FSH receptor polymorphisms and ovarian function

Les polymorphismes du récepteur FSH et la fonction ovarienne

Abstract. Follicle-stimulating hormone (FSH) is a key player in human reproduction. FSH activates the FSH receptor (FSHR) on granulosa cells in the ovary. The ovarian effects of endogenous and exogenous FSH can be modulated by polymorphisms of the FSHR gene. To date around 1,800 polymorphisms of the FSHR gene have been reported. This paper reviews the role of different polymorphisms for ovarian function, particularly in conjunction with the use of exogenous FSH in the course of controlled ovarian stimulation. There is currently only one polymorphism of the FSH receptor gene (codon 680) for which a sufficient number of studies have consistently identified a significant association to ovarian function. Polymorphisms of the FSHR gene may be used as markers to predict differences in FSHR function and ovarian response to FSH and may ultimately lead to stimulation protocols that are carefully personalized to each woman’s individual needs.

Key words: FSH, receptor, polymorphisms, reproduction, ovarian stimulation

Résumé. La follicle-stimulating hormone (FSH) est un acteur clé en reproduction humaine. La FSH active son récepteur (FSHR) au niveau des cellules de la granulosa de l’ovaire. Les effets ovariens de la FSH endogène et exogène peuvent être modulés par des polymorphismes du gène du FSHR. À ce jour, environ 1 800 polymorphismes du FSHR ont été rapportés. Cet article passe en revue le rôle de différents polymorphismes sur la fonction ovarienne, en particulier ceux interagissant avec l’utilisation de la FSH exogène dans le cadre de l’hypertimulation ovarienne contrôlée. Il n’y a, à ce jour, qu’un seul polymorphisme du FSHR (codon 680) pour lequel un nombre suffisant d’études ont identifié de façon cohérente une association significative avec la fonction ovarienne. Les polymorphismes du gène du FSHR peuvent être utilisés comme marqueurs prédictifs de différence dans la fonction du FSHR et de la réponse ovarienne à la FSH et pourraient in fine conduire à des protocoles de stimulation précisément adaptés au besoin individuel de chaque femme.

Mots clés : FSH, récepteur, polymorphisme, reproduction, stimulation ovarienne

Follicle-stimulating hormone (FSH) is a key player in human reproduction [12, 35, 36]. FSH stimulates growth and maturation of antral follicles and estradiol production by granulosa cells. It induces the synthesis of the androgen-converting enzyme aromatase, and plays a crucial role in the secondary follicle recruitment and selection of the dominant follicle [15]. The effects of endogenous and exogenous FSH on the ovary can be modulated by mutations or polymorphisms of the FSH receptor (FSHR) gene [33, 35]. Homozygous, inactivating mutations of the FSHR gene result in hypergonadotropic ovarian dysgenesis with primary ovarian failure [2]. Mutations, causing loss of binding specificity for FSH, result in human chorionic gonadotropin (hCG)-induced ovarian hyperstimulation syndrome (OHSS) [28, 37, 41]. However, mutations in the FSHR gene are rare, while sequencing showed that the FSHR gene contains over 1,700 single-nucleotide polymorphisms (SNPs). Only a small number of the SNPs of the FSHR are located in protein coding regions (exons), and very few of these result in amino acid changes. Recently,
SNPs in the FSHR gene have received a great deal of attention as they modify hormone action and influence the outcome of ovarian stimulation during assisted reproductive techniques (ART) by a differential response to exogenous FSH administration. The clinical meaning of SNPs in the FSHR gene for ovarian function, especially during controlled ovarian hyperstimulation (COH), is described in this review.

FSH and its receptor

The pituitary gonadotropin FSH consists of two subunits, the α- and the β subunit. FSH exerts its trophic and stimulatory effects on gametogenesis by binding to a specific receptor that is located in women exclusively on the surface of granulosa cells. FSHR belongs to the family of G protein coupled receptors and consists of 678 amino acids, grouped in an extracellular domain, an intracellular domain and seven transmembrane domains. Following the binding to FSH, the main signal-transduction mechanism involves activation of protein-kinase A (PKA). PKB and PKC are also involved [29, 35, 43]. The interaction is exerted through the N-terminal extracellular domain of the FSHR. It consists of 349 amino acids and is formed as a horseshoe-shaped pocket for hormone binding. Binding specificity is mediated by amino acids in both the α- and β-subunit. Binding to the receptor involves conformational changes in the hormone, with the α-subunit loops adjusting their shape to reach optimal interaction. The transmembrane domain consists of 264 amino acids and is formed by seven hydrophobic α-helix sequences of 20 to 25 amino acids each, connected by alternate extracellular and intracellular loops. The C-terminal intracellular domain is constituted of 65 amino acids. It is rich in Ser and Thr residues that can be phosphorylated by specific intracellular kinases and mediate the transduction of the signal originated from the FSH/FSHR binding [18].

