REPRODUCTION

Focus on Gonadotrophin Signalling

An update of the pathophysiology of human gonadotrophin subunit and receptor gene mutations and polymorphisms

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Abstract

New information about mutations and polymorphisms in the genes for the gonadotrophins and their receptors has become available in the last few years. In this short review mutations and polymorphisms in gonadotrophins, their receptors and their patho-physiological effects and implications are discussed. An increasingly clear picture about the structure–function relationships of gonadotrophin action is emerging from the combining the types and the locations of the mutations with their phenotypic effects and the information about the crystal structure of these molecules.

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Introduction

The most recent comprehensive overview of mutations in gonadotrophin subunit and receptor genes and their pathophysiological consequences was published some 5 years ago (Themmen & Huhtaniemi 2000). Since the knowledge in this field has expanded considerably during the past years I will present here an update on the information in the field that has accumulated in the last 5 years since the publication of the review.

The general principles of pathophysiological consequences of mutations and polymorphisms affecting gonadotrophin action will be introduced, followed by the recent advances in the field. New polymorphisms and mutations that have been detected in the genes for luteinising hormone (LH) and follicle-stimulating hormone (FSH) and their receptors (LH receptor and FSH receptor) will be discussed in detail. In Themmen and Huhtaniemi (2000) a comprehensive overview of the topic up to the year 2000 can be found. That paper contains extensive tables and figures specifying most of the mutations and variants of the common-α, LHβ, FSHβ, LH receptor and FSH receptor genes.

Functional consequences of mutations and polymorphisms affecting gonadotrophin action

Gonadotrophin receptors

The LH and FSH receptors together with their homologue the receptors for thyrotrophin-stimulating hormone (TSH), constitute the family of glycoprotein hormone receptors, a subfamily of the large group of G protein-coupled receptors (GPCRs). The TSH and FSH receptors (gene names: TSHR and FSH receptor) both have a single ligand, TSH and FSH respectively, whereas the LH receptor binds both LH and chorionic gonadotrophin (hCG in humans), an LH analogue produced by the placenta during pregnancy in primates and equine species. The LH receptor is therefore also known as the LH/CG receptor (gene name: LHCGR). These receptors have a rhodopsin-like transmembrane (TM) domain (Palczewski *et al.* 2000, Themmen & Huhtaniemi 2000, Dias & Van Roey 2001, Ascoli *et al.* 2002, Szkudlinski *et al.* 2002) in common with the superfamily of GPCRs. In addition, they have a large N-terminal extracellular domain (ECD; 359–414 amino acid residues; homology ~40%), which conveys specific hormone binding to the receptors (Braun *et al.* 1991, Cornelis *et al.* 2001, Remy *et al.* 2001). The TMs of individual glycoprotein hormone receptors appear to be functionally interchangeable and display high sequence homology (~70%) between the different glycoprotein hormone receptors.

Based on sequence analysis and comparison with other proteins (Kobe & Deisenhofer 1993), the ECDs of the glycoprotein hormone receptors were suggested to contain nine leucine-rich repeats (LRR) flanked by N- and C-terminal cysteine-rich regions. LRR motifs have been recognized in a large number of distinct proteins (Kobe & Kajava 2001) and are thought to form a horseshoe-like structure.

Together, these repeats form a flat concave surface consisting of short β turns to which the large ligand can bind. This structure is stabilized by short, parallel α-helical stretches that connect the beta-strands. The hormone binds to the β surface using multiple contact points and the helical segments are aligned to form the outer convex side of the ECD (Jiang *et al.* 1995, Kajava *et al.* 1995, Bhowmick *et al.* 1996).

Very recently, the report of the crystal structure of a complex of FSH with the ECD of the FSH receptor has corrected and further refined the existing models (Fan & Hendrickson 2005). This report shows that the β-sheet contains 9 parallel strands and a 10th that is contributed by the N-terminal cystine repeat. Furthermore, the curvature of the hormone-binding concave β-sheet is less strong (Fan & Hendrickson 2005). In the FSH–FSH receptor crystal, FSH is positioned in the FSH receptor tube formed by the concave β-sheet with its long axis perpendicular. The structure also suggests that the FSH receptor may either dimerize upon FSH binding, or may exist as a pre-formed dimer complex without ligand. In addition, the crystal reveals detailed information on the identity of the amino acids that are involved in the interaction between FSH and its receptor. The seat belt structure of FSH (see below) is directly involved in hormone–receptor interaction (Fan & Hendrickson 2005).

Gonadotrophin subunits

The glycoprotein hormones are heterodimers, each consisting of an α and a β subunit. The α subunit is identical in all hormones, whereas the β subunit confers the specificity to the hormone (Themmen & Huhtaniemi 2000). The crystal structures of deglycosylated hCG and FSH are known (Lapthorn *et al.* 1994, Fox *et al.* 2001) and, as expected, they are very similar. Although the α and β subunits show no amino acid sequence similarity, their three-dimensional structures are remarkably similar, having an elongated shape defining the long axis with two β-hairpin loops on one side and a single hairpin loop on the other side. The β-hairpins are stabilized by disulphide bridges. The α and β subunits form an elongated slightly curved structure and are non-covalently associated in a head-to-tail orientation by means of a cystine knot motif. Thus, the gonadotrophins are members of the superfamily of cystine knot growth factors. Other members include nerve growth factor, transforming growth factor-β and platelet-derived growth factor-β. The cystine knot motif is characterized by a cluster of three cystine disulphide bonds in each subunit. The cystine knot consists of an eight amino acid ring through which an intra-subunit disulphide bond is formed. The α–β dimer is held together by a 'seatbelt' structure formed by the C-terminal amino acids of the β subunit wrapped around the α subunit and stabilized by one of the disulphide bonds. Both the association of the subunits and the binding of the heterodimer to the receptor are dependent on this seatbelt.

