human reproduction update

Genetic predictors of controlled ovarian hyperstimulation: where do we stand today?

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BACKGROUND: Nowadays, the use of IVF has improved the prospects of infertility treatment. The expected outcome of IVF depends greatly on the effectiveness of controlled ovarian hyperstimulation (COH), where exogenous gonadotrophins are used to induce folliculogenesis. The response to stimulation varies substantially among women and is difficult to predict. Several predictive markers of COH outcome have been proposed (e.g. maternal age and ovarian reserve), but the search for optimal predictors is ongoing. Pharmacogenetic studies demonstrate the effects of individual genetic variability on COH outcome and the potential for customizing therapy based on the patient's genome.

METHODS: MEDLINE, EMBASE, DARE, CINAHL and the Cochrane Library, and references from relevant articles were investigated up to February 2011 regarding any common genetic variation and COH/IVF outcome.

RESULTS: Several polymorphisms in genes involved in FSH signalling, estrogen biosynthesis, folliculogenesis, folate metabolism and other aspects influence the response to exogenous gonadotrophin administration, resulting in differences in COH and IVF outcomes. Nevertheless, the most studied polymorphism *FSHR* Asn680Ser is practically the only genetic marker, together with *ESR1* PvuII T/C, that could be applied in clinical tests.

CONCLUSIONS: Although data are accumulating with evidence suggesting that the ovarian response to COH is mediated by various polymorphisms, the optimal biomarkers and the efficacy of the tests still remain to be evaluated.

Key words: controlled ovarian hyperstimulation / genetic variation / *in vitro* fertilization / polymorphism / pharmacogenetics

Introduction

The prevalence of infertility is increasing, affecting ∼15% of couples of reproductive age. It has significant medical, social and financial implications. It was not until the development of IVF that many diverse causes of infertility could be successfully overcome. Indeed, the Nobel Prize in Physiology or Medicine in 2010 was awarded to Prof. Robert G. Edwards for the development of IVF, a breakthrough that has helped millions of infertile couples worldwide to have children.

Today, 2–3% of all births in developed countries are estimated to be the result of IVF procedures (Gearhart and Coutifaris, 2011).

The demand for assisted reproductive techniques is steadily increasing, and there is a continual need to develop more effective and less harmful protocols. Regardless of the constant improvement of pregnancy rate, the success rate in IVF still remains around 30% per cycle (Macklon *et al.*, 2006). The expected outcome of IVF treatment depends greatly on the effectiveness of controlled ovarian hyperstimulation (COH), a routine procedure preceding IVF, where exogenous gonadotrophins are used to induce the growth and development of multiple follicles.

COH protocols involve the use of various gonadotrophins for ovarian induction, such as hMG and urinary or recombinant follicle-stimulating hormone (uFSH and rFSH, respectively). Downregulation of endogenous pituitary function using GnRH agonists or antagonists (pituitary suppression) is also a common treatment prior to COH, used to avoid premature ovulation and luteinization during folliculogenesis and to achieve better control of the ovaries (Salha and Balen, 2000).

Several studies have demonstrated high variability in clinical outcome in COH-treated women. This unpredictable variability in the response to gonadotrophins represents one of the most intractable problems of IVF treatment, with responses ranging from poor to high, leading to cycle cancellation and IVF failure or undesired complications related to iatrogenic ovarian hyperstimulation syndrome (OHSS). Thus, an understanding and prediction of the ovarian response to stimulation is of paramount importance to the success and safety of IVF treatment.

Various predictive markers of COH outcome have been proposed, such as age, ovarian reserve (ovarian volume and early antral follicle count), hormonal status [FSH, LH, estradiol, inhibin-B, anti-Mullerian hormone (AMH)], and cigarette smoking (Kligman and Rosenwaks, 2001; Coccia and Rizzello, 2008; Freour *et al.*, 2008; Haller *et al.*, 2008). Besides these parameters, genetic variability also seems to be an important factor in determining the ovarian response in COH and IVF. Differences in the human genome might alter cellular and tissue effects of FSH, thereby altering the response in COH.

The influence of polymorphisms in genes on the outcome of ovarian stimulation in IVF has been analysed by several groups, but the pharmacogenetic approach as regards FSH dosing is still in its infancy. Most studies have been focused on polymorphisms in the FSH receptor gene (*FSHR*). Patients with unfavourable *FSHR* genotypes have been shown to need higher doses of rFSH to overcome relative ovarian insensitivity (Behre *et al.*, 2005). A few teams have also examined the impact of variations in biochemical pathways involved in estrogen production and action (aromatase and estrogen receptor genes), folliculogenesis (*BMP15*, *GDF9* and

AMH) and other aspects. These pharmacogenetic studies illustrate the effects of individual variability on COH outcome and the potential for customizing therapy based on the patient's genome. The knowledge obtained from these studies offers the possibility to tailor the treatment protocol according to the patients' characteristics. A personalized approach in IVF helps in improving the prediction of the ovarian response to COH, which reduces the incidences of treatment cancellations and complications and lowers the number of treatment cycles required to achieve pregnancy. However, regardless of the continuous research into COH predictive factors, accurate prognosis of the ovarian response to exogenous gonadotrophins is currently not possible (Twigt *et al.*, 2011), and the search for optimal biomarkers is ongoing.

Several reviews regarding genetic factors in COH have been released (de Castro *et al.*, 2005; Moron *et al.*, 2007; Wunsch *et al.*, 2007; Loutradis *et al.*, 2008; Simoni *et al.*, 2008a; Overbeek and Lambalk, 2009a, b; Moron and Ruiz, 2010; Alfirevic *et al.*, 2010; Lalioti, 2011). However, the focus in these studies has mainly been on FSH receptor markers. With a new wave of studies on additional promising candidate genes recently published, we wish to give an update of the current status of pharmacogenetic analyses of COH in humans published to date.

Methods

Criteria for considering studies for this review

This systematic review is focused on studies of genetic variation and its influence on COH in IVF technology. Articles were eligible if they covered evaluation of the association between any genetic variation and ovarian stimulation outcome together with IVF outcome. Articles were selected if the target population consisted of women undergoing ovarian stimulation with exogenous gonadotrophins in fresh autologous IVF and ICSI procedures. The outcome measures were ovarian stimulation outcome (the amount of FSH administered, number of follicles and oocytes retrieved, poor responses, OHSS and other aspects) and IVF outcome if evaluated (such as implantation and pregnancy rates, and IVF failure).

Search strategy for the identification of studies

A systematic review of the literature was conducted up to February 2011 across the following databases: MEDLINE, EMBASE, DARE, CINAHL and the Cochrane Library. The reference lists of review articles and relevant studies were hand-searched to identify other potentially eligible studies. Abstracts from conference proceedings were also considered. No language or any other restrictions were applied. Keywords used for the searchers were: COH, controlled ovarian stimulation, ovarian induction, ICSI, IVF and OHSS. Each of these keywords was paired with several genetics-related terms, as summarized in Table I. In addition, a search of 'pharmacogenetics AND reproductive medicine' was conducted. We downloaded all references identified into EndNote software (version X2).

Inclusion and exclusion criteria

Articles were included if they studied associations between one or more genetic variations and COH outcome, and if evaluated, IVF outcome, and if the study group consisted of women undergoing fresh autologous IVF/ICSI cycles. Articles were excluded if the infertile women did not

undergo COH/IVF procedures, or if they reported only IVF outcome and no evaluation of polymorphisms in connection with COH outcome. Genetic studies focusing only on mutations in connection with COH and not common polymorphisms were not considered either. The focus of our study was solely on common variation (>1% frequency in the population) in order to identify COH predictive markers in the general population, and not in small subgroups.

Identification of eligible studies

The abstracts of all articles identified through the search were read by one researcher (S.A.), who selected the articles that were potentially eligible. In the next step, two researchers (S.A. and A.S.) carefully read and evaluated potentially eligible articles and decided on inclusion. The reference list of every selected article was carefully checked to identify other potentially eligible studies.

