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Isoforms and single nucleotide polymorphisms of the FSH receptor gene: implications for human reproduction

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The FSH receptor shows three single nucleotide polymorphisms (SNPs), one in the promoter and two in exon 10. In addition, the FSH receptor mRNA undergoes extensive alternative splicing. While no physiological role for the SNP in the promoter and for alternative spliced isoforms has been demonstrated so far, the SNPs in exon 10 result in four discrete allelic variants characterized by the amino acid combinations Thr^{307}-Asn^{680}, Ala^{307}-Ser^{680}, Ala^{307}-Asn^{680} and Thr^{307}-Ser^{680}. Several studies have demonstrated that the first two allelic variants are very frequent (~60 and 40% respectively) in the Caucasian population. The rarer Ala^{307}-Asn^{680} and Thr^{307}-Ser^{680} variants are much less frequent (<5%) in the Chinese. In males the FSH receptor variants are not related to testicular volume, serum FSH or serum inhibin B levels. The two most common receptor variants transiently transfected in COS-7 cells displayed similar functional characteristics. Frequency distribution of the two polymorphisms in normal women and patients with polycystic ovarian syndrome or premature ovarian failure is still under investigation. The homozygous Ala^{307}-Ser^{680} variant seems to be associated with significantly higher basal serum FSH levels and with a higher amount of FSH required for ovarian stimulation in women undergoing assisted reproduction. This suggests that the FSH receptor genotype can influence the ovarian response to FSH stimulation. The presence of SNPs in the FSH receptor gene capable of modifying FSH action paves the way for future patient-tailored, genotype-based hormone therapies.

Key words: FSH receptor/infertility/ovary/polymorphism/testis

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Introduction

FSH is a key player in human reproduction (Simoni and Nieschlag, 1995; Nieschlag *et al.*, 1999). This gonadotrophin exerts its trophic 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 is a G protein-coupled receptor and its main signal transduction mechanism involves activation of adenylate cyclase and elevation of intracellular cyclic (c)AMP (Simoni *et al.*, 1997). It has been suggested that other signal transduction pathways are also involved in FSH action. These include stimulation of intracellular calcium (Gorczynska *et al.*, 1994), activation of the mitogen-activated protein kinase (MAPK) pathway (Crépieux *et al.*, 2001; Seger *et al.*, 2001) and the stimulation of inositol triphosphate (IP_3) production (Tena-Sempere *et al.*, 1999). However, the physiological relevance of these pathways remains to be determined.

With its molecular mechanism still poorly understood, FSH plays an irreplaceable role in determining proliferation and differentiation of its target cells, thereby indirectly sustaining oocyte maturation and spermatogenesis. Descriptions of cases of naturally occurring mutations of the FSH receptor have highlighted the role of this gonadotrophin in male and female gonadal function (Gromoll *et al.*, 1996c; Simoni *et al.*, 1997; Themmen and Huhtaniemi, 2000), providing further evidence that when FSH action is disrupted, spermatogenesis is impaired and follicular maturation is impossible.

Following the description of the first mutation of the FSH receptor (Aittomäki *et al.*, 1995) the search for mutations in

several conditions of male and female infertility began. It was soon evident that, while naturally occurring mutations are extremely rare, the FSH receptor and its promoter bear some very common single nucleotide polymorphisms (SNPs) which seem to be important in determining the response to FSH stimulation (Perez Mayorga *et al.*, 2000). In this article, we will review the actual knowledge of the pathophysiological role of polymorphic FSH receptor variants in gonadal function.

Impact of SNPs on reproduction

With increasing knowledge of the human genome and its sequence it became evident that genetic variations due to SNPs are frequent in hormones and receptors of the hypothalamic–pituitary–gonadal axis (Cargill *et al.*, 1999; Gray *et al.*, 2000, Taylor *et al.*, 2001). In general, the incidence of such polymorphisms within a given gene ranges from 15 to 50% in the normal population, indicating that such genetic changes do not have pronounced effects on reproduction because otherwise, evolution would have exerted deleterious effects on them. However, it is likely that such genetic changes, as single SNPs or in various combinations, are capable of modifying or fine tuning endocrine feedback systems and hormone action, resulting in variable inter-individual reproductive performance ranging from fully functional gametogenesis to infertility.