Extensive studies on site-directed mutagenesis have unravelled the role of a number of amino acids in the tertiary structure of this subunit, including the structures necessary for glycosylation, proper folding, heterodimerization with the β subunit and receptor binding and signal transduction of the dimeric hormone.

Mutations and polymorphisms in human luteinizing hormone and its receptor
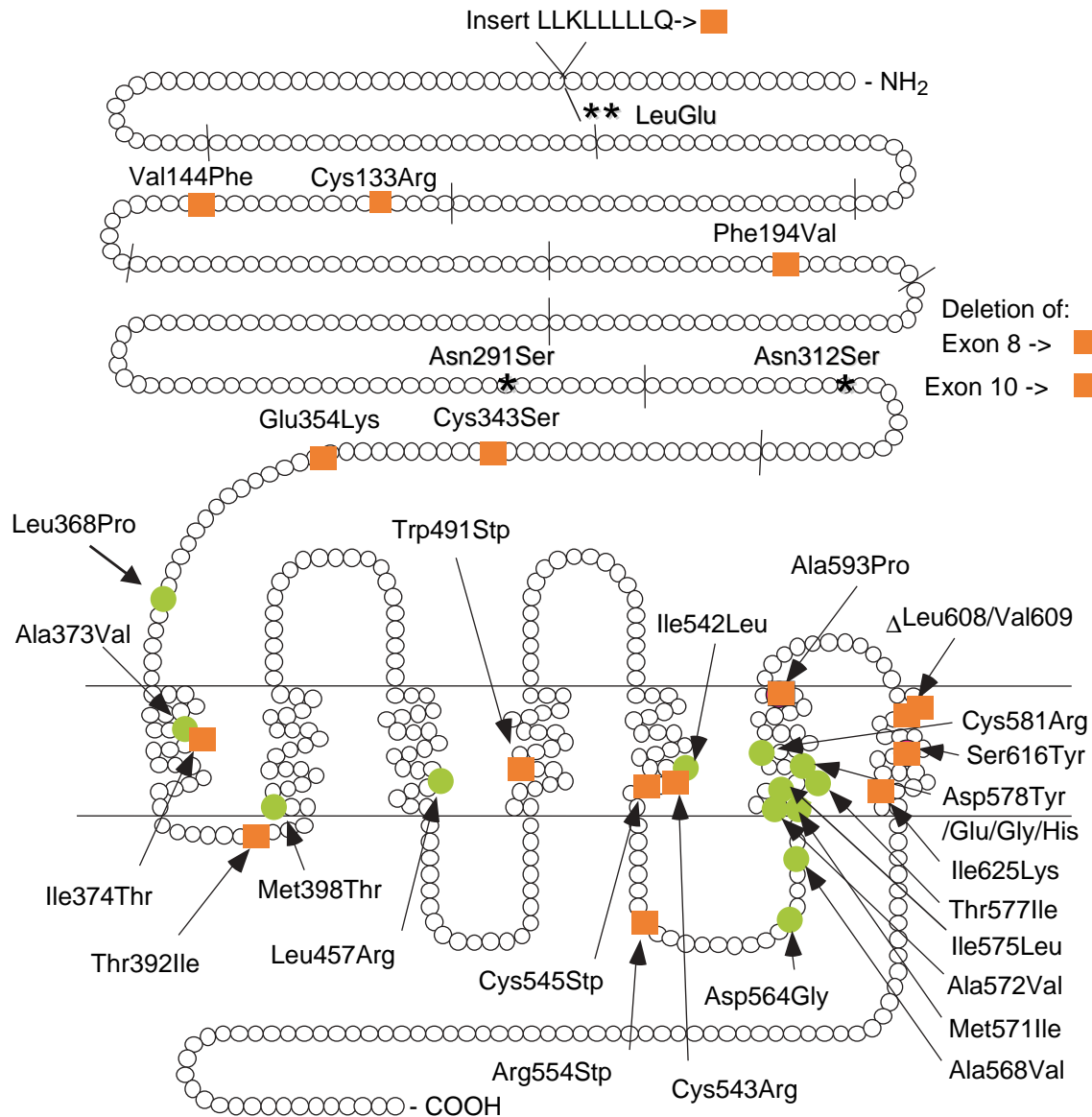
LH receptor

As reviewed previously (Themmen & Huhtaniemi 2000), LH receptor mutations (Fig. 1) can be activating, causing precocious puberty in boys, and inactivating, disrupting sex differentiation in men and causing anovulation in women. In the following sections, we will review the recent new mutations that have been described in the LH receptor gene.

Activating LH receptor mutations

A missense Leu368Pro mutation located in the first transmembrane helix of the transmembrane domain was found in two Brazilian brothers showing typical precocious puberty at an early age (2 and 3 years of age) (Latronico *et al.* 2000). *In vitro* expression of this mutant showed a similar pattern to many of the mutant LH receptors that are constitutively active (Themmen & Huhtaniemi 2000). Thus, cAMP production was increased in the absence of added hCG, and at higher hCG concentrations the receptor responded with further increases in cAMP, never reaching the levels attained at maximal response in the non-mutant LH receptor. As noticed in other families (Rosenthal *et al.* 1996), the mother of these two sons was also a carrier of the mutation, indicating that carriership of an activating mutation in the LH receptor gene has no adverse effects on female infertility (Latronico *et al.* 2000).