Results and discussion

Search results

Our search led us to 1936 articles (excluding duplicates). The process of paper selection is summarized in Fig. 1. Articles were removed at initial screening (based on the title or abstract) if they did not meet the inclusion criteria. Full-text versions of articles were obtained if eligibility could not be determined from reading the abstract. After screening titles and abstracts, we selected 72 articles for further reading. A total of 29 articles did not meet our inclusion criteria, and 43 studies were included in the review, 35 on COH and IVF parameters and 8 on OHSS. All the included studies are listed in Supplementary data, Table SI and summarized in a compact Table II (except for that by van Disseldorp et al., 2011, who conducted whole genome and not single-marker analysis). Figure 2 illustrates the hyperstimulation protocols used within all studies. The majority of the studies involved GnRH agonist stimulation protocols (72%) or

mixed protocols (19%), where patients undergoing GnRH agonist or antagonist protocols were included in the study as one group. Therefore, no further distinction in describing the results was made, except in Supplementary data, Table SI, where studies involving GnRH antagonist or mixed protocols are marked with asterisks.

## Follicle-stimulating hormone receptor

To date, the FSH receptor gene is the most studied genetic factor in regard to COH. It is well established that the physiological action of FSH depends on the activation of its receptor (FSHR), which is expressed by granulosa cells. The hormone FSH plays a pivotal role in ovarian function, where its main effects are related to granulosa cell proliferation, oocyte maturation and estrogen synthesis via activation of the aromatase gene. As the success of COH depends greatly on the efficacy of the FSH dose administered to the patient, the FSHR gene clearly is the primary candidate gene to explain differences in COH outcome.

The FSHR gene is located at chromosome region 2p21, and consists of 10 exons (Rousseau-Merck et al., 1993). A large number of single nucleotide polymorphisms (SNPs) in the gene have been identified (1488 SNPs, www.snpper.chip.org). The most common and well-studied SNPs in FSHR are Thr307Ala (rs6165) and Asn680Ser (rs6166). Both polymorphisms are located within exon 10, where Thr307Ala is located in the extracellular domain of the protein (the hormone-binding area) and Asn680Ser is located in the intracellular domain. These two polymorphisms are in near complete linkage disequilibrium (Simoni et al., 2002). This is why most studies have been focused solely on position 680, as genotyping of either of them permits genotype inference of the other. Several studies have concerned analysis of the effect of Asn680Ser and/or Thr307Ala on ovarian stimulation outcome and the data have been summarized in

Table I Search terms used for compilation of the literature.

Keywords	Paired terms
COH	Estrogen receptor
Controlled ovarian hyperstimulation	FSHR
Controlled ovarian stimulation	FSH receptor
ICSI	Genetics outcome
IVF	Genomics
OHSS	Mutation outcome
Ovulation induction	Pharmacogenetics
	Pharmacogenomics
	Polymorphism
	Progesterone receptor
	SNP
	Variant
	Variation

Each of the keywords was paired with each of the terms in the right-hand column in the search engines used: for example, 'COH AND estrogen receptor'.
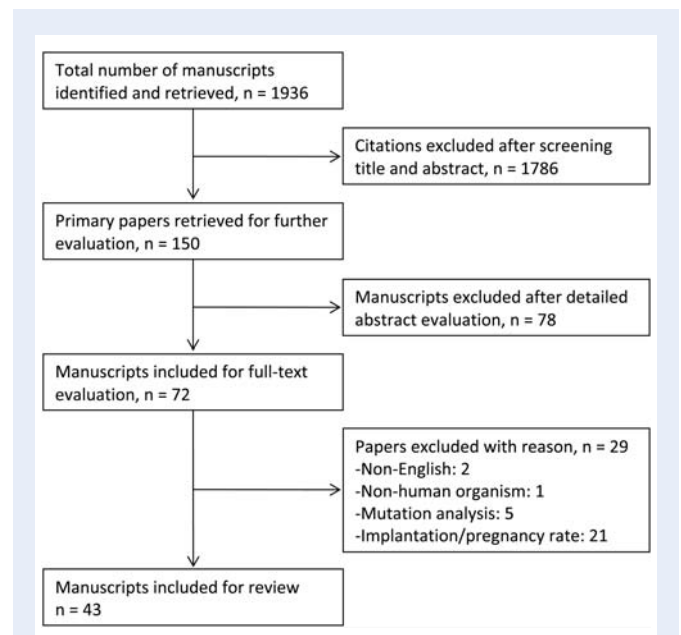
Figure 1 Process from initial search to final inclusion of the manuscripts.

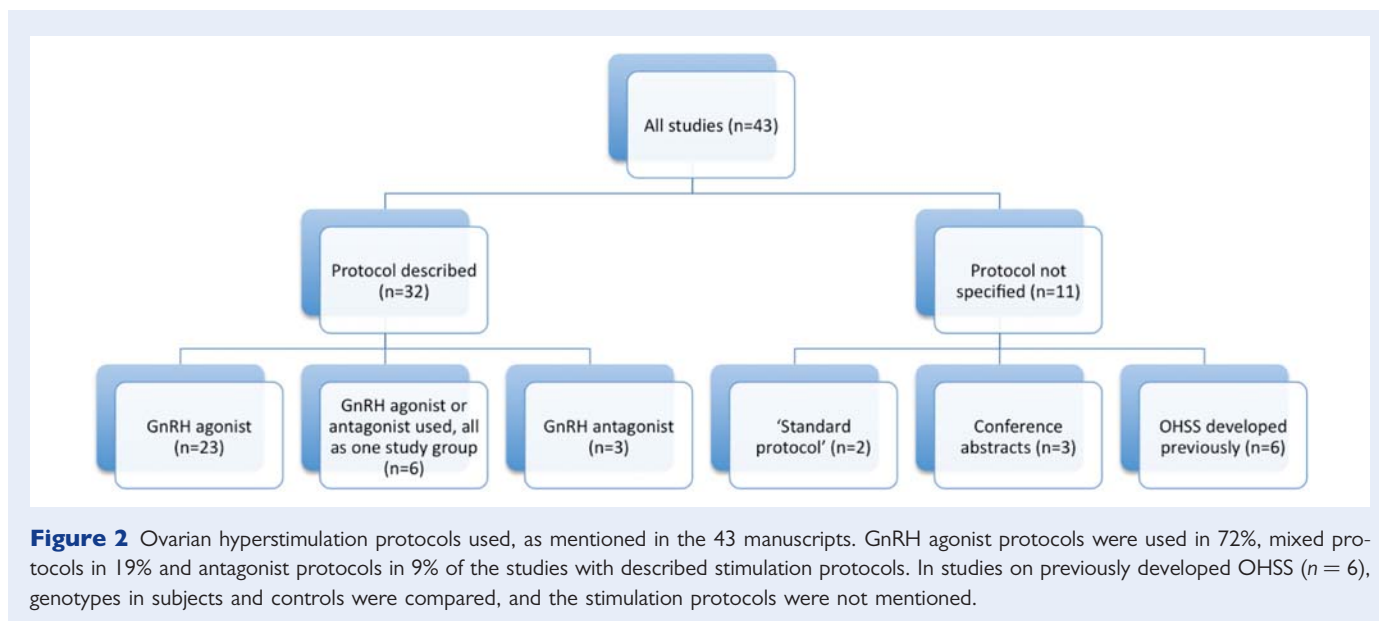**Table II** Polymorphisms studied in relation to COH outcome in infertile women undergoing IVF treatment.