As an example of genetic variations in a reproductive hormonal system, two common genetic variants in the β chain of LH have been described that alter the biological properties of the molecule (Lamminen and Huhtaniemi, 2001). One variant shows higher in-vitro bioactivity but has a shorter half-life in the circulation when compared with the 'wild-type' hormone. Expression of this variant can result in alteration of ovarian steroidogenesis and delayed progression of puberty depending on the hetero- or homozygous status (Raivio *et al.*, 1996). Moreover, studies have shown that the mRNA expression of the LH variant is influenced by polymorphisms in the core promoter region of the LH β subunit gene. The result is higher basal promoter activity of the variant LH β and a different response to hormonal stimulation compared with the wild-type LH β gene (Jiang *et al.*, 1999).

These studies demonstrate that polymorphisms can exert their effects via at least two mechanisms. One is by changing the biochemical properties of a certain gene product directly, and the other is by acting at the transcriptional level by changing the activity of a promoter. Since the gonadotrophins FSH and LH play such a crucial role in gonadal regulation and since the genes for FSH and LH and their receptors are highly conserved throughout evolution, it is reasonable to expect that seemingly minor alterations in the exon or in the promoter regions of the genes could have profound consequences on gonadal function, as is indeed the case.

The FSH receptor gene

The FSH receptor can be divided into three regions: the extracellular domain, a transmembrane region and the intracellular domain. The gene has been assigned to chromosome 2 p21 and spans 54 kbp (Rousseau-Merck *et al.*, 1993; Gromoll *et al.*, 1994b, 1996a). It consists of 10 exons and nine introns. Exons 1–9 encode the extracellular domain, while the C-terminal part of the extracellular domain, the transmembrane domain and the intracellular domain all are encoded by exon 10. This genomic structure very much resembles the genomic organization of the closely related LH and thyroid-stimulating hormone receptor genes, leading to the hypothesis that all three genes derived from one ancestral precursor by gene duplication.

The promoter of the FSH receptor gene in the rat, mouse and human is member of a class of promoters lacking a conventional TATA and CCAAT box and that have multiple transcriptional start sites. RNase protection assays and primer extension mapped the transcriptional start site in the human to position –99 relative to the start of translation, while studies on the rat gene identified two start sites at positions –80 and –98 relative to the translational start codon (Gromoll *et al.*, 1994a; Goetz *et al.*, 1996). The transcriptional start sites of the human FSH receptor mRNA are identical in the testis and ovary. Presently, little is known about transcriptional control of the FSH receptor gene, but deletion analysis of the rat FSH receptor promoter identified a region between –220 and 98, relative to the first transcriptional start site, to be important for full promoter activity (Heckert *et al.*, 1998). Similarly, in the human promoter, a region between –225 to –1 relative to the translational start site is capable of inducing transcriptional initiation (Gromoll *et al.*, 1994a). To date, only a single response element has been clearly identified within this core promoter region. Studies on the promoter for the rat FSH receptor gene (Goetz *et al.*, 1996) revealed an E box (CACATG) sequence located in position –124 to –119 upstream of the translational start site that is required for full promoter function of the rat FSH receptor gene. Mutations in the E box region resulted in strongly reduced activity of the promoter. The promoters of the human, sheep and mouse FSH receptor genes also contain an E box consensus sequence, which is known to interact with a family of basic helix–loop–helix transcriptional factors. Further studies to allocate elements involved in transcriptional regulation revealed an activator protein 1 binding site at position –214 in the rat FSH receptor gene (Xing and Sairam, 2001) that cannot be found in either the human or the mouse promoter. An initiator element (InR) that is critical for positioning RNA polymerase II is conserved only in the mouse and rat. In the mouse FSH receptor promoter two potential steroidogenic factor (SF)-1 like binding sites have been allocated to the –1110 to 1548 region. Mutations thereof resulted in reduced promoter activity, demonstrating that SF-1 is involved in the regulation of FSH receptor expression (Levallet *et al.*, 2001).

Naturally occurring mutations of the FSH receptor

The first inactivating mutation of the FSH receptor was described in 1995 in some Finnish families with primary ovarian failure, the so-called 'pure gonadal dysgenesis', as a phenotype (Aittomäki *et al.*, 1995). Since this mutation was relatively frequent in the Finnish population, it was originally thought that FSH receptor mutations in general could constitute a common defect in ovulation failure and amenorrhoea. An 'ad hoc' assay for the rapid detection of mutations was developed based on hybridization of a labelled probe, but the successive application of such an assay to DNA samples obtained from subjects of different ethnic origin revealed a selective enrichment of the mutation only in the Finnish population, while it turned out to be extremely rare in

other ethnic groups (Jiang *et al.*, 1998). It was also clear that mutations must occur in homozygous or compound heterozygous states in order to produce a reproductive phenotype (Simoni *et al.*, 1997). A sporadic, heterozygous Val241Ala mutation found in one infertile man did not impair receptor function *in vitro* (Simoni *et al.*, 1999) and was judged not to be responsible for the phenotype. After the description of a few other mutations occurring naturally in sporadic cases of primary or early secondary amenorrhoea (Beau *et al.*, 1998; Touraine *et al.*, 1999; Doherty *et al.*, 2002), there is now consensus that FSH receptor mutations must be regarded as a very rare cause of infertility.