Amino acid Asp578 in the LH receptor deserves special discussion with respect to activating LH receptor mutations. Asp578Gly is one of the most common activating mutations found in the LH receptor gene (Shenker *et al.* 1993, Laue *et al.* 1995), while tyrosine (Tyr) or glutamate (Glu) at this position also cause precocious puberty (Laue *et al.* 1995, 1996). However, Asp578His causes Leydig cells to undergo transformation and their increased growth results in adenoma formation (Liu *et al.* 1999). Interestingly, this mutation appears only to occur somatically, i.e. it is found only in the Leydig tumours themselves, but not in the germ line. Other case reports describe the same mutation (Canto *et al.* 2002, Richter-Unruh *et al.* 2002*b*), while another two studies on sex cord tumours failed to reveal any LH receptor mutations (Giacaglia *et al.* 2000, Vieira *et al.* 2002), indicating that not all Leydig cell adenomas are caused by the Asp578His somatic mutation in the LH receptor gene.

Another LH receptor mutation in the 2nd transmembrane α-helix, Met398Thr, has been reported in case reports (Ignacak *et al.* 2000, 2002). This mutation is of

Figure 1 The currently known mutations and amino acid altering polymorphisms in the human LH receptor gene. The green circles depict the activating mutations, the red squares inactivation mutations, and the asterisks the polymorphisms. Stp = nonsense (stop) codon.

special interest, since it shows incomplete penetrance, i.e. not all male family members that are carriers of the mutation show the precocious puberty phenotype (Evans *et al.* 1996). It will be of interest to study these families in more detail, since the incomplete penetrance may indicate interaction with a modifier gene that silences the activating effects of the amino acid change in the LH receptor.

Inactivating LH receptor mutations

Inactivating mutations can take many forms (Themmen & Huhtaniemi 2000) – large partial gene deletions to smaller deletions of just two amino acids and nonsense mutations resulting in truncated LH receptor protein have

been described. As an example, the homozygous insertion of one base pair (T) at codon 589 can be mentioned (Richter-Unruh *et al.* 2005). This insertion causes a frameshift in the LH receptor gene resulting in a truncated LH receptor protein. The functional consequences of this truncation were not tested *in vitro*. The patient, a 46XY patient with complete pseudohermaphroditism, showed lack of responsiveness to LH/hCG with concomitant undetectable serum testosterone (Richter-Unruh *et al.* 2005).

Inactivating LH receptor mutations have also been identified in the extracellular hormone-binding domain. In a complete 46XY pseudohermaphrodite patient a very low number of testicular Leydig cells was found accompanied by a low LH receptor expression (Gromoll *et al.* 2002). The patient was found to be homozygous

for a missense Phe194Val mutation located in exon 7 in a motif that is conserved in the glycoprotein hormone receptor family. The Phe194Val LH receptor did not respond at all to hCG *in vitro*. Phe194 is located in an interesting motif, AFNGT, conserved in the gonado-trophin receptors. N195GT is a glycosylation motif and the change of the homologous Ala189 in the FSH recep-tor to Val, identified in some patients with hypergonado-trophic ovarian dysgenesis (Aittomäki *et al.* 1995), causes complete inactivity of the FSH receptor due to intracellu-lar sequestration of the mutant receptor protein (Rannikko *et al.* 2002). An Asn191Ile change in the FSH receptor, which was found in a heterozygous patient without phenotype, leads to decreased coupling to the cAMP pathway (Gromoll *et al.* 1996*b*).

From the same group an interesting follow-up to the del(exon 10) LH receptor mutant has been published. This deletion was originally described in a patient who had nor-mal male sex differentiation, but did not show signs of pub-erty, apparently because the del(exon10) LH receptor did not respond to LH, while it was sensitive to hCG, hence the intact male sex differentiation (Gromoll *et al.* 2000). Indeed, *in vitro* expression of the del(exon10) LH receptor shows a nearly normal response to hCG whereas the dose–response relationship for LH is severely shifted to the right (Muller *et al.* 2003). The human del(exon10) LH receptor mutation has an interesting parallel in the marmo-set monkey. The marmoset uses CGβ rather than LHβ for LH receptor stimulation. The marmoset pituitary does not express LHβ (Muller *et al.* 2004). Concomitantly, the mar-moset monkey LH receptor always misses the amino acids equivalent to the exon 10 encoded amino acid residues (Zhang *et al.* 1997). The authors propose that owing to an unknown mutational event in evolution, expression of mar-moset LH was completely abolished, and marmoset CG – which, unlike LH, acts normally even when exon 10 is missing from the LH receptor – took over its function.

A homozygous Val144Phe mutation in the LH receptor gene was found in a 46XY patient with an almost com-plete female phenotype. *In vitro*, the mutant LH receptor protein was not transported to the plasma membrane. Inability of the protein to be transported to the cell surface has also been shown with some other missense or trunca-tion mutations such as Cys343Ser, Cys543Arg (Martens *et al.* 2002), Leu502Pro (Leung *et al.* 2004) and Tyr612-Stop (Salameh *et al.* 2005).

Both Val144 and Phe194 are thought to be located (based on a previously published model of the extracellu-lar domain of the LH receptor; Jiang *et al.* 1995) on the convex side of the leucine-rich repeat domain of the LH receptor protein (Val144 in the 4th helical segment; Phe194 in the 6th), so apparently are not directly involved in ligand binding.