Gene	Variation	Main findings
FSHR	• −29G/A (rs1394205) • Ile160Thr (rs121909659) • Thr307Ala (rs6165) • Asn680Ser (rs6166) • mRNA splice variant • mRNA, protein expression	Women with −29 AA genotype require highest amount of exogenous FSH for ovulation induction (Achrekar *et al.*, 2009b). 307Ala and 680Ser associated with reduced COH outcome and elevated FSH administration (Perez Mayorga *et al.*, 2000; Sudo *et al.*, 2002; de Castro *et al.*, 2003, 2004; Choi *et al.*, 2004; Behre *et al.*, 2005; Jun *et al.*, 2006; Loutradis *et al.*, 2006; Livshyts *et al.*, 2009). 680Ser associated with lower clinical pregnancy (Choi *et al.*, 2004; Jun *et al.*, 2006). Alternatively spliced FSHR products detected in 35% of IVF patients (Gerasimova *et al.*, 2010). Higher expression associated with better response to COH (Cai *et al.*, 2007)
LHB	• Trp8Arg (rs1800447) • Ile15Thr (rs34349826)	Variant βLH (8Arg−15Thr) more frequent among hypo-responders to rFSH (Alviggi *et al.*, 2009a), and in women with ovarian resistance to rFSH (Alviggi *et al.*, 2009b)
LHR	• 18insLeuGln	No association with OHSS (Kerkelä *et al.*, 2007)
CYP11A1	• (TTTTA)$_n$	No association with OHSS (Ferk *et al.*, 2006)
CYP19A1	• TCT Ins/Del • (TTTA)$_n$ • C/T (rs10046)	TCT Del/Del and shorter (TTTA)$_n$ associated with decreased ovarian FSH sensitivity in COH (Altmäe *et al.*, 2009)
ESR1	• (TA)$_n$ • PvuII T/C (rs2234693) • XbaI A/G (rs9340799) • BstUI G/C	Longer (TA)$_n$ repeats predict better COH outcome (Altmäe *et al.*, 2007). PvuII CC and XbaI GG demonstrate better COH and IVF outcome (Georgiou *et al.*, 1997; Sundarrajan *et al.*, 1999; Altmäe *et al.*, 2007; Ayvaz *et al.*, 2009)
ESR2	• Val328Val (rs1256049) • (CA)$_n$ • AluI G/A (rs4986938)	AluI GG in interaction with FSHR 680Ser and ESR1 PvuII T associated with poor response during COH (de Castro *et al.*, 2004)
PGR	• +331G/A (rs10895068)	+331 A allele associates with decreased pregnancy outcomes (Spandorfer *et al.*, 2006)
Folate pathway	• MTHFR 677C/T (rs1801133) • MTHFR 1298A/C (rs1801131) • MTHFR 1793G/A (rs2274976) • FOLR1 1314G/A (rs2071010) • FOLR1 1816C/delC (rs3833748) • FOLR1 1841G/A (rs1540087) • FOLR1 1928C/T (rs9282688) • TCN2 776C/G (rs1801198) • CTH 1208G/T (rs1021737) • SLC19A1 80G/A (rs1051266)	Women with MTHFR 677 CT have higher proportion of good quality embryos and increased chance of pregnancy (Laanpere *et al.*, 2011). MTHFR 677 CC women require less rFSH and produce more oocytes (Thaler *et al.*, 2006). Women with MTHFR 1298 C demonstrate reduced COH outcome (Rosen *et al.*, 2007). MTHFR 1793 GA and SLC19A1 80 GA associated with decreased number of previously failed IVF treatments, meanwhile CTH 1208 GT with increased chance of pregnancy (Laanpere *et al.*, 2011), FOLR1 1816 CdelC and FOLR1 1841 GA associated with raised risk of pregnancy loss (Laanpere *et al.*, 2011)
AMH/AMHR2	• AMH −649T/C (rs4807216) • AMH Ile49Ser (rs10407022) • AMHR2 −482A/G (rs2002555) • AMHR2 1749C/T (rs2071558) • AMHR2 4952G/A (rs3741664) • AMHR2 10A/G (rs11170555)	No association with low or high response in COH stimulation (Hanevik *et al.*, 2010)
GDF9	• 169G/A • 447C/A (rs254286) • 546G/A (rs10491279)	546 A allele associated with poor COH and IVF outcomes in women with diminished ovarian reserve (Wang *et al.*, 2010)
BMP15	• −673C/T (rs58995369) • −9C/G (rs3810682) • Asn103Ser (rs41308602) • IVS1 + 905A/G (rs3897937)	−673 T, −9 G and IVS1 + 905 G alleles associated with high response in COH (Moron *et al.*, 2006; Hanevik *et al.*, 2011). Haplotype TGGA (−673, −9, IVS1 + 905, Asn103Ser) serves as a risk factor for OHSS (Moron *et al.*, 2006)
SOD2	• Val16Ala (rs4880)	AlaAla genotype associated with better COH and IVF outcome (Ruiz-Sanz *et al.*, 2010)
SHBG	• (TAAAA)$_n$	Women with shorter repeats present higher number of small follicles (Hatzi *et al.*, 2010)

a number of review articles (de Castro *et al.*, 2005; Moron *et al.*, 2007; Wunsch *et al.*, 2007; Loutradis *et al.*, 2008; Simoni *et al.*, 2008a; Overbeek and Lambalk, 2009a, b; Moron and Ruiz, 2010; Alfirevic *et al.*, 2010; Lalioti, 2011).

Although there is some discordance (Klinkert *et al.*, 2006; Hanevik *et al.*, 2010, 2011), there is sufficient evidence to state that genetic variation in *FSHR* has a role in COH outcome. In short, the *FSHR* 680Ser variant was originally associated with elevated basal levels of

FSH (a key marker of ovarian reserve and the best-known predictor of COH response) and elevated gonadotrophin requirements during COH (Perez Mayorga *et al.*, 2000). Several investigators have confirmed these original findings in different populations (Sudo *et al.*, 2002; de Castro *et al.*, 2003, 2004; Choi *et al.*, 2004; Jun *et al.*, 2006; Loutradis *et al.*, 2006; Livshyts *et al.*, 2009). However, such associations were not observed by Klinkert *et al.* (2006) or Hanevik *et al.* (2010), and instead a positive association with pregnancy rates

Figure 2 Ovarian hyperstimulation protocols used, as mentioned in the 43 manuscripts. GnRH agonist protocols were used in 72%, mixed protocols in 19% and antagonist protocols in 9% of the studies with described stimulation protocols. In studies on previously developed OHSS (*n* = 6), genotypes in subjects and controls were compared, and the stimulation protocols were not mentioned.

and the 680Ser allele in IVF-treated women was found (Klinkert et al., 2006). In addition, a recent study revealed an association between the Ser allele and a higher frequency of ongoing pregnancies (Valkenburg et al., 2009). The only clinical trial on gene variants and COH outcome conducted so far has confirmed the previous findings of an Asn680Ser polymorphism effect, indicating that lower FSH sensitivity of 680Ser homozygote carriers can be overcome by higher FSH doses during COH protocols (Behre et al., 2005). Furthermore, a recent meta-analysis of four main studies in different European populations, where patients were divided into poor responders or good responders, confirmed the role of the 680Ser variant in poor responses during COH treatment (Moron and Ruiz, 2010). Another recently published meta-analysis of COH/IVF outcomes in patients with different Asn680Ser genotypes was focused on: (i) basal FSH levels, (ii) total FSH doses used during COH, (iii) oocytes retrieved and (iv) pregnancy rates (Yao et al., 2011). This extensive meta-analysis concluded that carriers of the SerSer genotype have significantly higher basal FSH levels and tend to require higher exogenous FSH doses for COH compared with Asn carriers (Yao et al., 2011). However, they did not find any remarkable differences in peak estradiol level, in the number of oocytes retrieved and in the pregnancy rate, which could have been influenced by the high heterogeneity across the eight studies eligible for the meta-analysis (Yao et al., 2011).

FSHR gene variants have also been associated with the extreme phenotype of COH, OHSS. The Asn680 allele has been found to be a risk factor and predictor of severity of symptoms among OHSS patients (Daelemans et al., 2004; Macek et al., 2010). Also, Ile160Thr, specifically the Thr allele, has been detected more frequently among OHSS patients (Binder et al., 2008). However, other authors have not replicated these findings (d'Alva et al., 2005; Kerkelä et al., 2007; Binder et al., 2008). Unexpectedly, enrichment of the 680Ser allele and the 307Ala allele in OHSS patients has also been reported (Daelemans et al., 2004; Achrekar et al., 2009a), which obviously contradicts the general findings (of 'less active alleles'). However, in both studies, the unexpected results could, in part, be explained by women's age. In the Achrekar et al. study,

women with the 307Ala allele were 2 years younger than other women (28.7 versus 30.8 years), and in the Daelemans et al. study, the group of OHSS women were on average 31 years old and were compared with all IVF-women. The connection between the 680Ser allele and a higher pregnancy rate (Klinkert et al., 2006; Valkenburg et al., 2009) is also unclear. Again, one explanation could be woman's age, since, in the Klinkert et al. study, these women were younger than women with the Asn allele. Another explanation could be the existence of two opposite functional variants in linkage disequilibrium with these two common variants. Alternatively, the existence of an U effect of these alleles could explain these opposing results, given the age-dependent penetrance of the 680Ser allele observed by de Castro et al. (2003). It has been hypothesized that in younger women, 680Ser carriers could have a 'follicle-burning phenotype', recruiting a higher number of follicles during every natural cycle and therefore being at a higher risk of OHSS in an eventual COH cycle. Meanwhile, in the same individuals, ovarian reserve exhaustion could occur earlier, leading to an age-dependent poor responder phenotype in later life (de Castro et al., 2005). Indeed, the FSHR 680AsnAsn genotype has been associated with delayed age at menarche, however, not with age at natural menopause (Zerbetto et al., 2008). Our considerations are only speculative, and further research is clearly needed to clarify the role of FSHR variants in the extreme phenotypes of COH outcome, also taking age into account.

