SOD2 is a mitochondrial enzyme that catalyses the detoxification of superoxide free radicals, playing a crucial role in protection against redox harm. COS produces a disequilibrium in the oxidant–antioxidant status [72]. SOD2 may have a role in different responses to COS. The *SOD2* gene is located at 6q25. The A16V (rs4880) polymorphism has been identified. A valine at position 16 produces a conformational change in the protein structure, which may decrease efficiency. Interestingly, the AA genotype was associated with higher number of total oocytes following COS [59]. Larger population studies are needed to validate this finding.

p53

The p53 tumor suppressor protein plays a crucial role in maintaining genomic stability in somatic cells. The p53 tumor suppressor gene contains 11 exons and is located on chromosome 17p13. A SNP located at the second position of codon 72 in the *p53* gene consists of an ancestral C allele derived from a G allele. Presence of the C allele results in a proline in codon 72, and presence of the G allele results in an arginine. Significant differences in the codon 72 polymorphic form of p53 might affect the biological activity of p53 (rs1042522). The R72 variant of the p53 protein is markedly more efficient than the P72 form. Previous studies have shown that the P72 polymorphism is a risk factor for recurrent implantation failure and pregnancy loss [73]. Even though functional impact of p53 on oogenesis has not yet been investigated, one can hypothesize that low p53 activity is associated with greater DNA damage during folliculogenesis. Recently, a study has shown that women who were homozygous for A72 had greater number of oocytes than women who were PP homozygous or heterozygous [3]. The impact of the p53 polymorphism on ovarian response must be confirmed in larger studies.

Genome-wide association studies

Genome-wide association studies (GWAS) are powerful tools used to identify genetic factors

that influence drug response. To our knowledge only one genome-wide analysis has been performed in the field of ovarian stimulation [15]. This study was performed in a homogeneous group of 102 healthy, Caucasian, regularly cycling, nonsmoking women aged 38 years or less with a BMI <30 kg/m² with a regular indication for IVF. Genetic profiles were associated with the number of oocytes obtained. After correction for multiple testing, no SNPs were observed to be significantly correlated to ovarian response, embryo quality or pregnancy [15]. The GWAS limitation is the small sample size. GWAS require multiple correction testing. In order to assess more subtle genetic effects with a greater power and to find new biomarkers in COS further studies in larger cohorts are clearly needed.

Conclusion & future perspective

In order to tailor the amount of FSH used in IVF protocols an important effort has been made in the identification of a genetic profile. More data

Executive summary

Follicle-stimulating hormone receptor

- The N680S polymorphism determines ovarian response to follicle-stimulating hormone (FSH) stimulation in patients undergoing *in vitro* fertilization (IVF) treatment.
- Patients with the S680 allele need more FSH during the stimulation phase.
- In fertile egg donors, S/S patients showed that the gonadotropin dosage is higher and fewer oocytes are retrieved than in other genotype groups in controlled ovarian stimulation (COS).

Luteinizing hormone β subunit

- Women that carry the variants W8R and I15T in the *LHB* gene require higher recombinant FSH consumption while having fewer oocytes retrieved.

Luteinizing hormone receptor

- The rs4073366 +28G>C polymorphism on the *LHR* gene was associated with a threefold increased risk of developing OHSS in women who are C variant carriers.

Estrogen receptors

- In *ESR1*, the T>C SNP at position -397 in intron 1: patients carrying the CC genotype had higher number of follicles, mature oocytes, fertilization rate and good quality embryos.
- The rs9340799 SNP in intron 1 on *ER1* gene: patients carrying the GG genotype had higher number of mature oocytes and fertilization rate.
- Longer TA repeats in the *ER1* promoter region was associated with better COS.

Anti-Mullerian hormone & receptor

- The I49S (rs10407022) SNP in the *AMH* gene and the -482 A>G (rs2002555) polymorphism in the *AMHR2* gene were associated with an increase in estradiol concentrations during the menstrual cycle.

Sex hormone-binding globulin

- An increase in the follicle and oocyte number was observed in women carrying long *SHBG* $(TAAAA)_n$ allele homozygotes compared with short *SHBG* $(TAAAA)_n$ allele homozygotes.

CYP19

- Women carrying shorter TTTA repeats in *CYP19* intron 4 need higher gonadotropin administration during COS in order to achieve follicle numbers as high as those observed in long *CYP19A1* allele carriers.

MTHFR

- The common 677C>T *MTHFR* polymorphism (rs1801133) gives rise to an unstable enzyme with reduced activity. Heterozygote individuals seems to have more favorable outcomes compared with homozygotes.

BMP15

- Four SNPs (-673C>T, rs58995369; -9C/G, rs3810682; IVSI+905, rs3897937; N103S, rs6165) have been identified in the *BMP15* gene that could predict over-response to recombinant FSH and OHSS.

GDF9

- In the *GDF9* gene 546G>A polymorphism (rs10491279), the A allele was correlated with poor COS and *in vitro* fertilization outcomes in women with diminished ovarian reserve.

SOD2

- The A16V (rs4880) polymorphism on the *SOD2* gene has been identified. The AA genotype was associated with higher number of total oocytes following COS.

p53

- Women who were homozygous for A72 on the *p53* gene had a greater number of oocytes than women who were PP homozygous or heterozygous.

Genome-wide association studies

- Genetic profiles were associated with the number of oocytes obtained.

are being published, with evidence suggesting that the ovarian response to COS is mediated by various genetic variants. However, the specificity and sensitivity of a single genetic marker will be too low for it to be employed as a predictive biomarker [22]. The development of testing panels that analyze interactions among different polymorphism could increase the clinical value. Indeed, a multilocus rather than a gene by gene statistical analysis has been shown to be a promising predictive tool [28]. Further GWAS and high-throughput sequencing strategies in women undergoing COS are waiting to be employed in COS pharmacogenomics.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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