LH receptor polymorphisms

The LH receptor gene carries a large number of single nucleotide polymorphisms (SNPs). According to the SNPper website (http://SNPper.chip.org) 282 SNPs are found in the LH receptor gene, resulting in an average distance between SNPs of 306 base pairs. Exact allele fre-quencies are not available for most of these polymorph-isms. The most frequent LH receptor polymorphisms that involve an amino acid change are the absence or pre-sence of a two amino acid insertion at position 18 in exon1 (insLQ; allele frequency 29%), and two variable amino acids at position 291 and 312 respectively: N291S (S allele: 10%) and N312S (N allele: 45%) (Richter-Unruh *et al.* 2002*a*). In addition, an R124Q has been described as an SNP but with low frequency. Powell and coworkers (2003) recently reported that breast cancer patients have a significantly worse overall survival when they are either homozygous or heterozygous carriers of the insLQ allele (Hazard Ratio 2.4; $P = 0.006$) (Powell *et al.* 2003). In addition, trends were observed for associ-ations between the insLQ carriers and nodal involvement or larger tumour size. These results indicate that the insLQ polymorphic insertion probably has an effect on LH receptor protein function, although one report showed that *in vitro* hCG signal transduction of the two LH recep-tor variants was not different (Rodien *et al.* 1998*b*). The finding of an association of breast cancer disease with insLQ needs independent confirmation. A plausible explanation for the observed association of insLQ with breast cancer could be through an effect of LH through the LH receptor, with or without insLQ, causing an increase in the levels of oestrogens in the serum, which, in turn, may stimulate breast cancer cells and therefore cause recurrence of the disease. Alternatively, direct effects of LH on breast cancer cells themselves have been suggested (Guo *et al.* 2004). Since LH is a major regula-tor of ovarian steroid hormone production, further investi-gations are needed into the possible role of the polymorphism in other steroid hormone-related diseases. These include prostate cancer in the male and oestrogen-related disease endpoints such as bone density or frac-tures in elderly women.

LHβ subunit

As yet, only a few mutations are known in the gonado-trophin subunit genes (Fig. 1). None of these mutations are activating the genes, all appear to be loss-of-function mutations. Two subjects with LHβ and 7 with FSHβ inac-tivation have been described in the literature (Themmen & Huhtaniemi 2000, Layman *et al.* 2002, Valdes-Socin *et al.* 2004). Conspicuously, no germ line mutations in the common-α subunit or hCGβ subunits are known. The reason may be that such mutations would inactivate chor-ionic gonadotrophin (hCG), which may be embryo lethal, or even incompatible with implantation.
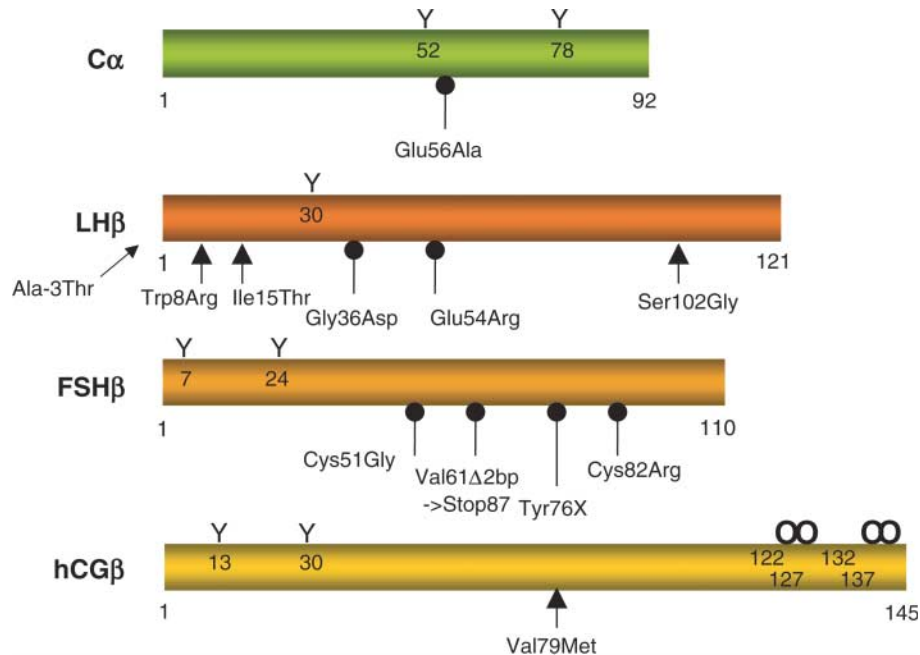
Figure 2 The currently known mutations and amino acid altering polymorphisms in the common α-subunit (Cα), LHβ, FSHβ and hCGβ genes. For references and further details, see text. Arrow, polymorphism; line with closed circle, inactivating mutation.

LHβ mutations

Compared with the rare loss-of-function LH receptor mutations ($n = 17$), mutations in the LHβ gene (Fig. 2) have proven to be very rare. Only the second report of a homozygous carrier of a missense inactivating mutation in the LHβ gene was published recently (Valdes-Socin *et al.* 2004). In the hypogonadal 30-year-old 46XY patient, low circulating LH was found with concomitant reduced spermatogenesis and hypoplastic Leydig cells, hallmarks of absent LH signalling as found in patients with inactivating LH receptor gene mutations (Valdes-Socin *et al.* 2004). The patient's mutant Gly36Asp LHβ was expressed *in vitro* together with the common-α subunit, and showed absence of α/β heterodimerization and therefore no LH bioactivity (Valdes-Socin *et al.* 2004). The Gly36Asp in the LHβ subunit disrupts a 5-amino acid motif that allows the formation of a cystine knot (see above). In the absence of the cystine knot structure no dimerization can occur, and no bioactive LH can be produced.