SNPs in the FSH receptor gene (FSHR)

The FSHR gene is located on chromosome 2 p21-p16 and consists of 10 exons and 9 introns. The first nine exons encode for the extracellular domain of the receptor, whereas exon 10 encodes for the C-terminal end of the extracellular domain, the entire transmembrane domain and the intracellular domain of the FSHR [35]. Exon 10 is fundamental for signal transduction, but it is not necessary for ligand binding [14].

To date around 1,800 SNPs of the FSHR gene have been reported in the National Centre for Biotechnology Information (NCBI) SNP database (http://www.ncbi.nlm.nih.gov). Only eight SNPs are located in the coding regions (exons), with the rest being intronic. One SNP is located in the 5′untranslated region of the FSHR mRNA position -29 (rs2189241). Seven of the eight SNPs within the coding region are found in exon 10 at codon positions 307, 329, 449, 524, 567, 665 and 680, and six of these result in amino acid substitution (non-synonymous). The Ala307Thr (rs6165) and the Ser680Asn (rs6166) polymorphisms are well characterized with respect to frequency and ethnic distribution. These SNPs have been confirmed to be in linkage disequilibrium (http://www.hapmap.org), i.e. linked to each other during recombination in a way that does not follow a random pattern.

The two most common SNPs in the coding region of FSHR gene occur at nucleotides 919 and 2039 in exon 10, which correspond to amino acid positions 307 and 680, respectively, of mature protein (figure 1) [19, 34]. Codon 307 is located in the hinge region of the receptor, connecting the hormone binding domain to the transmembrane domain, which is highly variable in the three glycoprotein hormone receptors (FSHR, luteinizing hormone receptor and thyroid-stimulating-hormone receptor). Codon 680 is located in the intracellular C-terminal domain in a region that also varies between the three receptors. The allelic structure at the codons 307 and 680 are claimed to affect the woman’s reproductive potential via influencing some functional properties of the FSHR. The two codons are in linkage disequilibrium generating several allelic variants at these positions, which may be found with specific frequencies inside a population. Some of these variants are quite rare, whereas the combinations of Ala$^{307}$-Ser$^{680}$ (named AS) and Thr$^{307}$-Asn$^{680}$ (named TN) are the most frequently observed in different ethnic groups, representing about 40% of all FSHR alleles worldwide [2]. The two other SNP combinations (i.e. Thr$^{307}$-Ser$^{680}$ and Ala$^{307}$-Asn$^{680}$) are possible, but account for less than 5% of the FSHR alleles.

Codon 680 SNP and COH

Ovarian stimulation is usually performed by administering exogenous FSH following pituitary down-regulation. Ovarian response to FSH, however, varies widely among women undergoing ovarian stimulation [16] with widespread clinical consequences. If ovarian response to stimulation is insufficient, the cycle may need to be cancelled. Similarly, if ovarian response is excessive, cycle cancellation may be necessary to avoid the risk of OHSS, a potential life-threatening complication. The ovarian response to gonadotropin stimulation depends primarily on the woman’s ovarian reserve [4] and is a major determinant
of the success of IVF [31]. Although chronological age is the major determinant of ovarian reserve, there is considerable individual variability in the rate of ovarian ageing [40]. Serum FSH level and antral follicle count (AFC) assessed by transvaginal sonography (TVS) are often used as test for ovarian reserve [4]. The AFC correlates with the number of oocytes retrieved and pregnancy rates and is currently considered to be the best available single predictor of ovarian stimulation [20]. Serum anti-Müllerian hormone (AMH) levels may accurately identify patients at risk of an extreme ovarian response or premature ovarian failure (POF) [5, 27]. However, studies on the predictive values of ovarian reserve assessment for the selection of the appropriate stimulation scheme and FSH dose have yielded contradictory results [11].

As the interaction between FSH and its receptor plays a key role in ovarian stimulation, a number of groups have investigated the effect of SNPs in the FSHR gene on ovarian response. SNPs in exon 10 reportedly modulate FSHR function and the ovarian response to FSH. This effect was first observed in a partly retrospective, non randomized study involving women undergoing COH for assisted reproduction. It was shown that the amount of FSH needed for COH to achieve similar peak estradiol levels is significantly lower in women who are homozygous for the Asn680 variant compared with women who are either homozygous for Ser680 or are heterozygous, indicating a lower ovarian sensitivity to FSH in vivo for the Ser680 variant [30]. This data have been corroborated in a prospective interventional study in which a randomized, controlled trial design showed a differential response of estradiol to FSH because of the SNP at codon 680 of FSHR gene. In this study, women undergoing COH were administered equal dosages of FSH resulting in significantly lower serum levels of estradiol in women homozygous for Ser680 compared with women homozygous for Asn680. This lower response is overcome by increasing the FSH dose [3]. Despite differences in estradiol levels, no significant differences were detected in either the number of follicles or retrieved oocytes, fertilization rate, cumulative embryo score and pregnancy rate. This indicates that, according to the current protocols, some women might receive too much FSH, thus putting them a risk of OHSS, which depends indirectly on excessive stimulation by FSH. Indeed, a recent retrospective association study has demonstrated that the Ser680 variant occurs significantly more often in women that develop iatrogenic OHSS, but that the Asn680 variant is associated with the severity of OHSS [7]. Therefore, women with the Asn680 genotype that are undergoing COH might be at

