Polymorphism of the FSH receptor and ovarian response to FSH
Polymorphisme du récepteur de la FSH et réponse ovarienne à la FSH

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Résumé

L’hormone folliculostimulante (FSH) joue un rôle crucial dans la reproduction humaine. Son récepteur (FSHR) est exclusivement localisé dans les cellules de la granulosa de l’ovaire ainsi que dans les cellules de Sertoli des testicules. Il existe deux SNP dans l’exon 10 du gène du FSHR qui entrainent la formation de deux formes alléliques d’une fréquence quasiment similaire. Au codon 307, situé dans la région charnière, se trouve ou bien une thréonine (Thr) ou bien une alanine (Ala), tandis qu’en position 680, dans le domaine intracellulaire, se trouve une asparagine (Asn) ou une sérine (Ser). Des études cliniques ont bien montré que le polymorphisme p.N680S détermine la réponse ovarienne chez des patientes soumises à un traitement inducteur de l’ovulation. Les patientes portant l’allèle Ser680 nécessitent plus de FSH pour atteindre le même taux d’estradiol comparées aux patientes porteuses de l’allèle Asn680. Une étude analysant des femmes ayant un cycle menstruel normal a révélé que le génotype Ser680/Ser680 entraîne un taux élevé de la FSH et une prolongation du cycle mensuel. Le mécanisme moléculaire responsable de la “résistance” partielle du Ser680-FSHR par rapport à la FSH est inconnu jusqu’à présent. Des expériences futures devraient contribuer à notre compréhension concernant l’effet de la FSH sur la sélection et la dominance folliculaire, et ainsi, permettre des traitements sur mesure de l’infertilité et de la préservation de la fertilité.

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Abstract

Follicle-stimulating hormone (FSH) is a key factor in human reproduction. FSH activates its receptor (FSHR) located exclusively on Sertoli cells in the testis and granulosa cells in the ovary. Two common single nucleotide polymorphisms (SNP) within exon 10 of the human FSHR gene result in two almost equally common allelic variants exhibiting threonine (Thr) or alanine (Ala) at position 307 in the hinge region, respectively, asparagine (Asn) or serine (Ser) at codon 680 of the intracellular domain. Clinical studies have demonstrated that p.N680S polymorphism determines the ovarian response to FSH stimulation in patients undergoing IVF-treatment. Patients with the Ser680 allele need more FSH during the stimulation phase to reach the serum estradiol levels of Asn680 patients. A study investigating women with normal, mono-ovulatory menstrual cycles revealed that the Ser680/Ser680 genotype leads to higher FSH serum levels and a prolonged cycle. To date, the molecular mechanism underlying the partial “resistance” of the Ser680-FSHR to FSH remains unclear. Future experiments should extend our current understanding of FSH action on follicular selection and dominance, thereby permitting novel, patient-tailored therapies for infertility and fertility preservation.

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1. Introduction

Ovarian response to stimulation therapy and the timing of follicular depletion vary substantially between individual patients. Both factors are of great importance for the performance and outcome of infertility therapy. Besides the established clinical markers, analysis of genetic variants may be of use as a prognostic tool in the near future. Follicle-stimulating hormone (FSH) is fundamental for fertility and activates its receptor (FSHR) located exclusively on granulosa cells in the ovary. Clinical studies on the p.N680S-polyorphism of the FSH receptor gene have demonstrated the homozygous Ser/Ser variant to be less sensitive to endogenous or exogenous FSH in terms of estradiol production. Prospective genotyping of FSH receptor gene have demonstrated the homozygous Ser/Ser variant to be less sensitive to endogenous or exogenous FSH in terms of estradiol production. Prospective genotyping before stimulation therapy could therefore individualise the FSH dose according to the patients’ requirements and improve the balance between adequate ovarian response and unwanted side effects. Furthermore, the identification and analysis of other candidate genes could shed light on the variable timing of follicular reserve and depletion.

Controlled ovarian hyperstimulation (COH) is an integral part of all assisted reproduction therapies. The goal is to achieve multiple follicular development in order to optimise the chances of conception by in vivo or in vitro fertilisation (IVF). Ovarian response to stimulation therapy varies substantially between individual patients and is difficult to predict. It is nevertheless of great importance for the performance and outcome of infertility therapy. Sufficient numbers of mature oocytes are a substantial requisite for high pregnancy rates, compensating for inescapable losses during follicular puncture, fertilisation, embryo development and implantation. Conversely, relative overdosage can lead to life-threatening conditions known as ovarian hyperstimulation syndrome (OHSS). In the ovary, follicular growth occurs through continuous recruitment from the primordial follicle pool up to the primary and secondary follicle. The growth of subsequent antral follicles is crucially dependent on the cyclic action of FSH.