This effect of the mutation is different from the other published inactivating LHβ mutation (Weiss *et al.* 1992). In this case the Gln54Arg abolished interaction of the LHα/β dimer with the LH receptor, resulting in delayed puberty and absent testosterone production and spermatogenesis in the patient (Weiss *et al.* 1992). Both LHβ mutations illustrate the role of LH during sex differentiation. Apparently, LH is not necessary for male differentiation before birth, since both patients had a male phenotype and descended testes. The fetal activation of Leydig cell proliferation, differentiation and testosterone production is taken care of by placental hCG, allowing these patients to undergo fetal male sex differentiation and insulin-like factor 3-mediated testis descent.

LHβ polymorphisms

The best-studied polymorphism in the LHβ gene is a combination of two SNPs (T82C/T104C) that are in complete linkage disequilibrium and results in a combination of two amino acid changes: Thr8Arg/Ile15Thr (Furui *et al.* 1994, Pettersson *et al.* 1994). This polymorphism (V-LH) is found worldwide at highly variable frequencies in cohorts studied from different countries and different ethnic groups. Most recent association studies of the V-LHβ gene polymorphism were carried out using relatively small cohorts or were case reports (Okuno *et al.* 2001, Takahashi *et al.* 2000*a*, 2001). The V-LH polymorphism can be studied in two ways: using PCR-based techniques that analyse the polymorphisms at the DNA level, and using an ELISA based on a specific antibody that does not recognize V-LH and therefore reports lower LH levels than do other assays (Pettersson *et al.* 1992). Several studies were conducted to investigate possible effects of the two amino acid changes on LH function and to identify possible associations with disease endpoints (reviewed in Themmen & Huhtaniemi 2000). No association of the V-LHβ allele with infertility was found in cohorts of 95 male infertility patients (Lee *et al.* 2003), in a comparison of 145 infertile with 200 fertile men (Ramanujam *et al.* 2000), or in association studies of several endocrine-related cancers (Cramer *et al.* 2000, Powell *et al.* 2003). Nevertheless, V-LH appears to have functional effects, since in a study of 40 healthy Japanese women, V-LHβ carriers appeared to respond to

a gonadotrophin-releasing hormone challenge with a higher maximal LH response (Takahashi *et al.* 2000*b*). Further evidence for the functional differences of V-LH from normal LH was obtained by comparing the *in vitro* and *in vivo* behaviour of recombinant forms of the two hormones. V-LH displayed higher biopotency *in vitro*, whereas its half-life in the circulation was shorter than that of normal LH (Manna *et al.* 2002). Moreover, the carbohydrate side chain composition of V-LH was clearly different, suggesting different pathways in its intracellular processing.

A second polymorphic variant of the LHβ gene is an SNP that causes a Gly102 to Ser amino acid change (Lamminen *et al.* 2002). The frequency of this LHβ polymorphism appears to be low, and in cohorts from Finland, India, Denmark, Rwanda and Korea the Ser allele was absent (Kim *et al.* 2001, Lamminen *et al.* 2002, Lee *et al.* 2003). In one study (Singaporean Chinese), the Ser allele polymorphism was detected in infertile men (5 out of 145) but not in the fertile control group ($n = 200$) (Ramanujam *et al.* 2000).

A third polymorphic variant of the LHβ gene was identified by Jiang and colleagues (2002). This SNP causes an Ala to Thr change three amino acid residues before the signal peptide cleavage site (Ala3Thr). The Thr variant appears to have slightly different *in vitro* signal transduction properties compared with the Ala-containing LHβ protein, although the mature proteins are essentially identical with respect to their amino acid composition. Thus, Ser^{-3} LHβ was more potent in stimulating cAMP in treated cells, whereas the Thr^{-3} LHβ appears to be more effective in stimulating phosphatidylinoside (PI) turnover (Jiang *et al.* 2002).

One possible polymorphism in the hCGβ gene has been reported, an SNP that causes a change from Val to Met at position 79 (Fig. 2) (Miller-Lindholm *et al.* 1999). In this report the frequency of the Met allele appeared to be quite high (4.2%). Upon *in vitro* expression in cultured cells Met79-LHβ was unable to fold correctly and this resulted in impaired ability to associate with the α subunit, suggesting that the Met79 allele may have deleterious effects. However, when the same polymorphism was searched for in 580 DNA samples from 4 European populations, not a single case was found (Jiang *et al.* 2004). The real frequency and significance of this polymorphism thus remains unknown.