Recent findings are providing novel molecular insights into the critical role of FSHR in COH. One study demonstrated that altered expression of FSHR mRNA and/or protein in granulosa cells results in different ovarian responses: lower expression accounts for a poor response and higher expression gives the best response to gonadotrophin stimulation (Cai et al., 2007). Furthermore, a new polymorphism in the 5′-UTR of the FSHR gene (position −29 A/G; rs1394205) has been associated with altered transcriptional activity of the FSHR gene (Nakayama et al., 2006). Subsequently, the −29 AA genotype was associated with a poor COH response (Achrekar et al., 2009b). However, these results were obtained in a small sample size, and in a bigger study, no association of −29 A/G with clinical parameters

(basal serum FSH, peak estradiol levels) in IVF patients was observed (Wunsch et al., 2005). Further, a new study suggests another molecular mechanism involved in COH outcome (Gerasimova et al., 2010). Namely, aberrant splicing variants of *FSHR* mRNA (deletion of exon 2, deletion of exon 6) in granulosa cells were identified, which could partially explain the difference in response to FSH in those women undergoing COH treatment (Gerasimova et al., 2010; Lalioti et al., 2010).

Taken together, the data indicate that the *FSHR* gene may have an important role in the success of ovarian stimulation. Women with the 680Ser allele comprise up to 75% of women undergoing IVF treatment, and are characterized by higher basal FSH serum concentrations, higher administered amounts of FSH required and higher risks of hypo- or hyper-responses (Simoni et al., 2008a). Genotyping the *FSHR* Asn680Ser SNP, together with some additional novel markers (e.g. transcript levels), may therefore provide a means of identifying a group of poor responders before infertility treatment is initiated (Simoni et al., 2008a). This personal stimulation approach would lead to higher starting doses in these women, which—in a short-term—means higher costs, but ultimately is cheaper and safer to the patient, as it will prevent poor response and/or cycle cancellation.

The question of whether genetic variation in *FSHR* is associated with pregnancy rates remains controversial (Choi et al., 2004; Behre et al., 2005; Jun et al., 2006; Klinkert et al., 2006; Loutradis et al., 2006; Achrekar et al., 2009a) and requires further studies in larger populations. Previous studies have included relatively small numbers of patients (see Supplementary data, Table SI), and thus have not been powered to address the clinical end-points of fertilization and pregnancy rates.

Luteinizing hormone beta polypeptide

Luteinizing hormone beta (LHB) polypeptide encodes the β subunit of the hormone. LH is a heterodimeric glycoprotein that consists of α and β subunits. FSH and LH share a common α subunit, while the β subunit is hormone-specific and includes the receptor-binding domain. LH stimulates the production of androgens serving as substrates for estrogen synthesis in the follicles. LH co-operates with FSH in promoting follicular development and steroidogenesis, maturation, ovulation and luteinization of the leading follicle (Hillier et al., 1994).

The *LHB* gene is located at chromosome region 11p13 and it has three exons. A number of polymorphisms in the gene have been identified (179 SNPs; www.snpper.chip.org), and three polymorphisms in the coding area have been found to lead to decreased LH activity (Haavisto et al., 1995): polymorphisms Trp8Arg and Ile15Thr have been associated with slightly suppressed fertility (Berger et al., 2005), menstrual irregularities causing infertility (Furui et al., 1994) and recurrent pregnancy loss (Okuda et al., 1994), and the Gly102Ser SNP has been associated with infertility and menstrual disorders (Liao et al., 1998; Ramanujam et al., 1999). In women undergoing IVF treatment, the variant 8Arg-15Thr has been found to be more frequent among hypo-responders to rFSH (Alviggi et al., 2009a) and among women with ovarian resistance to rFSH, who require higher rFSH consumption while having fewer oocytes retrieved (Alviggi et al., 2009b). This short-acting LH variant seems to be less

efficient, with reduced capacity to sustain multiple follicle growth under COH. Further studies with larger study groups and different populations are needed to clarify whether Trp8Arg−Ile15Thr could represent a COH marker in singling out a subgroup of women who could benefit from exogenous LH supplementation during ovarian stimulation (having less bioactive LH). It has been demonstrated that among women with low COH responses and higher rFSH consumption, supplementation with exogenous LH improves ovarian outcome and reduces total rFSH consumption (De Placido et al., 2004).

Luteinizing hormone receptor

LH exerts its actions by binding to its cell surface receptor, LHR (also known as LHCGR, as LH and hCG bind to the same receptor). LHR is critical for maintenance of the theca, maturation of follicles and ovulation. The *LHR* gene is located at chromosome region 2p21, consisting of 11 exons and several common polymorphisms have been identified (over 520 SNPs according to www.snpper.chip.org database). Polymorphisms 18insLeuGln, Asn291Ser and Ser312Asn in the *LHR* gene have been associated with increased receptor activity (Piersma et al., 2006, 2007; Simoni et al., 2008b) and their possible effects in steroid-hormone-related diseases have been suggested (Powell et al., 2003; Piersma et al., 2007). Our group analysed 18insLeuGln polymorphisms in the coding region of *LHR* in a relatively small group of OHSS patients, and we found no association between these variants and the development of OHSS (Kerkelä et al., 2007). To our knowledge, this has been the only study so far in which *LHR* variation in IVF-treated women has been analysed, and further studies are clearly needed to unravel its role in different responses to COH.

Cholesterol side-chain cleavage enzyme (*CYP11A1*)

The cytochrome P450, family 11, subfamily A, polypeptide 1 gene (*CYP11A1*) product (cholesterol side-chain cleavage enzyme) catalyses the conversion of cholesterol to pregnenolone—the first and rate-limiting step in the synthesis of steroid hormones. The *CYP11A1* gene is one of the most promising candidates as regards abnormalities in androgen biosynthesis and therefore the androgen pathogenic mechanism of OHSS could be related to genetic predisposition (Ferk et al., 2006). The *CYP11A1* gene is located at chromosome region 15q23−q24, with 9 exons, and several SNPs have been identified (241 SNPs, www.snpper.chip.org). In COH-treated women, including those at a high risk of OHSS, a polymorphism in the promoter region of the gene (TTTTA$_n$) was analysed. Although this polymorphism is suggested to regulate the transcriptional activity of *CYP11A1* and has been strongly related to hyperandrogenaemia and polycystic ovary syndrome (Gharani et al., 1997), no association with the pathogenesis of OHSS was detected, although the study was of limited sample size (Ferk et al., 2006). To our knowledge, this is the first study in which *CYP11A1* gene variation in ovarian stimulation patients has been analysed, and the results must be confirmed by other groups.