2. FSH and its receptor

FSH is a key player in human reproduction [7,26,27]. This pituitary gonadotropin exerts its tropic and stimulatory effects on gametogenesis by binding to a specific receptor located exclusively on the surface of Sertoli cells in the testis and granulosa cells in the ovary. The FSH receptor (FSHR) is a G protein-coupled receptor and its main signal transduction mechanism involves activation of protein kinase (PK) A; however, PKB and PKC are also involved [23,27,36]. Recently, the nature of the FSH–FSHR interaction has been resolved by crystallising FSH in a complex with the extracellular domain of its receptor [9]. According to this, the extracellular FSHR domain is shaped as a slightly curved tube. FSH, consisting of an α and β subunit, binds to the inner, slightly concave face of the tube in a hand-clasp fashion. Binding specificity is mediated by amino acids in both hormone subunits. Binding to the receptor involves conformational changes in the hormone, with the α subunit loops adjusting their shape to reach optimal interaction. The extracellular FSHR domain occupied by the hormone forms dimers in the crystal, suggesting that receptor dimerisation may participate in signal transduction. Dimerisation of the FSHR has also been demonstrated by biochemical approaches [9,15,34].

3. Single nucleotide polymorphisms (SNP) in the FSH receptor gene (FSHR)

Several SNP of the FSHR are known. To date, the National Centre for Biotechnology Information (NCBI) SNP database indicates 744 SNPs in the FSHR gene (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=DisplayFiltered&DB=snps), of which only eight are located in the coding region (exons), with the rest being intronic. One SNP is located in the 5’ untranslated region of the FSHR mRNA at 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 are non-synonymous. The p.T307A (rs6165; heterozygosity: 0.499) and the p.N680S (rs6166; heterozygosity: 0.470) polymorphisms are those best characterised with respect to frequency (approaching 50%) and ethnic distribution (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=6166) and have been confirmed to be in linkage disequilibrium by the hapmap project (http://www.hapmap.org/cgi-perl/gbrowse/hapmap/hapmap_B35/).

Exon 10 encodes the C-terminal end of the extracellular domain, the entire transmembrane domain and the intracellular domain of FSHR [27]. Exon 10 is fundamental for signal transduction but is not necessary for ligand binding. The transmembrane domain, however, might make contact with the hormone bound to the extracellular domain, in particular to the loops L1 and L3 of the α subunit [10]. The two most common SNP in exon 10 are found at nucleotide positions 919 and 2039 (numbered according to the translation start codon with ATG as “1”) corresponding to amino acid position 307 and 680, respectively, of the mature protein (Fig. 1) [11]. Codon 307 is located in the hinge region of the receptor, connecting the hormone binding domain to the transmembrane domain, which is highly variable among the three glycoprotein hormone receptors, i.e. the FSHR, luteinising hormone receptor (LHR) and the thyroid-stimulating hormone receptor. The corresponding position is not conserved in the other glycoprotein hormone receptors. Codon 680 is in the intracellular C-terminal domain in a region which is also variable between the three glycoprotein hormone receptors. No special role for the amino acids occupying position 307 and 680 is known so far. The two SNPs within exon 10, in linkage disequilibrium, result in two major, almost equally common allelic variants in the Caucasian population: Thr307-Asn680 and Ala307-Ser680 [29] with the Ala307-Ser680 variant representing about 40% of all FSHR alleles worldwide. The other two SNP combinations (i.e. Thr307-Ser680 and Ala307-Asn680) are possible, but constitute less than 5% of the FSHR alleles.

Studies in women with normal ovarian function demonstrate that SNPs in exon 10 modulate FSHR function and the ovarian response to FSH. This effect was first observed by our group in a partly retrospective, non-randomised study involving women undergoing COH for assisted reproduction. We showed that the amount of FSH needed for COH to achieve similar peak oestradiol levels was significantly lower in women with the Ser/Ser allele variant (group I, n = 24) compared to the Asn/Asn allele variant (group III, n = 44) of the FSH receptor (lower panel: *significant difference between groups I and III). This difference in ovarian response could be overcome by increasing the daily FSH dose from 150 to 225 U/day (upper panel: *significant higher total FSH dose) in women with the Ser/Ser allele variant (group II, n = 25); lower panel: no significant difference between groups II and III]). From: Behre et al.; Pharmacogenet Genomics, 2005; 15:451–456.