Only one naturally occurring activating mutation of the FSH receptor has been reported in a hypophysectomized man who had normal spermatogenesis under testosterone substitution despite undetectable serum gonadotrophin levels (Gromoll *et al.*, 1996b). No activating mutations of the FSH receptor have been reported in the presence of normal pituitary function, suggesting that such mutations might not result in any phenotype if the pituitary–gonadal axis is otherwise normal (Simoni, 1998).

The experience of the last decade has clearly shown that mutations of the FSH receptor are very rare. This finding is consistent with the indispensable role of FSH in human reproduction, so that mutations abolishing gonadotrophin activity are self-eliminating. However, screening of several hundreds of patients and controls world-wide led to the discovery of SNPs in the FSH receptor gene and to the subsequent study of the correlation between polymorphism and gonadal function.

FSH receptor isoforms and polymorphisms

Alternatively spliced isoforms

Cloning the FSH receptor from various animal species demonstrated the expression of several alternatively spliced mRNA transcripts both in the ovary and in the testis (Gromoll *et al.*, 1992, 1993; Kahn *et al.*, 1993, 1997; O'Shaughnessy and Dudley, 1993; O'Shaughnessy *et al.*, 1994; Rajapaksha *et al.*, 1996; Sairam *et al.*, 1996, 1997; Yarney *et al.*, 1997a,b; Kraaij *et al.*, 1998; Yaron *et al.*, 1998; Babu *et al.*, 1999; Tena-Sempere *et al.*, 1999; Song *et al.*, 2002). Therefore, it appears that the FSH receptor gene undergoes extensive alternative splicing, potentially giving rise to a number of different isoforms. By exon skipping or usage of alternative splice donor or acceptor sites, FSH receptor variants are generated in which the open reading frame is usually preserved (Gromoll *et al.*, 1992). This leads to potentially functional receptor protein isoforms. It has been speculated that such isoforms could modulate FSH action by interfering with the intracellular processing of the native receptor and receptor expression or, if they are expressed at the cell surface, by altering hormone binding affinity or by subtracting the hormone from binding to the full length receptor. However, the expression *in vitro* of FSH receptor variants lacking transmembrane domains showed altered post-translational processing and suggested that a physiological role for such isoforms is unlikely (Peterson *et al.*, 2000). In addition, the co-expression of the wild-type receptor together with alternatively spliced variants did not affect signal transduction through the full length FSH receptor (Kraaij *et al.*, 1998). Thus, a postulated modulating or dominant negative effect of such truncated variants is not confirmed by the existing in-vitro data.

The only naturally occurring FSH receptor isoform which seems to be expressed at the protein level is a growth factor type I-like FSH receptor isolated from the ovine testis, in which the typical seven-segment transmembrane domain is replaced by a one-segment transmembrane domain similar to that found in the growth factor receptors (Sairam *et al.*, 1997; Yarney *et al.*, 1997b). Such a growth factor type I-like FSH receptor has been localized in mouse granulosa cells at the protein level (Babu *et al.*, 2001), but it is not known whether such an isoform is expressed in the human gonads. In-vitro experiments using granulosa cell lines have shown that this FSH receptor isoform binds FSH, which thereby induces calcium-mediated signalling (Touyz *et al.*, 2000) and MAPK activation (Babu *et al.*, 2000), while the stimulation of the 'classical' FSH receptor would not result in such activation. Therefore, the pleomorphism of FSH signal transduction might be mediated by the expression of different FSH receptor isoforms on the cell surface and it would be interesting to investigate whether the expression of such variants is developmentally regulated and/or can account for the switch from the proliferation- to differentiation-inducing effects of FSH. However, there is no evidence at present that such a FSH receptor isoform has any clinical relevance for humans and these interesting results await confirmation by other working groups and further investigation in human gonadal tissue.