Aromatase (*CYP19A1*)

The aromatase gene (*CYP19A1*) is another candidate as regards a pharmacogenetic approach in COH. Aromatase is one of the key enzymes in ovarian steroidogenesis, catalysing the final stage of

conversion of the androgens testosterone and androstenedione to estradiol and estrone, respectively (Ryan, 1982). The *CYP19A1* gene is located at chromosome region 15q21.1 and comprises 10 exons, where the last 9 (II−X) are coding exons and the first exon, one of nine alternative untranslated first exons, regulates tissue-specific expression (Sebastian and Bulun, 2001). Over 1080 SNPs in the aromatase gene have been identified according to www.snpper.chip. org. However, a tetranucleotide repeat polymorphism $(TTTA)_n$ in intron 4 has attracted major attention, since overrepresentation of the $(TTTA)_{12}$ allele in breast cancer patients with excessive aromatase activity has been reported (Kristensen *et al.*, 1998; Haiman *et al.*, 2000). Concurrently, women carrying shorter *CYP19A1* $(TTTA)_n$ repeats exhibit lower estrogen concentrations (Haiman *et al.*, 2000; Tworoger *et al.*, 2004). Fifty nucleotides upstream from the $(TTTA)_n$ repeat polymorphism, a TCT trinucleotide Ins/Del variation has been identified (Kurosaki *et al.*, 1997), where the Del allele refers to lower ovarian aromatase activity (Baghaei *et al.*, 2003). Our group has demonstrated that women with TCT Del homozygosity and shorter $(TTTA)_n$ repeats exhibit decreased ovarian FSH sensitivity in COH (Altmäe *et al.*, 2009). However, two other groups have found no influence of *CYP19A1* polymorphism 1673C/T (rs10046) on ovarian response to exogenous FSH (de Castro *et al.*, 2004) or on the aetiology of OHSS (Binder *et al.*, 2008). The 1673C/T SNP is in linkage disequilibrium with $(TTTA)_n$ repeats, where the T allele and the long $(TTTA)_{12}$ allele are associated with elevated aromatase transcript levels in breast cancer tissue (Kristensen *et al.*, 2000), and the C allele is related to poor pituitary suppression in premenopausal women (De Castro *et al.*, 2005). Clearly, further studies are needed to clarify the effect of aromatase gene variants on COH outcome.

Estrogen receptors

Estrogen receptors (ESRs) are important candidates in ovarian responsiveness to FSH, since direct effects of estrogens on follicle growth, maturation and oocyte release are well established (Goldenberg *et al.*, 1972). In addition to folliculogenesis, estrogens play an important role in endometrial preparation for implantation (Speroff and Fritz, 2005). Estrogen signalling is mediated by estrogen receptors, which are ligand-activated transcription factors composed of several domains important for hormone binding, DNA binding and activation of transcription (Kuiper *et al.*, 1996). Two estrogen receptors have been identified in humans, ERα (6q25) and ERβ (14q22), encoded by *ESR1* and *ESR2* genes, respectively. In folliculogenesis, the proliferative actions of estrogens are mediated by ERα (predominantly expressed in the theca layer), while the differentiation and antiproliferative effects required for reaching the antral stage require ERβ (expressed in granulosa cells of growing follicles at all developmental stages; Pelletier and El-Alfy, 2000; Britt and Findlay, 2002).

Previous findings have demonstrated that genetic variability in *ESR* genes is involved in the outcome of controlled ovarian stimulation (Georgiou *et al.*, 1997; Sundarrajan *et al.*, 1999; de Castro *et al.*, 2004; Altmäe *et al.*, 2007). In fact, the first pharmacogenetic approach applied in COH/IVF in 1997 was focused on *ESR1* gene polymorphisms (Georgiou *et al.*, 1997).

The *ESR1* gene is highly polymorphic, with more than 2200 SNPs, while around 720 SNPs in *ESR2* have been identified (www.snpper. chip.org). The most studied polymorphisms in *ESR1* are rs2234693

(T/C, defined by the cleavage site of restriction enzyme PvuII) and rs9340799 (A/G, defined by restriction enzyme XbaI) in intron 1 and a $(TA)_n$ dinucleotide repeat polymorphism in the promoter region. The PvuII TT genotype has been associated with decreased pregnancy rates in women undergoing IVF, when 2−3 consecutive cycles were studied (Georgiou *et al.*, 1997; Sundarrajan *et al.*, 1999). In other studies, where single cycles were followed, no influence of *ESR* genotypes on pregnancy rates was detected (Altmäe *et al.*, 2007; Ayvaz *et al.*, 2009; Choi *et al.*, 2009). These results refer to the impact of ESR1 PvuII polymorphism on the cumulative pregnancy outcome in COH/IVF, rather than per single embryo transfer.

In line with PvuII TT and a decreased pregnancy rate, IVF patients carrying the PvuII CC genotype demonstrate improved follicular quality (Georgiou *et al.*, 1997), and higher numbers of follicles, mature oocytes, fertilization rate and good quality embryos following COH/IVF (Sundarrajan *et al.*, 1999; Altmäe *et al.*, 2007; Ayvaz *et al.*, 2009). In addition, we detected an association between the C allele and longer $(TA)_n$ repeats, and consequently with a better COH response (Altmäe *et al.*, 2007). Further, the PvuII C allele frequency has been found to be lower among poor responders ($\leq$3 follicles) compared with normal COH responders (de Castro *et al.*, 2004). The other common SNP in the *ESR1* gene, XbaI A/G, has also shown association with COH outcome: oocyte maturation and fertilization rates were higher in women with the XbaI GG genotype (Ayvaz *et al.*, 2009). Additionally we have demonstrated the association between the GG genotype and higher estradiol levels achieved during COH (Altmäe *et al.*, 2007).

A previous study by de Castro *et al.* group has presented an original multilocus analysis, where an oligogenic model of *FSHR* 680Ser − *ESR1* PvuII T − *ESR2* AluI G explained the poor response to FSH in COH (de Castro *et al.*, 2004). The result of this analysis could be of importance because, apparently, a negative result in a marker-by-marker approach cannot rule out the involvement of the selected gene (Marchini *et al.*, 2005). In fact, the same study and our previous study at a single gene level did not reveal any effect of *ESR2* RsaI G/A (Altmäe *et al.*, 2007) and AluI G/A polymorphisms (de Castro *et al.*, 2004) on COH outcome.

Viewed together, these publications support the notion that estrogen receptor signalling pathways have a role in COH outcome. Nevertheless, additional extensive and independent analyses using bigger sample size, and other populations are necessary to confirm or rebut previous observations.

Progesterone receptor

Progesterone receptor (PGR), a member of the steroid receptor superfamily, mediates the physiological effects of progesterone. Progesterone is an important hormone in the complex regulation of normal female reproductive functions. The major physiological role of progesterone in the ovary and uterus is the release of mature oocytes, facilitation of implantation and maintenance of pregnancy (Graham and Clarke, 1997). Progesterone enhances the effect of FSH on granulosa cells by increasing cAMP (Goff *et al.*, 1979) and it inhibits FSH-induced estradiol production (Schreiber *et al.*, 1981).

Human PGR is encoded by a single-copy gene *PGR* (11q22−23). *PGR* has two separate promoters and translational start sites, producing two isoforms—PGR-A and PGR-B (Giangrande *et al.*, 2000).

Although the two isoforms differ only in that PGR-B contains an additional 164 amino acids at the amino terminus, they are two functionally distinct transcription factors that mediate their own response genes and physiological effects, with little overlap (Giangrande et al., 2000). The receptors start to be expressed in large follicles, and expression is increased by the LH surge (Drummond, 2006).

Several polymorphic variants in the PGR gene have been described, and over 910 SNPs have been identified (www.snpper.chip.org). The most commonly studied polymorphisms in PGR are +44C/T and +331G/A in the promoter area, and a 306 bp Alu insertion polymorphism (PROGINS) in intron 7. Among IVF-treated women, no association between +331G/A and COH outcome has been detected (Spandorfer et al., 2006). As expected, this SNP had an effect on IVF pregnancy outcome, the +331 A allele being associated with decreased pregnancy outcomes (Spandorfer et al., 2006). In a previous study also, a relationship between the +331 A allele and an elevated risk of implantation failure was suggested (Cramer et al., 2003). It is proposed that the +331 A allele increases transcription of the PGR gene, which favours the production of PR-B isoforms, thus affecting the PGR-A/PGR-B ratio (De Vivo et al., 2002).

In conclusion, polymorphisms in the PGR gene seem to be attractive candidates as regards IVF pregnancy outcome, rather than COH outcome.