Fig. 2. Total FSH dose (mean ± S.E.M., upper panel) and oestradiol levels (lower panel) in three study groups. Serum levels of oestradiol before ovulation induction were significantly lower in women with the Ser/Ser allele variant (group I, n = 24) compared to the Asn/Asn allele variant (group III, n = 44) of the FSH receptor (lower panel: *significant difference between groups I and III). This difference in ovarian response could be overcome by increasing the daily FSH dose from 150 to 225 U/day (upper panel: *significant higher total FSH dose) in women with the Ser/Ser allele variant (group II, n = 25); lower panel: no significant difference between groups II and III]. From: Behre et al.; Pharmacogenet Genomics, 2005; 15:451–456.

In another study [13,14], a non-randomised trial, normogonadotropic anovulatory women who failed to ovulate or conceive after clomiphene citrate administration were treated by low-dose FSH for ovulation induction [20]. No significant difference in the FSH dose needed for mono-follicular development was detected between women with different allele variants of the FSHR. It appears, therefore, that so far the differential oestradiol response caused by different FSHR allele variants is more prevalent compared with normal controls [30]. No different allelic distribution was found in women with polycystic ovary syndrome, the Asn/Ser allele variant was more prevalent compared with normal controls [30]. Moreover, other genetic factors such as polymorphisms in the ER1 and ER2 genes [5] as well as in the antimüllerian hormone (AMH) and AMH type II receptor genes [17] are involved in modulating ovarian sensitivity to FSH. Most recently it was shown that in women undergoing IVF-treatment the clinical pregnancy rate in Asn/Asn women is significantly higher compared to Ser/Ser women [16]. However, another study showed opposite results, with higher pregnancy rates in women with the Ser/Ser genotype using a similar study design [18]. These contrasting data should be interpreted with caution and larger, well-designed and properly powered studies should be conducted before drawing conclusions about the effects of the FSHR genotype on pregnancy rate.

Studies in women with pathological ovarian function did not find any association between FSHR polymorphisms and premature ovarian failure [2,31]. In Japanese women with polycystic ovary syndrome, the Asn/Ser allele variant was more prevalent compared with normal controls [30]. No different allelic distribution was found in women with polycystic ovary syndrome in the UK [2] and Singapore [33]. Similarly, no association could be found between FSHR polymorphisms and twinning [13,14]. In a non-randomised trial, normogonadotropic anovulatory women who failed to ovulate or conceive after clomiphene citrate administration were treated by low-dose FSH for ovulation induction [20]. No significant difference in the FSH dose needed for mono-follicular development was detected between women with different allele variants of the FSHR. It appears, therefore, that so far the differential oestradiol response caused by different FSHR allele variants is more prevalent compared with normal ovarian function. Another study demonstrated that the Ser<sup>680</sup> allele is significantly more frequent in women with normal menstrual cycles and elevated
FSH levels compared to those with normal FSH levels, corroborating the idea of a higher FSH threshold [6]. An intriguing association between the Ala<sup>307</sup> - Ser<sup>680</sup> variant and ovarian cancer susceptibility was recently reported [35].

5. Prognostic value of FSH receptor genotyping in COH

We performed a prospective interventional randomised controlled study in which we showed a differential estradiol response to FSH caused by the SNP at codon 680 of the FSHR gene. In this study, the same FSH dose for COH resulted in significantly lower serum levels of estradiol in women with the Ser/Ser allele variant at position 680 compared to women with the Asn/Asn allele variant. This lower response could be overcome by increasing the FSH dose [1] (Fig. 2). Despite differences in estradiol levels, no significant differences were detected in the number of follicles or retrieved oocytes, fertilization rate, cumulative embryo score or pregnancy rate, suggesting that, according to the current protocols, FSH might be overdosed in individual women, thus putting them at risk for OHSS, which indirectly depends on excessive FSH stimulation. Indeed, a recent retrospective association study by another group demonstrated that the FSHR Ser<sup>680</sup> variant was significantly more often represented in women developing iatrogenic OHSS but the Asn<sup>680</sup> allele was significantly associated with the severity of OHSS [3]. Therefore, women with the Asn<sup>680</sup> genotype undergoing COH might be at risk for excessive stimulation with FSH, possibly increasing the risk of severe iatrogenic OHSS. This study, however, was based on rather limited number of cases and remains an isolated observation awaiting confirmation.