SNPs in the FSH receptor gene

Inspection of the FSH receptor gene using databases and the results of our own mutational screening programme led to the identification of several SNPs located in the promoter and coding region. In the core promoter region a frequent (>30%) G→A polymorphism is located at position –29. This position corresponds to a potential binding domain GGAA (underlined is the location of the polymorphism) for an ETS transcription factor, which is altered by the base exchange (J.Gromoll unpublished data). Future studies have to show whether this changes the DNA binding capacity for this transcription factor, thereby leading to altered receptor mRNA expression.

During the screening for FSH receptor mutations, several groups described polymorphisms in this gene and tried to correlate them with particular reproductive phenotypes. By analysing women with premature ovarian failure (POF), Whitney *et al.* described the occurrence of polymorphisms in *Pst*I and *Hind*III Southern blot digests hybridized with a FSH receptor probe and in PCR products of the 3′ region of exon 10 of the FSH receptor analysed by denaturing gradient gel electrophoresis (DGGE) (Whitney *et al.*, 1995). These polymorphisms were found to occur both in normal controls and in POF patients and were not analysed further, but the DGGE polymorphism was probably due to the single nucleotide exchange at position 2039 now well characterized, while the variation documented by Southern blot reflects some other yet uncharacterized polymorphism.

Today two SNPs, originally described by Aittomäki *et al.*, are well known (Aittomäki *et al.*, 1995). These two non-synonymous SNPs with frequencies of >30% in the normal population have been identified in the coding region of exon 10 of the FSH receptor gene. The first is located at position 919 (numbering

Table I. Frequency distribution of the FSH receptor alleles TN, AS, TS and AN in men and women

Author	TN (%)	AS (%)	TS (%)	AN (%)	Subjects (*n*)	Gender	Ethnic origin
Simoni *et al.*, 1999	60	40	0	0	86	Males	Caucasian
Conway *et al.*, 1999	60	40	0	0	51	Females	Caucasian
Tong *et al.*, 2001	61	29	5	5	236	Females	Chinese
Asatiani *et al.*, 2002	52.5	46	1.5	0	288	Males	Caucasian
Sudo *et al*, 2002	64	36	0	0	522	Males	Japanese
J.Laven *et al.* (unpublished)	34	40	20	6	227	Females	Mixed

according to the translational start codon with ATG as 1) in which A is substituted by G, changing codon 307 from threonine (ACT) to alanine (GCT). The second is located at nucleotide position 2039 in which G is replaced by A. This leads to an amino acid change at position 680 from serine (AGT) to asparagine (AAT). There are some indications that there might be more SNPs, either synonymous or non-synonymous, present in the FSH receptor gene, albeit at a frequency below 5%. Database searches for the presence of additional SNPs are hampered by the fact that all human databases are currently undergoing massive data re-arrangements, leading to frequent gene sequence changes and making a reliable statement on the presence of further SNPs in the FSH receptor gene virtually impossible. To date only the three above-mentioned SNPs have been proven by us and by others to occur in clinical samples. Concerning the two SNPs in exon 10, they can be associated in four possible allelic combinations, i.e. Thr^{307}-Asn^{680} (allele TN), Ala^{307}-Ser^{680} (allele AS), Ala^{307}-Asn^{680} (allele AN) and Thr^{307}-Ser^{680} (allele TS). As described below, the alleles TN and AS are very common, with a frequency of ~60 and ~40% respectively, while the alleles AN and TS are found only sporadically (<5%) in of populations of various ethnic origin (Figure 1, Table I).

SNPs in the FSH receptor of other animal species

It is difficult to assess the meaning of these four possible FSH receptor alleles in evolutionary terms. Looking at the FSH receptor structure in the animal species in which it has been cloned can offer some hints about the functional evolution of the receptor. As shown in Figure 1, Ala-Asn is the most frequent combination in animal species relatively close to the human. However, this amino acid combination is presently rare in the human, suggesting that human variants might have further evolved towards form(s) better suited for optimal interaction with the human hormone in order to ensure optimal reproductive fitness. In fact, the cynomolgus monkey carries the TN variant (Gromoll *et al.*, 1993). At position 680 Asn is highly conserved among the species, while Ser is found only in the human. Whether this indicates some subtle functional advantage for receptor function is presently unknown.