Mutations and polymorphisms in human follicle-stimulating hormone and its receptor

FSH receptor

As with the LH receptor gene, FSH receptor gene mutations exist, in principle, in gain-of-function or activating and in loss-of-function or inactivating forms (Fig. 3). Until now only one case of an activating FSH receptor mutation has been described in a case of persistent spermatogenesis of a hypophysectomized man (Gromoll *et al.* 1996*a*). The number of inactivating FSH receptor mutants is slowly increasing.
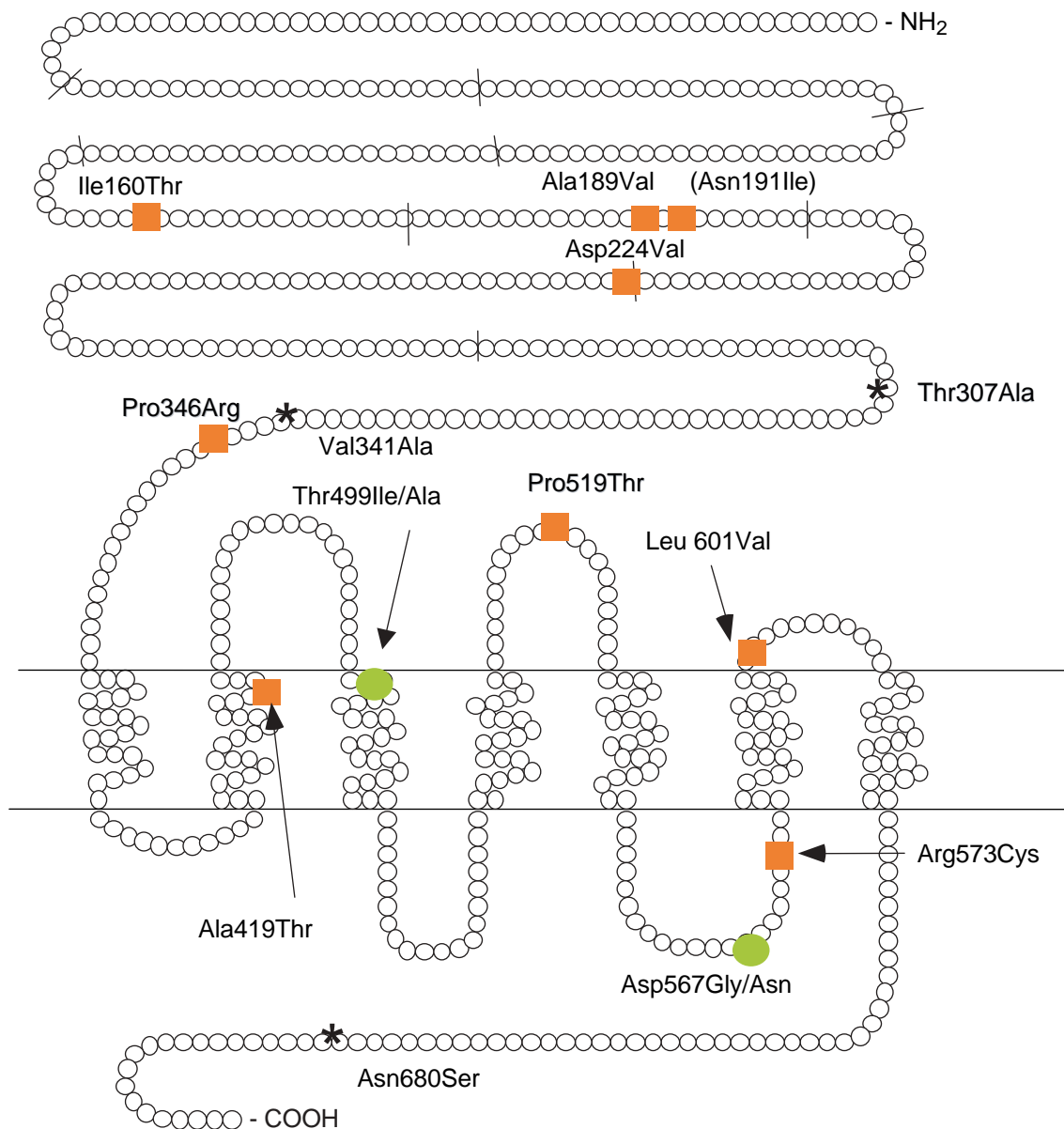
Activating FSH receptor mutations

Whereas no additional activating FSH receptor mutations have been described, *in vitro* FSH receptor mutagenesis showed that such amino acid changes are certainly possible (Tao *et al.* 2000). It remains to be established whether activating FSH receptor mutations cause a phenotype other than the very special case of the hypophysectomized man in which the single FSH receptor mutation was detected (Gromoll *et al.* 1996*a*) and searches for activating FSH receptor mutations in candidate diseases, such as premature ovarian failure, ovarian tumours, megalotestes, precocious puberty and twin pregnancies have been unsuccessful (Giacaglia *et al.* 2000, Montgomery *et al.* 2000, de la Chesnaye *et al.* 2001, Takakura *et al.* 2001, Tong *et al.* 2001, Sundblad *et al.* 2004). An animal model for an activating FSH receptor mutation would be seminal in resolving this intriguing question.

The similarity of the glycoprotein hormones makes the receptors vulnerable to mutations that would relax ligand specificity, especially during early pregnancy when hCG levels in women are extremely high. Indeed, such mutations have been described in the TSH receptor gene causing hCG-induced hyperthyroidism during pregnancy (Rodien *et al.* 1998*a*). Similarly, the FSH receptor may become sensitive to hCG during pregnancy causing ovarian hyperstimulation syndrome (OHSS). Such FSH receptor mutations were recently reported in women who had recurrent OHSS during consecutive pregnancies (Kaiser 2003, Smits *et al.* 2003, Vasseur *et al.* 2003, Montanelli *et al.* 2004). Expression of the mutant FSH receptors showed stimulation of cAMP production by hCG through the mutant FSH receptors (Kaiser 2003, Smits *et al.* 2003, Vasseur *et al.* 2003). In two of the cases (Asp567Asn and Thr449Ala), low levels of constitutive receptor activation and response of the mutated receptor to TSH were also found (Montanelli *et al.* 2004). The location of the mutations in the FSH receptor protein was surprising. Neither of the changed amino acids (Thr449Ile, Ala and Asp567Asn) are located in the extracellular hormone binding domain, but rather in the transmembrane part of the FSH receptor. In addition, no high affinity hCG binding to the mutant receptors could be demonstrated. In one of the papers it is suggested that weakening of interhelical locks between the transmembrane helices by the mutations might make the receptor more vulnerable to low affinity activation by other glycoprotein hormones (Montanelli *et al.* 2004).