Folate-metabolizing pathway genes

There is growing evidence that folate status and variation in folate-metabolizing genes are involved in female reproductive functions [reviewed in (Laanpere et al., 2010)]. The water-soluble B vitamin folate participates in one-carbon biosynthetic and epigenetic processes that facilitate the synthesis and methylation of nucleic acids and proteins. Folate is thus indispensable during periods of rapid cell growth and proliferation, which occur during follicular and embryonic development. A very recent study in IVF-treated women suggests that the effect of folic acid is most prominent during early follicle development, affecting immature follicles (Twigt et al., 2011). Insufficient folate intake impairs fertility in animal models (Willmott et al., 1968; Mohanty and Das, 1982; Mooij et al., 1992), and causes early spontaneous abortions and other adverse pregnancy outcomes in humans (George et al., 2002). Folate-deficient women undergoing COH have lower oocyte quality, lower pregnancy rates and impaired ovarian function (Ebisch et al., 2006; Forges et al., 2007; Rosen et al., 2007; Boxmeer et al., 2009; Hecht et al., 2009).

Folate deficiency increases deoxyuridine monophosphate misincorporation into DNA, disrupts DNA integrity (Blount et al., 1997), slows DNA replication and causes apoptosis and necrosis of the affected cells (Huang et al., 1999; Kimura et al., 2004). Both dietary folate deficiency (Jacob et al., 1998) and a common 677C/T polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene (Stern et al., 2000; Friso et al., 2002) cause defective DNA methylation, which may alter gene expression (Ingrosso et al., 2003) and lead to chromosome fragility (Lukusa and Fryns, 2008). Furthermore, dietary or genetically determined folate deficiency may lead to elevated serum homocysteine (Hcy) concentrations (Jacques et al., 2001). High levels of Hcy (hyperhomocysteinaemia) have been associated with several pathological conditions, including pregnancy

complications [summarized in Tamura and Picciano (2006)]. In IVF, negative correlations between Hcy levels and oocyte maturity (Szymanski and Kazdepka-Zieminska, 2003), in vitro embryo quality (Ebisch et al., 2006; Berker et al., 2009) and pregnancy and implantation rates have been shown (Haggarty et al., 2006; Pacchiarotti et al., 2007). Thus, Hcy has been suggested to be a mediator of the negative effect of insufficient folate.

Several variations have been identified in genes involved in folate absorption and folate-mediated one-carbon metabolism. These polymorphisms may alter the beneficial effects of folates and other B vitamins that play a role in the metabolism of methyl groups and change the flux of folate cofactors between methylation and nucleotide synthesis pathways (see Fig. 3 for the folate-metabolizing pathway) (Narayanan et al., 2004). The MTHFR 677C/T polymorphism is the most influential and prevalent genetic variation affecting folate metabolism. It results in an amino acid change at codon Ala222Val, giving rise to an unstable enzyme with reduced activity of 50–60% (Frosst et al., 1995), which results in the accumulation of Hcy and impaired methylation reactions (Harmon et al., 1996).

Our group has recently conducted an extensive study concerning 10 polymorphisms in folate-metabolizing pathway genes, and COH/IVF outcome (Laanpere et al., 2011), being the first to focus on genetic variability and COH outcomes in this complex pathway. The SNPs analysed in the study are indicated in Fig. 3. We demonstrated that MTHFR 677 CT heterozygotes have a higher proportion of good quality embryos and an increased chance of pregnancy (Laanpere et al., 2011). Previously, the 677 CT heterozygous genotype, rather than the homozygous CC genotype, was associated with an increased chance of having had a previous IVF pregnancy, and a live birth in the current IVF cycle (Haggarty et al., 2006). Further, we found the 677 CT genotype more frequently found among control individuals than in women with unexplained infertility undergoing infertility treatment (Altmäe et al., 2010). Better COH responses have been observed in women older than 35 years with the wild-type 677 CC genotype; these patients required less FSH and produced more oocytes (Thaler et al., 2006). However, another study revealed no correlation between 677C/T variation and COH outcome, but instead, with the MTHFR 1298A/C polymorphism, where the wild-type A allele was associated with better COH outcomes (Rosen et al., 2007). Unexpectedly, in a study of women with extreme COH responses, though in a very small sample size, the variant 677 T allele was associated with OHSS (Dulitzky et al., 2002). In another study of bigger sample size, this finding was not replicated (Machac et al., 2006). It should be noted that the majority of women undergoing infertility treatment take folate supplements, which could be one explanation for the negative or contradictory results. It is commonly known that individuals carrying the MTHFR 677 T allele have increased plasma Hcy concentrations and with additional folate administration, they are able to achieve normal Hcy concentrations (Fohr et al., 2002). No data about folic acid intake in studies by Dulitzky et al. and Machac et al. were presented. All patients in the studies by Laanpere et al., Thaler et al. and Rosen et al. were counselled to take 0.4 mg of folic acid per day, which might have augmented ovarian response, particularly in homozygous TT patients. Nevertheless, it is not known whether the effects of MTHFR gene variants on ovarian sensitivity can be reversed within a short period of adequate folate fortification. Chronic effects of these variants could be one explanation for
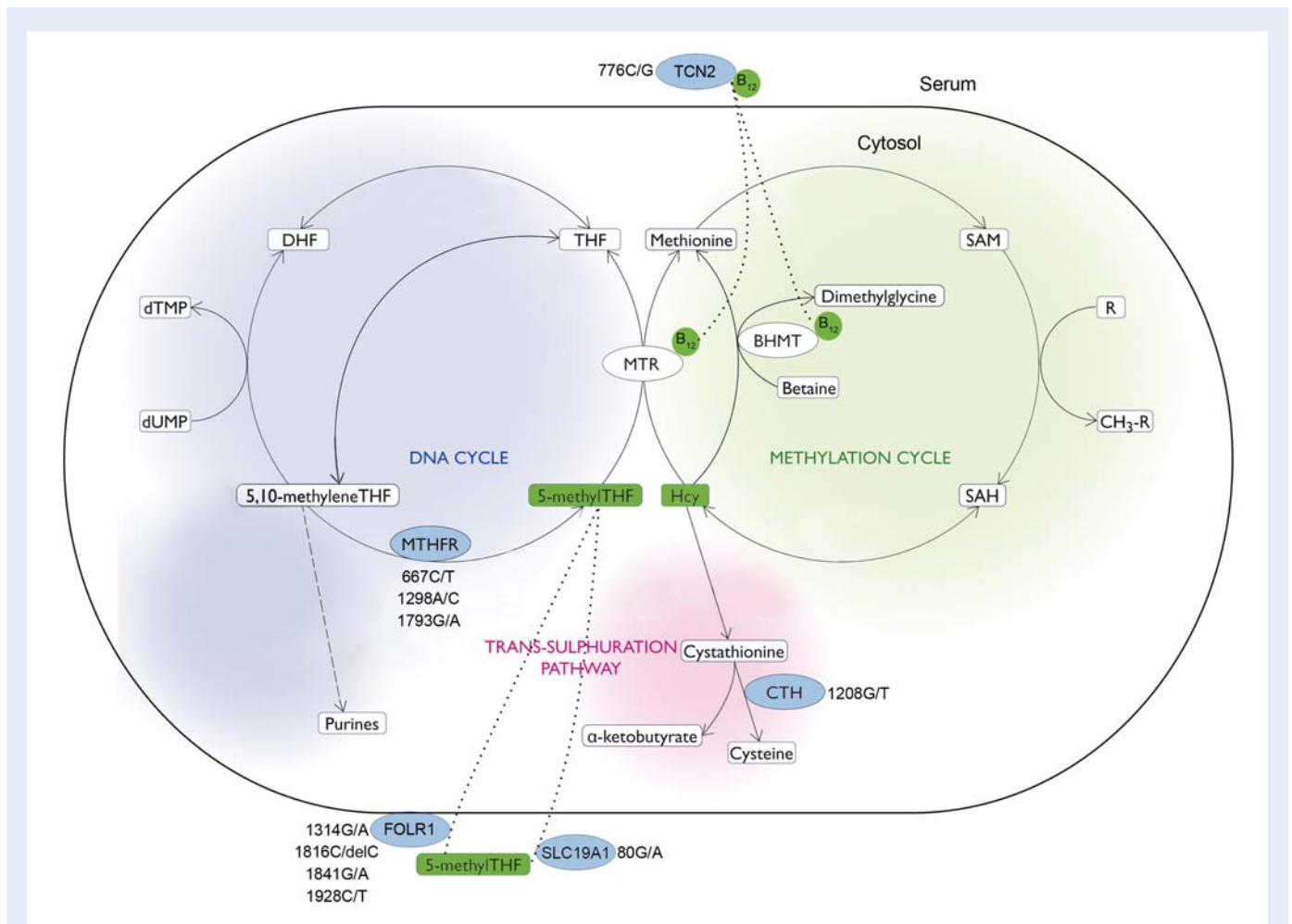
Figure 3 Folate-mediated one-carbon metabolism, consisting of two cycles that compete for folate cofactors: DNA biosynthesis (blue) and methylation (green). Homocysteine is catabolized in the trans-sulphuration pathway (pink). Genes analysed in the study by Laanpere *et al.* (2011) are indicated as blue ellipses with studied polymorphisms listed next to the gene, and biochemical markers folate, homocysteine and vitamin B12 are shown in green. The dotted line represents a compound transport into the cell. The dashed line indicates biochemical reactions where not all steps are shown. Abbreviations: 5,10-methyleneTHF, 5,10-methylenetetrahydrofolate; 5-methylTHF, 5-methyltetrahydrofolate; B12, vitamin B_{12}; BHMT, betaine-homocysteine methyl-transferase; CTH, cystathionase; DHF, dihydrofolate; FOLR1, folate receptor α; Hcy, homocysteine; MTHFR, methylenetetrahydrofolate reductase; MTR, methionine synthase; R, methyl acceptor, such as DNA or histones; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SLC19A1, solute carrier family 19 member 1; THF, tetrahydrofolate; TCN2, transcobalamin II (from Laanpere *et al.* 2011; published with permission from Reproductive BioMedicine Online).