Concerning other animal species, the occurrence of polymorphisms has not yet been studied in detail. In the bovine, two synonymous SNPs have been allocated to positions 1506 and 1593 of exon 10 (Rahal *et al.*, 2000). Nothing is known about the frequencies of these SNPs. It is to be expected that SNPs of the FSH receptor gene are also present in other species. For example,

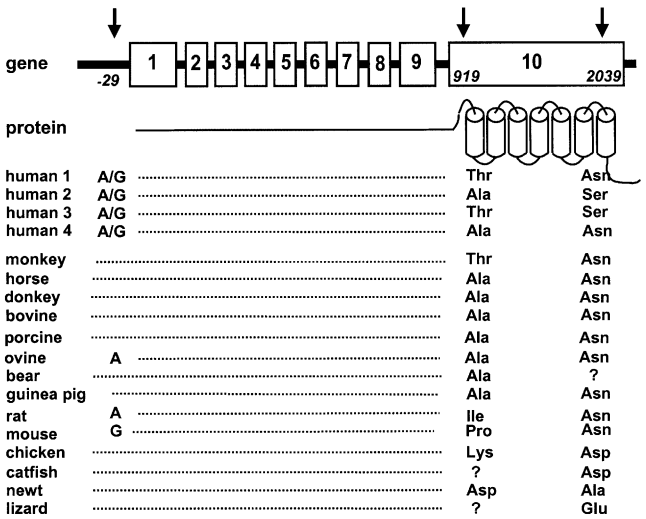

Figure 1. SNPs (arrows) in the promoter region and allelic variants of the human FSH receptor gene. A possible linkage between the promoter SNP to the other SNPs within the coding region is not yet known. The corresponding sequence in other animal species is reported below the human variants. The FSH receptor has been cloned in the cynomolgus monkey (Gromoll *et al.*, 1993), horse (Robert *et al.*, 1994), donkey (Richard *et al.*, 1997), bovine (Houde et al., 1994), pig (Remy *et al.*, 1995), sheep (Yarney *et al.*, 1993), guinea pig (NCBI gene bank accession no. AY082514), bear (Howell-Skalla *et al.*, 2000), rat (Sprengel *et al.*, 1990), mouse (Tena-Sempere *et al.*, 1999), chicken (Wakabayashi *et al.*, 1997), newt (Nakayama *et al.*, 2000), catfish (Kumar et al., 2001) and lizard (Borrelli *et al*., 2001).

a mouse SNP database is currently being established and it will be highly interesting to see whether genetic variations similar to those described in the human can also be found in this species. For the SNP –29 in the promoter region, a sequence comparison with the FSH receptor promoter from rats, mice and sheep (Sairam and Subbarayan, 1997) revealed the presence of either A (rat and ovine) or G (mouse) compared with either G or A in the human. A putative linkage of the promoter SNP to the other SNPs in the coding region is currently not known.

Detection of SNPs in the FSH receptor

Several PCR-based detection methods for the identification of SNPs in the FSH receptor gene are available. The two SNPs in exon 10 can be easily detected after PCR amplification of genomic DNA extracted from peripheral leukocytes by restriction analysis (Simoni *et al.*, 1999; Tong *et al.*, 2001; Sudo *et al.*, 2002), DGGE (Whitney *et al.*, 1995; Da Fonte Kohek *et al.*, 1998)

or single-strand conformation polymorphism (SSCP) (Tuerlings *et al.*, 1998; Simoni *et al.*, 1999). A detailed description of these techniques including sequence information for the design of oligonucleotides, staining procedures etc. has been published by our laboratory (Gromoll *et al.*, 2000).

Basically, all methods rely on the nucleotide change caused by the SNP. Such genetic variation either introduces or changes a recognition site for a restriction enzyme or leads to a conformational change in the secondary structure of a PCR amplicon covering this part of the gene. In the case of a restriction site, the most appropriate method would be restriction fragment length polymorphism analysis, whereas for the latter SSCP gel electrophoresis would be the method of choice. Common to all these methods is a somewhat cumbersome technology which requires much hands-on time, making them suitable if only a limited number of samples has to be screened.

We have recently developed a method based on the TaqMan technology (AB prism, Applied Biosystem, Weiterstadt, Germany) suitable for allelic discrimination. In allelic discrimination assays, the PCR includes two specific probes labelled with two different fluorescent dyes to differentiate the amplification of each allele. During PCR, normally producing amplicons ranging from 100 to 150 bp, each labelled probe anneals specifically to complementary sequences between the forward and reverse primer sites. Taq polymerase can cleave only probes that hybridize perfectly to the allele. Cleavage separates the reporter dye from the quencher, which results in increased fluorescence by the reporter dye. Thus, the type of fluorescent signal generated by PCR amplification indicates which alleles are present in the sample. We have established allelic discrimination assays for the SNPs located at positions –29, 919 and 2039 of the FSH receptor gene. A detailed description of these assays, including oligonucleotide sequence information, can be obtained from the authors upon request. Using this technology, ~70 samples can be screened for the presence of one SNP in ~2 h.