Inactivating FSH receptor mutations

Altogether 9 inactivating mutations are known in the human FSH receptor gene. The first one, Ala189Val,

Figure 3 The currently known mutations and amino acid altering polymorphisms in the human FSH receptor gene. The green circles depict the activating mutations, the red squares inactivation mutations, and the asterisks the polymorphisms.

was described about 10 years ago (Aittomäki *et al.* 1995) in subjects of multiple Finnish families with a phenotype of hypergonadotrophic hypogonadism, primary or early onset secondary amenorrhoea, variable development of secondary sex characteristics and arrest of follicular maturation between the primordial and preantral stages. This mutation appears to be unique to the Finnish population. The mutant FSH receptor protein does not traffic to the plasma membrane, rendering the gene inactive (Rannikko *et al.* 2002). In addition, when homozygous Ala189Val FSH receptor patients were treated with massive doses of recombinant FSH, no functional responses of the ovaries or testes were observed (Vaskivuo *et al.* 2002). A similar patient was described

who was homozygous for a Pro419Thr mutation (Meduri *et al.* 2003). The affected female presented with hypergonadotrophic premature ovarian failure, very low oestrogen and inhibin B levels, and total lack of response to high doses of recombinant FSH. Pro419 is located in the 2nd extracellular loop of the transmembrane domain in a conserved motif in the glycoprotein hormone receptors. Histological analysis of ovarian biopsies showed a similar pattern to that found in patients with the Ala189-Val FSH receptor mutations: arrest of follicular maturation beyond the primary stage. Also this receptor was completely unable to traffic to the plasma membrane, reminiscent of many of the inactivating LH receptor mutants (see above).

Other patients with FSH receptor gene mutations had less severe phenotypes, with secondary amenorrhoea, gonadotrophin resistance, normal ovarian size and presence of follicles up to the antral stage (Beau *et al.* 1998, Touraine *et al.* 1999). These patients were carriers of combinations of complete and partially inactivating mutations. Several additional *FSH receptor* mutations have recently been discovered, all in women. It appears that the male phenotype of inactivation of the FSH receptor gene is quite weak, with normal androgen production, reduced sperm quality but maintained fertility (Tapanainen *et al.* 1997).

A patient with a compound heterozygous mutation of Ala189Val and Ala419Thr was recently reported (Doherty *et al.* 2002). She appeared to be less affected than the homozygous Ala189Val patients described before (Aittomäki *et al.* 1995). At 17 years of age she had primary amenorrhoea, normal secondary sex characteristics, very low oestrogen levels but clear signs of endometrial oestrogen stimulation in transvaginal ultrasound investigation, and a normal progestin challenge test. The Ala419Thr FSH receptor bound FSH normally, but did not signal *in vitro* (Doherty *et al.* 2002).

The last mutation reported was a Pro348Arg substitution just outside the transmembrane domain of the receptor protein completely abolishing FSH binding, although it was not investigated whether this was due to intracellular sequestration of the receptor or to a genuine lack of ligand binding to a plasma membrane-expressed receptor (Allen *et al.* 2003). The female patient was hypergonadotrophic with delayed puberty and primary amenorrhoea.

Despite the rarity of the currently known FSH receptor mutations there is good correlation between the phenotype and the degree of receptor inactivation, as well as the site of mutation and its functional consequences, in the same fashion as with the larger number of LH receptor mutations (Themmen & Huhtaniemi 2000). Mutations in the extracellular domain (Ile160Thr, Ala189Val, Asn191Ile, Asp224Val and Pro346Arg) completely abolish FSH receptor function and are found in the patients with the severest phenotypes. Transmembrane domain mutations (Ala419Thr, Arg573Cys and Leu602Val) have decreased but not completely abolished signalling and these patients have a less severe phenotype. Patients with milder mutations may still respond to high doses of FSH stimulation, and the molecular diagnosis of these rare patients may help in the design of a rational treatment for their infertility. All heterozygotes for the mutations so far studied have been free from phenotype, indicating that a single functional FSH receptor gene allele is sufficient for normal reproductive function.

FSH receptor polymorphisms

Besides the inactivating and activating mutations, a large number of SNPs have been identified in the human FSH receptor gene (http://www.ncbi.nlm.nih.gov/SNP/). The number is large, >700, but can be explained by the large size of the FSH receptor gene (191 kb), and the average SNP distance (282 bp) is similar with that of the LH receptor (306 bp). The majority of the polymorphisms are intronic, 5 are located in the FSH receptor coding region and one in the promoter. The exonic polymorphisms are all in the transmembrane domain encoding exon 10 and all cause an amino acid change (Ala307Thr, Arg524Ser, Ala665Thr and Ser680Asn), except the SNP at codon 392 that is silent.