the obtained differences in ovarian responsiveness to the stimulation in spite of folic acid supplementation (Thaler *et al.*, 2006).

In addition to the *MTHFR* 677C/T polymorphism, the other studied folate pathway SNPs in our recent study had some effect on IVF outcome. *MTHFR* 1793 GA, *SLC19A1* 80 GA and *CTH* 1208 GT heterozygotes showed a lower probability of previously failed IVF treatments, while women heterozygous for *FOLR1* 1816 C/delC and linked 1841 G/A showed an increased risk of pregnancy loss (Laanpere *et al.*, 2011). The conclusion in our study was that polymorphisms in folate-metabolizing genes affect COH and pregnancy outcome in IVF, and heterozygous individuals, rather than the wild-type homozygotes, appear to have more favourable outcomes. The concept of heterozygous advantage that our study results reflect is not new in reproduction (Gemmell and Slate, 2006). The theory of

heterozygote advantage, or overdominance, suggests that genetic variation is maintained in natural populations as a result of greater viability and reproductive fitness among heterozygotes (Hansson and Westerberg, 2002).

Given the involvement of folate, Hcy and variations in folate-metabolizing genes in folliculogenesis, these polymorphisms are interesting biomarker candidates and their importance in COH response variation should be studied further.

Transforming growth factor beta superfamily

A number of genes belonging to the transforming growth factor beta (TGFβ) superfamily have been suggested to be instrumental in determining the outcome of ovarian stimulation. The TGFβ superfamily is a

large group of proteins that share common structural motifs. Many of these proteins are produced in the ovary and are important as local regulators of follicular development and oocyte maturation (Knight and Glister, 2006). So far, the variation in *BMP15*, *GDF9*, *AMH* and *AMHR2* genes in relation to COH outcome has been studied (Moron et al., 2006; Hanevik et al., 2010; Wang et al., 2010; Hanevik et al., 2011).

AMH (also known as Mullerian inhibiting factor) is one of the most studied peptides in this family. In contrast to the other members, AMH is suggested to have an inhibitory role in follicle recruitment (Durlinger et al., 1999). AMH is expressed in the granulosa cells in primary- to antral-stage follicles (Weenen et al., 2004), and it has been shown that it inhibits primordial follicle recruitment and granulosa cell mitosis and therefore might diminish FSH sensitivity (Knight and Glister, 2006). AMH exerts its influence via AMH receptor, type II (AMHR2). The gene encoding AMH is located at chromosome region 19p13.3, and the AMH receptor gene is located at 12q13. A previous study revealed SNPs in the *AMH* (Ile49Ser) and *AMHR2* (−482A/G) genes that were associated with higher follicular phase estradiol levels in serum, thus representing an indirect measure of increased follicular sensitivity to FSH (Kevenaar et al., 2007). So far, only one study has been carried out on *AMH* and *AMHR2* gene polymorphisms in women with high and low responses to COH, but no association was detected (Hanevik et al., 2010).

Growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) are expressed in oocytes from early-stage follicles (Aaltonen et al., 1999). Both proteins play crucial roles in determining follicle growth and ovulation rates. The gene for *GDF9* is located at chromosome region 5q31.1, and that for *MPB15* is at Xp11.2. In sheep, naturally occurring mutations have been identified in both genes, affecting ovulation rate and fecundity (Hanrahan et al., 2004; Gemmell and Slate, 2006). In humans, polymorphisms in *GDF9* and *BMP15* are also associated with fecundity and an increased incidence of dizygotic twinning (Palmer et al., 2006); premature ovarian failure has also been reported (Kovanci et al., 2007).

With respect to COH phenotypes, *GDF9* and *BMP15* alleles have been associated with stimulation outcome (Moron et al., 2006; Wang et al., 2010; Hanevik et al., 2011). In the *GDF9* gene 546G/A polymorphism, the A allele was correlated with poor COH and IVF outcomes in women with diminished ovarian reserve (Wang et al., 2010). Variant alleles of *BMP15* (−673C/T, −9C/G, IVS1+905A/G) were associated with increased follicle production in COH. Although these individual *BMP15* polymorphisms did not show strong association with the risk of OHSS, the TGGA haplotype (−673, −9, IVS + 905, Asn103Ser) was a risk factor and the CCAA haplotype was found to confer protection against OHSS (Moron et al., 2006). In addition, in a newly published study, an association between the −9 G allele and a high response to ovarian stimulation was detected (Hanevik et al., 2011).

Given the important role of these TFGβ superfamily members (*AMH*, *GDF9* and *BMP15*) in folliculogenesis and the additional data obtained from animal models, polymorphisms in these genes are promising candidates in COH outcomes, whose involvement in different responses to FSH should be investigated further. Genetic variation in other growth factors in this family, such as inhibins, follistatin, activins and other bone morphogenetic proteins could also influence the outcome of COH.

Other genes

Recent studies have revealed two new candidate genes in relation to COH/IVF outcome: that for sex-hormone-binding globulin (SHBG) and that for superoxide dismutase 2 (SOD2).

SHBG is the main transport protein for sex steroids, regulating their access to target tissues, and a role for it in local regulation of ovarian function has been suggested (Forges et al., 2004, 2005). Levels of SHBG vary among individuals, and genetic variation seems to contribute to this. A pentanucleotide repeat polymorphism $(TAAAA)_n$ in the 5′ promoter area of the *SHBG* gene (at region 17p13) has been shown to influence its transcriptional activity (Hogeveen et al., 2001), and shorter repeats correlate with higher protein levels (Ferk et al., 2007). A recent study of women undergoing ovarian stimulation demonstrated an association between $(TAAAA)_n$ shorter repeat polymorphism and a higher number of small follicles as well as higher follicular SHBG concentrations (Hatzi et al., 2010). It was concluded that this polymorphism may influence follicle size (and maturation) during follicle recruitment in IVF at early stages (Hatzi et al., 2010).

The other candidate gene, that for SOD2, is a mitochondrial enzyme that catalyses the detoxification of superoxide free radicals within mitochondria, hence playing a crucial role in protection against damage (Beyer et al., 1991). In the *SOD2* gene (at 6q25), the Ala16Val polymorphism has been identified, where the variant Val allele produces a conformational change in the protein structure that may decrease the efficiency of transport into mitochondria (Hiroi et al., 1999). As COH therapy produces a disturbance in the oxidant−antioxidant balance, causing the serum to be less protected against oxidants (Aurrekoetxea et al., 2010), *SOD2* gene variants may have a role in different responses to COH. Interestingly, in a recent study, the *SOD2* AlaAla genotype was associated with a higher number of total and fertilized oocytes following COH, and it was an independent predictor of pregnancy (Ruiz-Sanz et al., 2010).

This candidate gene approach, where marker-by-marker analysis is conducted, has limited power in the identification of novel biomarkers, as it requires a set of previously known or suspected genes. New studies on the pharmacogenetics of COH, applying novel high-throughput techniques, are on their way.