![Figure 1. Schematic representation of the FSH receptor protein and sites of amino acid changes due to SNPs. At codon 307 threonin (Thr, ACT) is changed to alanine (Ala, GCT). At codon 680 asparagin (Asn, AAT) is changed to serine (Ser, AGT).](image)
risk for excessive stimulation with FSH increasing their risk of severe iatrogenic OHSS.

In a clinical study monitoring menstrual cycle in women with normal mono-ovulatory cycles it could be demonstrated that during the luteo-follicular transition, serum levels of estradiol, progesterone and inhibin A were significantly lower and FSH started to rise earlier in women homozygous for the Asn680 variant. In addition, FSH levels were steadily and significantly higher during the follicular phase in this group, whereas no differences were observed between groups in estradiol and inhibin B levels and growth velocity of the dominant follicle, showing that higher levels of endogenous FSH are necessary to achieve physiological ovulation in carriers of the homozygous Ser680 genotype. Menstrual cycles were significantly longer, with a difference of about three days in these women. Thus, the homozygous Ser680 genotype results in a higher ovarian threshold for FSH, decreased negative feedback to the pituitary and longer menstrual cycle [17].

Recently it was shown in a IVF treatment group, that the clinical pregnancy rate in women who are homozygous for the Asn680 variant is significantly higher compared with those with the Ser680 genotype [22]. However, another study of similar design showed opposite results, with higher pregnancy rates in women with the Ser680 genotype [23]. These contradictory results should be interpreted with caution, and larger, well-designed and properly powered studies seem to be necessary before drawing conclusions about the effects of the FSHR genotype on pregnancy rates.

Other studies have observed that the genotype for the codon 680 polymorphism was associated with differences in basal FSH levels [13, 22, 24, 25, 38], and follicular/oocyte number [24].

In a recent clinical study it could be shown that women with a homozygous A genotype at the -29 position in the 5’ untranslated region of the FSHR undergoing COH and IVF required the highest amount of exogenous FSH for ovulation induction. Furthermore estradiol concentrations before the day of hCG administration were significantly lower and the number of pre-ovulatory follicles and retrieved oocytes were lowest in women with the homozygous A genotype. The authors suggested an association of this SNP with poor ovarian response [1].

Overall, the presented data indicate a possible role of FSHR polymorphism for successful ovarian stimulation. The Ser680 genotype occurs in up to 75% of all women undergoing IVF treatment. These women are characterized by higher basal FSH serum concentrations, the need for a higher amount of exogenous FSH and a higher risk of hypo- or hyper-response. Genotyping of the codon 680 polymorphism may therefore help to indentify a population of potential poor responders in IVF procedures before ovarian stimulation is initiated. An adaptation of the stimulation protocol designed to overcome the partial resistance to FSH response should be sufficient to improve significantly the success of IVF in these women [32]. However, randomized controlled trials are required to confirm the clinical effectiveness of such an approach.

It is important to note that studies in women with ovarian dysfunction did not find any association between FSHR polymorphisms and ovarian response to FSH; e.g. POF [6, 39]. Another study demonstrated that the Ser680 allele is significantly more frequent in women with a normal menstrual cycle and elevated FSH levels than in women with normal FSH levels [10]. Finally, an intriguing association between the Ala307-Ser680 haplotype and ovarian cancer susceptibility was reported in different populations [26, 42]. Another study group could not confirm the FSHR Ala307-Ser680 haplotype as a risk factor for epithelial ovarian cancer [21]. The authors concluded, that the modification of tumor risk may be affected by the ethnology of the patient collective, however, a possible association between ovarian cancer and FSHR polymorphisms remains to be further elucidated.

**Conclusion**

There is currently only one polymorphism of the FSH receptor gene (codon 680) for which a sufficient number of studies have consistently identified a significant association to ovarian function. Studies in women from various ethnic backgrounds with normal ovarian function demonstrate convincingly that SNPs in exon 10 of the FSH receptor gene can be used as markers to predict differences in FSHR function and ovarian response to FSH [8, 9, 13, 22, 25, 30, 38], although ovarian response is a polygentic trait and the interaction with other gene polymorphisms remains to be investigated. Strong evidence for an association could ultimately lead to stimulation protocols that are carefully personalized to each woman’s individual needs, according to the particular combination of polymorphisms inherited.

**References**