Frequency distribution of SNPs and allelic variants of the FSH receptor

Our group was the first to report that, at least in the Caucasian population, the two FSH receptor polymorphisms in exon 10 almost invariably occur in two combinations, giving rise to two discrete allelic variants: Thr^{307}-Asn^{680} (allele 1, TN) and Ala^{307}-Ser^{680} (allele 2, AS) (Simoni *et al.*, 1999) (Figure 1). Other combinations (alleles TS and AN) are possible, but are far less frequent in both Caucasian (Da Fonte Kohek *et al.*, 1998; Asatiani *et al.*, 2002) and Chinese populations (Tong *et al.*, 2001). Table I shows the frequency distribution of the four possible FSH receptor alleles in men (Simoni *et al.*, 1999; Asatiani *et al.*, 2002) and women (Conway *et al.*, 1999; Tong *et al.*, 2001; Sudo *et al.*, 2002). Ninety percent or more of the FSH receptor alleles are constituted by the two variants TN and AS, as was previously suggested (Da Fonte Kohek *et al.*, 1998), with the TN variant being the most frequent. The AS variant is less frequent among Singapore Chinese compared with Caucasians. In addition, the two alternative variants, TS and AN, are more frequent in Chinese individuals (Tong *et al.*, 2001) than in Caucasians (Conway *et al.*, 1999; Asatiani *et al.*, 2002). Very recently, we confirmed and extended this finding in a group of 231 women including subjects of Caucasian, Arabian, Asian and Latin American origin (J. Laven *et al.*, unpublished data). Therefore, it seems that ethnic background should be considered when comparing frequency distribution of FSH receptor polymorphic variants between infertile and control subjects.

Functional consequences of FSH receptor polymorphisms in vitro

The functional activity of the two most common FSH receptor variants, TN and AS, was investigated *in vitro* in transient transfection experiments using COS-7 (Simoni *et al.*, 1999) and HEK293 cells (Sudo *et al.*, 2002). No significant differences between the two receptor variants were observed in binding affinity and FSH-stimulated cAMP production (Simoni *et al.*, 1999; Sudo *et al.*, 2002) or IP_3 production (Sudo *et al.*, 2002). However, since important functional differences have been documented *in vivo* in normal women upon ovarian stimulation (see below), it might well be that the experimental setting chosen was not adequate to demonstrate functional changes. These experiments need to be repeated using cells which are the natural endocrine target cells for FSH, such as granulosa and Sertoli cells. In addition, the possibility that the receptor variants affect alternative signal transduction pathways (Ca^{2+}, MAPK) differently requires further consideration.

FSH receptor polymorphism and testicular function

We investigated the occurrence of FSH receptor mutations and polymorphisms in infertile men. As in anovulation, FSH receptor mutations are rare in male infertility (Leifke *et al.*, 1997; Tuerlings *et al.*, 1998; Simoni *et al.*, 1999; Song *et al.*, 2001) and the only cases reported to date were observed in five male relatives of Finnish women with primary amenorrhoea carrying the Ala189Val mutation in the extracellular domain of the FSH receptor (Tapanainen *et al.*, 1997). Intriguingly, some of these men had fathered children and had some residual ongoing spermatogenesis. Recent in-vitro data have shown that the Ala189Val mutation is compatible with some residual activity of the FSH receptor, but most of the mature protein remains trapped inside the cell when expressed in COS-7 cells or in a granulosa cell line (Rannikko *et al.*, 2002). Therefore, the observed inter-individual phenotypic variability in men with such a mutation might be due to different numbers of functional FSH receptors expressed at the cell surface.

By performing mutation screening, we and others showed that the two common FSH receptor variants, TN and AS, are similarly distributed in infertile men and normal controls, thereby excluding the possibility that one of the two isoforms is involved in the pathogenesis of idiopathic male infertility (Simoni *et al.*, 1999; Song *et al.*, 2001). In addition, we analysed some clinical parameters which might be regarded as indirect indicators of FSH receptor bioactivity *in vivo*, such as testicular volume, serum FSH levels and serum inhibin B and testosterone concentrations. FSH receptor variant-related differences could not be detected in either infertile or fertile men, suggesting that the occurrence of FSH receptor isoforms has no functional consequences in men (Simoni *et al.*, 1999; Asatiani *et al.*, 2002), at least under basal conditions. A significant difference in the