Genome-wide association studies

Genome-wide association studies (GWAS) are powerful tools used to identify genetic factors that influence drug responses, with no a priori assumptions. From the effect of individual variation on drug response, i.e. pharmacogenetics, GWAS has given rise to pharmacogenomics: whole genome impact on drug responses. A genome-wide pharmacogenomic approach has already proved useful in predicting responses to some treatments, such as in multiple sclerosis and HIV (Byun et al., 2008; Bertrand et al., 2009). A website reporting all GWAS has been created (www.genome.gov/GWAStudies), where currently 905 publications and 4514 SNPs have been reported. For instance, there are seven GWAS in the database focusing on the age at menarche and/or age at menopause.

In COH, all the studies so far have involved analysis of the effect of single genetic markers on exogenous gonadotrophin administration. Now a new study represents the first genome-wide approach in the investigation of pharmacogenomic influence on ovarian stimulation

(van Disseldorp *et al.*, 2011). This preliminary study was conducted in a homogeneous Caucasian population, albeit in a relatively small group (*n* = 102), and no SNPs were identified that were significantly associated with parameters reflecting oocyte yield, ovarian sensitivity to COH or oocyte quality after IVF (van Disseldorp *et al.*, 2011). The hindrance of a small sample size in GWA studies is that with the required multiple correction testing, significant findings concerning genes with smaller effects could be obscured. GWAS is a sensitive method for detecting the multiple small gene effects often found in complex traits, requiring relatively big study groups (Overbeek and Lambalk, 2009a). Additionally, through analysis of the whole genome, a GWAS approach would enable identification of new genes that influence drug responses in COH. Further studies in larger cohorts of IVF-treated women are clearly needed, in order to assess more subtle genetic effects with a greater power and to find new biomarkers in COH.

Conclusions and future perspectives

The scope of this systematic review was to overview the literature addressing the influence of common genetic variations on COH outcome in IVF treatment.

Previous studies have focused on one or a few polymorphisms in genes known to be involved in ovarian stimulation in IVF treatment (Table II and Supplementary data, Table SI), including those for FSH receptor, estrogen receptors, aromatase and other genes in FSH signalling. In the last 2 years a wave of new pharmacogenetic studies in IVF patients has occurred, demonstrating promising COH outcome biomarkers among genes such as *LHB*, *SHBG*, *SOD2* and folate-metabolizing pathway and TGFβ superfamily genes (Alviggi *et al.*, 2009a, b; Hatzi *et al.*, 2010; Ruiz-Sanz *et al.*, 2010; Wang *et al.*, 2010; Hanevik *et al.*, 2011; Laanpere *et al.*, 2011). Recent studies also provide novel molecular insights into the role of the FSH receptor in COH, including variation in mRNA levels (Cai *et al.*, 2007). Further, the first genome-wide analysis in IVF patients was recently published (van Disseldorp *et al.*, 2011). Although data are accumulating with evidence suggesting that the ovarian response to COH is mediated by various polymorphisms, the optimal biomarkers and the efficacy of the tests still remain to be evaluated.

The genetic marker *FSHR* Asn680Ser is by far the most studied in relation to ovarian stimulation, and is practically the only marker that could currently be explored in the clinical field in terms of testing its utility in the routine analysis of women undergoing COH protocols. However, the specificity and sensitivity of a single genetic marker will be too low for it to be employed as a predictive biomarker (Moron *et al.*, 2007). Therefore, in order to develop real predictive genetic testing panels for COH outcome, it is important to increase the number of validated markers (for instance, polymorphisms in *ESR1*, *CYP19A1*, *MTHFR*, *GDF9*, *BMP15*, *SHBG* and other genes, including novel biomarkers such as *FSHR* splice variants or mRNA levels), and to analyse interaction among them. Indeed, a multilocus rather than a marker-by-marker statistical analysis has been shown to be a promising predictive tool, as a combined model of *FSHR-ESR1-ESR2* variants predicted 10–15% of poor responders to rFSH in IVF treatment (de Castro *et al.*, 2004).

Recent advances in molecular biology, the publication of the human genome and the development of high-throughput technologies, make it possible to expand our knowledge to identify additional variants that modify the effects of exogenous gonadotrophin administration in COH protocols. For instance, variation in *FSHR* mRNA levels and splicing variants has been demonstrated to predict COH outcome (Cai *et al.*, 2007; Gerasimova *et al.*, 2010). Further, in addition to the first proof-of-principle GWAS in women undergoing COH (van Disseldorp *et al.*, 2011), this genome-wide analysis tool involving thousands of anonymous SNPs could identify new candidate genes and interactions. In addition, high-throughput sequencing strategies are waiting to be employed in COH-pharmacogenomics research.

Although, to date, some results obtained by different research groups are promising, research involving useful predictive panels of genetic markers in COH is still in its infancy. The obstacles in pharmacogenetic studies applied in IVF-treated women so far are the small sample sizes and the lack of independent replication of the results. It has been calculated that, when assuming a common SNP variant to explain 3.4% of COH response variation, 1000 samples are needed in order to reach 80% power to identify such a variant, with a *P*-value of $< 5 \times 10^{-7}$ (assuming that the SNP has a minor allele frequency of 26%; van Disseldorp *et al.*, 2011). In addition to the large sample size, these genetic association studies should focus on optimal selection of study samples, which are controlled for ethnicity and geographic origin in order to minimize phenotype heterogeneity (McCarthy *et al.*, 2008). A well-designed study requires also power calculations for the sample size optimization and optimal marker selection (Kyzas *et al.*, 2007). Therefore, there is clearly a need for a series of large and well-designed clinical studies, taking into account different COH protocols, in order to be able to identify markers useful for adjusting the doses during COH treatment.

In addition to the small sample sizes in association studies, the fact that studies with positive associations are more favourably published is a hindrance to drawing the real picture. Publication of association studies with negative findings should be encouraged and more accepted. Isolated statistical significance is not necessarily a guarantee for association; neither does a lack of normal significance exclude the possibility of a true association (Wunsch *et al.*, 2005). Further, even though a polymorphism may show no association with COH outcome, such a variant could have an impact on cumulative IVF outcome, rather than on per single treatment cycle. The predictive value of genetic variation on cumulative IVF treatment outcomes has not been thoroughly evaluated so far and needs further investigation.

Ideally, pharmacogenetic/genomic studies will eventually lead to an era in which an individual's DNA sequence is regarded as an integral determinant of drug therapy (Giacomini *et al.*, 2007). Probably, the most predictive COH test will involve a combination of phenotypic (patient's age, weight, ovarian morphology, basal FSH level and other blood serum ovarian reserve markers) and genetic markers, which will be applied in routine diagnostic tests before starting ovulation stimulation in order to be able to predict and achieve an 'appropriate' response, i.e. obtain a balance between efficacy (retrieval of an adequate number of oocytes) and risks (avoidance of OHSS and cycle cancellation because of an inadequate response) while maximizing the chance of pregnancy (Fauser *et al.*, 2008; Overbeek and Lambalk, 2009b).

The relatively slow uptake of pharmacogenetics in clinical practice is a result of several factors, including the (additional) cost of genetic tests, the need for specific laboratory equipment, the lack of proof of the utility of pharmacogenetic tests, difficulty in interpretation of test results and a lack of knowledge amongst health professionals and also patients regarding pharmacogenetics (Alfirevic *et al.*, 2010). Nevertheless, in today's genomic era, where the first genomic diagnostic tools in reproductive medicine have been developed (Altmäe and Salumets, 2011; Diaz-Gimeno *et al.*, 2011), there is the opportunity and hope to make individualized ovarian stimulation protocols a reality.

Supplementary data

Supplementary data are available at http://humupd.oxfordjournals.org/.

Authors' roles

S.A. was involved in the study design, conducting literature review, the analysis of identified data and manuscript drafting; O.H. took part in the study design and manuscript drafting; A.S.-E. played a role in manuscript drafting; A.S. was involved in the study design, analysis of identified data and manuscript drafting.

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Conflict of interest

The authors declare that there is no conflict of interest as defined by the guidelines of the International Committee of Medical Journal Editors (ICMJE; www.icmje.org).

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