The best-studied polymorphisms in the FSH receptor gene are Ala307Thr and Ser680Asn, which have been found to be in linkage disequilibrium in most of the studies. The two most common allelic variants, Thr307/Asn680 and Ala307/Ser680, are almost equally distributed in Caucasian populations (Simoni *et al.* 2002). The other alleles represent less than 5% of the total. In several studies the association of the Ser680Asn polymorphism with endpoints that relate to FSH sensitivity have been investigated. Thus, the amount of FSH needed to reach similar oestradiol levels in *in vitro* fertilization (IVF) cycles was higher in women that carried the Ser680 FSH receptor allele, suggesting that the Ser680 FSH receptor allele encodes a less active FSH receptor protein that is less sensitive to FSH than the Asn680 allele (Perez Mayorga *et al.* 2000, Sudo *et al.* 2002, de Castro *et al.* 2003, 2004), although this finding could not be confirmed in all studies (Laven *et al.* 2003). In an association study of the Ser680 allele with OHSS (Daelemans *et al.* 2004), it was found that the Ser680 allele was enriched in a control IVF population compared with the general population, but that the OHSS population had even higher enrichment of this allele in comparison with controls (57 vs 39%), which is difficult to reconcile with the suggested lower sensitivity of the Ser680 FSH receptor.

Some studies have shown an association of the Ser680 polymorphism with amenorrhoea or anovulation (Sudo *et al.* 2002, Laven *et al.* 2003) but no association with premature ovarian failure (Conway *et al.* 1999, Sundblad *et al.* 2004). Association studies with polycystic ovarian syndrome have yielded conflicting results in different populations (Conway *et al.* 1999, Tong *et al.* 2001, Sudo *et al.* 2002). Neither is there an association with twinning and the *FSH receptor* allele (Hasbargen *et al.* 2001), and no relationship to male fertility parameters has been found (Simoni *et al.* 1999, Asatiani *et al.* 2002). Recently, in a study of menstrual cycle parameters in women homozygous for either allele, an association of the Ser680 FSH receptor allele was found with higher FSH and lower inhibin A, oestradiol and progesterone in the luteo-follicular transition phase of the cycle, and a longer menstrual cycle (Greb *et al.* 2005). The Ser680 and Asn680 FSH receptor proteins appear to have identical characteristics upon *in vitro* expression, which leaves a molecular explanation for the observed clinical associations open for further research (Simoni *et al.* 1999, Sudo *et al.* 2002).

FSHβ subunit

FSHβ mutations

A total of four different FSHβ gene mutations have been published (Fig. 2). One of these involves a two base-pair deletion at codon 61 of the gene, causing a frame shift, premature stop and a truncation of the FSHβ protein, rendering it completely inactive (Matthews *et al.* 1993). The second mutant, Tyr76X, also causes truncation of the protein (Layman *et al.* 2002) and *in vitro* expression of the mutant causes complete abrogation of both immuno- and bio-activity. The other two mutations, Cys51Gly and Cys82Arg (Themmen & Huhtaniemi 2000) both affect cysteines involved in the cystine knot structure which is essential for dimerization and bioactivity of the FSHβ/α dimer (see above). The deletion mutants, in addition, lack the 'cystine noose' necessary for receptor recognition, and the seat belt domain needed to stabilise the FSHβ/α dimer. Hence, from the molecular point of view, the current knowledge about the genetic and crystalline structure of FSH provides sufficient background information to explain the hormone inactivation at the molecular level.

The patients with these inactivating FSHβ mutations present with a phenotype that can be expected on the basis of the physiological function of FSH. Thus, the women all show sexual infantilism and infertility as a result of a lack of follicle growth and differentiation in the absence of bioactive FSH. However, the three men with FSHβ mutations are all normally masculinized but azoospermic (Lindstedt *et al.* 1998, Phillip *et al.* 1998, Layman *et al.* 2002). This phenotype is at variance with the male phenotype caused by FSH receptor mutations where, notwithstanding reduced sperm quality, fertility is maintained (Tapanainen *et al.* 1997).

The siblings with the Tyr76X mutation showed some evidence of puberty, whereas no residual bioactivity could be found in the studies by Layman *et al.* (2002) suggesting that other factors might preserve gonadal steroidogenesis in the absence of FSH or that current bioassays cannot discriminate among very low FSH levels.

All 4 women with inactivating FSHβ gene mutations have largely similar phenotypes, which in the complete form includes absent puberty and infertility due to lack of follicular maturation. Other features are absence of breast development, primary amenorrhoea, low oestrogen production, undetectable serum FSH, and increased LH. As expected, a large number of undeveloped follicles, usually in the primordial stage but sometimes more advanced, can be found in the ovaries of the affected individuals. The two previously characterized men had azoospermia - one had normal puberty and in the other puberty was delayed.

FSHβ polymorphisms

Somewhat surprisingly, and in contrast to LHβ, the FSHβ subunit appears to be highly conserved. Only a few silent polymorphisms in the exons and totally conserved promoter region were found in the FSHβ gene when studied recently in 50 Danish and 50 Finnish DNA samples (I T Huhtaniemi, personal communication).

Concluding remarks

In the coming years one of the focuses of pathophysiological research in the gonadotrophin field will be on the association studies of gene polymorphisms with endpoints of disease in cohorts of sex differentiation and infertility patients, and other patients with aberrations of hormonal homeostasis. In this way also one of the important questions in the LH receptor field may be answered i.e. what, if any, is the role of the extra-reproductive system expression of the gonadotrophin receptors, especially the LH receptor. The author declares that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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