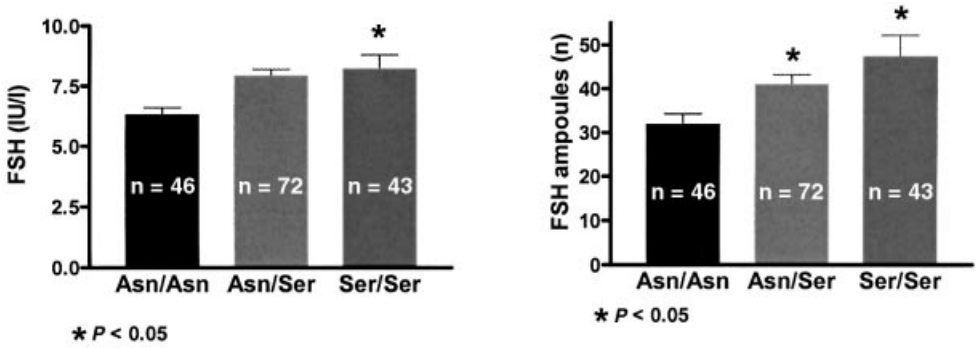

Figure 2. Serum day 3 FSH levels (left panel) and number of FSH ampoules (75 IU each) required in a controlled ovarian stimulation trial involving 161 ovulatory women subdivided into three groups according to the FSH receptor genotype at position 680 (modified from Perez Mayorga *et al.*, 2000). Perez Mayorga, M., Gromoll, J., Behre, H.M., Gassner, C., Nieschlag, E. and Simoni, M. Ovarian response to FSH stimulation depends on the FSH receptor genotype *J. Clin. Endocrinol. Metab.*, **85**, 3365–3369 (2000) copyright owned by The Endocrine Society.

frequency distribution of the SNP at position 919 between infertile men with high (>12 IU/l) and low (<12 IU/l) FSH levels has been described in Korea. However, a cut-off of 12 IU/l seems rather arbitrary and no differences were observed when infertile men with high FSH were compared with normal controls (Song *et al.*, 2001). In addition, serum FSH concentrations were significantly higher and testis volume significantly lower in infertile patients with Ala^{307}. However, no consideration was given to the SNP at position 2039 and no data from controls were reported (Song *et al.*, 2001). While data from women suggest that FSH receptor variants are indeed associated with significant differences in frequency distribution and FSH levels (see below), these contradictory data from men should be considered with caution.

Three alternatively spliced FSH receptor variants have recently been isolated from human testicular tissue: an exon 9-deleted isoform, a variant containing a short extra exon and an exon 6-deleted variant. Whether these isoforms are expressed at the protein level was not investigated. In any case, there was no association between the three variants and spermatogenic defects (Song *et al.*, 2002).

FSH receptor polymorphism and ovarian function

Several studies have attempted to correlate the frequency distribution of FSH receptor polymorphisms and ovarian function. In most studies no association between FSH receptor variant and pathological ovarian function was shown in women with POF or polycystic ovaries (PCO) compared with control subjects (Da Fonte Kohek *et al.*, 1998; Liu *et al.*, 1998; Conway *et al.*, 1999; Tong *et al.*, 2001). However, recent studies based on larger numbers of subjects identified a significant correlation between the heterozygous TN/AS genotype and PCO (Sudo *et al.*, 2002) and between the homozygous Ser at position 680 and World Health Organization type II amenorrhoea (J.Laven *et al.*, unpublished data). Therefore, it is still unclear whether the polymorphisms in exon 10 play a pathogenic or even only a permissive role in chronic anovulation. Significantly higher serum FSH levels in women with homozygous Ser at position 680 have been reported both in normal ovulatory subjects (Perez Mayorga *et al.*, 2000) and in anovulatory patients (Sudo *et al.*, 2002; J.Laven *et al.*, unpublished data), suggesting that this receptor genotype might result in a mild 'resistance' to the gonadotrophin. In any case, since FSH receptor variants appear to respond differently to FSH stimulation *in vivo* (see below), they might play some role in determining ovarian response to pharmacological stimulation with FSH.

Spontaneous dizygotic twinning

Natural multiple pregnancy resulting in dizygotic twins is often familial, suggesting a genetic predisposition. Dizygotic twinning is related to multiple ovulation, the cause of which has not been identified with certainty so far. Since follicle recruitment and maturation is FSH-dependent, it was speculated that multiple follicle development might be favoured by mutations or polymorphisms conferring increased activity to the receptor. However, no activating mutations of the FSH receptor have been described in women with repeated dizygotic twinning and the suggestion of an association with the Ser^{680} receptor variant (Al-Hendy *et al.*, 2000) has been strongly refuted (Derom *et al.*, 2001; Gromoll and Simoni 2001; Hasbargen *et al.*, 2001; Liao *et al.*, 2001). It should be kept in mind that the Ser^{680} polymorphism is rarer than the so-called 'wild-type' Asn^{680} (Table I), especially in certain ethnic groups (De la Chesnaye *et al.*, 2001) and an adequate number of control subjects should be analysed before drawing conclusions about receptor genotype–phenotype correlations. In addition, the current evidence suggests that, if any difference exists, the Ser^{680} receptor variant should have lower bioactivity than the Asn^{680} variant and that would be difficult to reconcile with multiple ovulation.