Another study involved menstrual cycle monitoring in women with normal, mono-ovulatory cycles. We were able to show that during the luteo-follicular transition, serum levels of estradiol, progesterone and inhibin A and inhibitin B were significantly lower and FSH started to rise earlier in women homozygous for the Ser<sup>680</sup> allele compared with women homozygous for the Asn<sup>680</sup> FSHR (Fig. 3). 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, inhibin B and growth velocity of the dominant follicle, showing that higher levels of endogenous FSH are necessary to achieve physiological ovulation in carriers of the Ser<sup>680</sup>/Ser<sup>680</sup> genotype. Menstrual cycles were significantly longer, with a difference of about 3 days in these women. Thus, this study demonstrated that the FSHR Ser<sup>680</sup>/Ser<sup>680</sup> genotype results in a higher ovarian threshold for FSH, decreased negative feedback to the pituitary and longer menstrual cycle [12].


The molecular mechanism of the Ser<sup>680</sup>-FSHR “resistance” to FSH is completely unclear. In vitro studies based on transiently transfected COS7 and HEK cells failed to demonstrate any difference between different SNPs in FSH binding and signal transduction both in terms of cyclic adenosine monophosphate (cAMP) and inositol triphosphate 3 (IP<sub>3</sub>) stimulation [28,30]. FSH-dependent events in granulosa cells, such as progesterone and estradiol production, are differentially regulated by poorly understood mechanism(s) not involving (or involving only in part) cAMP production and PKA stimulation. Some nuclear transcription factors such as the liver receptor homolog-1 (LRH-1) and possibly steroidogenic factor-1 (SF-1) or the dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome 1 (DAX-1) are proposed to be involved in the differential control of estrogen and progesterone production by granulosa cells [25]. However, no differences are seen when cAMP (i.e. the earliest measurable effect of FSH stimulation in the signal transduction cascade) is measured after stimulation of the two FSHR variants [28,30]. Future studies, possibly in human granulosa cells, should aim to ascertain whether the two variants activate different signal transduction pathways downstream of cAMP/IP3 and/or whether quantitative differences are observed. For example, the SNP at codon 680 results in an amino acid substitution involving a serine (i.e. a potential site of phosphorylation) in a region important for receptor recycling [19]. The C-terminal truncation of the human FSHR involving the amino acid at position 680 results in rerouting of the internalised receptor to lysosomes and increased FSH-induced downregulation without affecting cAMP or IP3 production [19]. It should be considered that, in vivo, receptors are usually activated at a low level of occupancy. If the Ser<sup>680</sup> FSHR protein is constitutively less present at the cell surface, both promoter activity and intracellular fate of the receptor protein could play a role in determining quantitatively lower stimulation in response to FSH, an issue rather difficult to address in vitro.

Alternatively, other factors not directly related to the FSHR polymorphism may be responsible for the observed effect and should be considered. For example, it is now well established...
that ovarian follicle development is crucially dependent on members of the transforming growth factor β superfamily and that FSH and Smad homologue (Smad)-3 interact in the downstream signalling of the FSHR in the mouse ovary. Likewise, several other factors such as the insulin-like growth factor system are known to modulate intraovarian FSH action by autocrine or paracrine mechanisms. In this respect, the 680 polymorphism of the FSHR could represent only a marker of some other, still unrecognized factor in linkage disequilibrium which could be responsible for the effect observed in vivo.

8. Conclusions and outlook

The importance of the FSHR genotype for ovarian response in the physiological and therapeutic setting has clearly been demonstrated in vivo. The molecular mechanism of FSHR variant activity is now the key to understanding follicular selection and dominance in the human and to design novel, patient-tailored therapeutic approaches, not only to ovarian stimulation, but also to infertility and, possibly, fertility preservation, e.g. after chemo- or radiotherapy for cancer or related to age. Should, for instance, a particular cellular pathway be preferentially stimulated by one FSHR variant, this might be exploited for prognostic and therapeutic purposes and selectively designed agonists/antagonists. These aspects should be investigated in the future by comparing the effects of the two FSHR variants in human granulosa cells. Meanwhile the FSHR genotype should be considered, together with age and parameters of ovarian reserve, among the factors influencing ovarian response in COH, thereby helping the clinician to select the proper FSH starting dose for safe and effective multiple follicular growth stimulation.

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