Normal ovarian function

We speculated that SNPs in the FSH receptor gene could result in subtle differences in receptor function which would become evident only in cases of receptor stimulation. We therefore analysed whether the FSH receptor polymorphism at position 680 could be responsible for the variable ovarian response observed during ovarian stimulation in assisted reproduction (Perez Mayorga *et al.*, 2000) In this study, we retrospectively selected 161 ovulatory women who underwent ovarian stimulation in our ICSI–IVF programme. The indications for ICSI or IVF were male infertility, tubal factor or both. The FSH receptor polymorphism at position 680 was determined by restriction analysis. The overall

frequency distribution of the polymorphism was similar to the data reported in the literature, with 29% Asn/Asn, 45% Asn/Ser and 26% Ser/Ser. No differences could be found among these three groups concerning number of follicles, number of oocytes and serum estradiol concentration on the day of hCG injection. This suggested that the treatment was equally successful, independent of the FSH receptor isoform. However, the number of FSH ampoules required for achieving this effect was significantly different among the groups. The number of ampoules was 31.8 ± 2.4, 40.7 ± 2.3 and 46.8 ± 5.0 for the Asn/Asn, Asn/Ser and Ser/Ser groups respectively ($P < 0.01$). In addition, the basal FSH levels on day 3 of the menstrual cycle were significantly different among the three groups: 6.4 ± 0.4, 7.9 ± 0.3 and 8.3 ± 0.6 IU/l for the Asn/Asn, Asn/Ser and Ser/Ser groups respectively ($P < 0.01$) (Figure 2). These data showed for the first time that the FSH receptor genotype is an important determinant of the ovarian response to FSH in normal ovulatory women (Perez Mayorga *et al.*, 2000). More recently, data obtained in 58 IVF–embryo transfer patients undergoing ovulation induction confirmed our finding that women with the Ser/Ser genotype require significantly more hMG before hCG administration (Sudo *et al.*, 2002).

The different allelic variants of the FSH receptor show slightly different activity *in vivo* which has not so far been demonstrated in in-vitro experiments (Simoni *et al.*, 1999; Sudo *et al.*, 2002), indicating that the choice of the appropriate in-vitro model system might be crucial for the functional characterization of the receptor variants. The mechanism of such different activity is presently unknown but might involve subtle differences in receptor expression, down-regulation and desensitization, since Ser at position 680 might become phosphorylated and thereby contribute to receptor turnover. Alternatively, the two receptor isoforms could interact slightly differently with the cAMP and/or other signal transduction pathways.

The clinical implications of this finding are very important and innovative. Since the Ser^{680} group needed significantly more ampoules of FSH to reach the same degree of ovarian stimulation, in the future it might be possible to preselect women according to their FSH receptor genotype and to design stimulation protocols tailored to their individual needs. This might lead to a decrease in the total amount of FSH needed to achieve optimal ovarian stimulation and to a reduction of side-effects such as ovarian hyperstimulation syndrome, which is indirectly dependent on the amount of gonadotrophins administered.

Outlook

The complete sequencing of the human genome has shown the existence of thousands of SNPs which determine the individuality of each human being and, most probably, his/her proneness to contract illnesses and respond to therapy. In the field of reproductive endocrinology, polymorphisms have been described in all the peptide hormones and receptors of the hypothalamo-pituitary–gonadal axis (Cargill *et al.*, 1999). Future studies should analyse how these different polymorphisms contribute to the individual hormonal profile and fertility. The Asn680Ser polymorphism of the FSH receptor represents the first example of genetic analysis which might have important therapeutic implications for fertility treatment. It is presently unknown whether FSH receptor variants play any role in men under FSH stimulation. In women, prospective studies should identify the best stimulation protocol based on the individual's genetic profile in order to reduce side-effects and costs and improve efficacy. Finally, elucidation of the mechanism by which different FSH receptor isoforms respond to different FSH isoforms might provide new therapeutic concepts in reproductive endocrinology.

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