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ACTUALITIES IN MUTATIONS OF LUTEINIZING HORMONE (LH) AND FOLLICLE-STIMULATING HORMONE (FSH) RECEPTORS

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INTRODUCTION

Pituitary gonadotropins (FSH and LH) act through G protein coupled receptors, encoded by genes located on 2p21-p16 chromosome. FSH, LH, hCG, TSH are heterodimeric glycoprotein hormones with a common α subunit and a specific β subunit (of note, there is a 82% homology of LH with placental hCG). The gene encoding LH receptor (LHR), also known as LHCGR, has 11 exons, while the FSH receptor (FSHR) gene has 10 exons. The receptors have 3 domains: a long extracellular amino-terminal domain, a 7- loop transmembrane domain and a short carboxy-terminal intracellular domain. They work through Gαs activation and cAMP pathway, protein kinase A recruitment, extracellular-regulated kinase (ERK1/2) and cAMP-responsive element binding-protein (CREB) phosphorylation, subsequently stimulating steroidogenesis in target cells (1, 2). LHR may also activate Gq/11 and inositol triphosphate pathway (1).
Gonadal expression of gonadotropin receptors. LHR is expressed in testicular Leydig cells and ovarian granulosa and thecal cells. LHR stimulates the differentiation of fetal Leydig cells and testosterone production; it is essential for male sexual maturation and spermatogenesis. LHR in women stimulates folliculogenesis, ovulation and progesterone secretion (3).

FSHR is expressed in ovarian granulosa cells of developing follicles and in testicular Sertoli cells lining the seminiferous tubules. FSHR is involved in follicular growth and estradiol production in female and regulation of Sertoli cells function and spermatogenesis in male (2).

Extragonadal expression of FSHR has also been identified in human osteoclasts, monocytes, female reproductive tract and the developing placenta, umbilical vein endothelial cells, liver and adipose tissue, blood vessels from malignant tumors and metastase, probably playing a role in osteoclast-mediated bone resorption and angiogenesis (2). The expression of FSHR in umbilical vein endothelial cells could not be reproduced by other authors (4). Identified in human, monocytes and osteoclasts seem to be receptor isoforms or alternative splicing variants that do not act through the classical Gs protein pathway, but probably, through Gi2 which in turn triggers MEK/Erk, NF-kB, and Akt activation (2).

Extragonadal expression of LHCGR was described in the uterus, sperm, seminal vesicles, prostate, skin, breast, adrenals, thyroid, neural retina, neuroendocrine cells, but the physiological role of some of them has remained largely unexplored (1).

To date, several genetic mutations as well as polymorphisms have been demonstrated to significantly affect the pathophysiology of gonadotropins' interaction with their receptors, although the underlying molecular mechanisms are not always clear:

- activating (gain of function) mutations, usually transmitted autosomal dominant and phenotypically manifest in homozygote as well as in heterozygote condition;

- inactivating (loss-of-function) mutations, more rare, mostly transmitted autosomal recessive, more frequent for LHR than for FSHR, which have phenotypical expression only in homozygote or compound heterozygote condition;

- single nucleotide polymorphisms, better studied for FSHR (3, 5).

A mutation can affect the intracellular transport of the receptor (more rare for FSHR), the hormone binding and activation, transduction of the signal, receptor's desensitization internalization, degradation or recycling, or regulation of gene expression (in case of polymorphisms) (2).

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Activating mutations of LHR

In women they are not symptomatic. In men the described mutations are located in exon 11, the most frequent being aspartic acid in 578 position replaced with glycine (D578G). They are heterozygote, autosomal dominant, and produce familial male precocious puberty (testotoxicosis). The disease has variable penetrance, onset around 4 years old, and consists of hyperplasia of Leydig cells, with precocious puberty, increased serum testosterone, prepubertal (low) LH, not responsive to GnRH, increased stature and growth rate, with short adult stature if not treated. Fertility is usually normal. The D578G mutation was described in 62% of familial cases and in 29% of sporadic cases (6, 7). The treatment includes an antiandrogenic drug (as spironolactone, flutamide) or/and an aromatase inhibitor, with good effect on final stature (8); 5-alpha reductase inhibitors or ketoconazole may also be used.

Aspartic acid 578 mutation to histidine (D578H) is a somatic mutation described in boys with non-familial precocious puberty and Leydig cells adenomas (only in adenomas' cells) (6).

Inactivating LHR mutations in men

They produce Leydig cells hypoplasia (LCH), an autosomal recessive disease where the phenotype correlates with mutation's severity. Several mutations have been described, either homozygous or compound heterozygous (7, 9). Complete form (type 1 LCH) displays female phenotype, blind-ending vagina, no uterus/tubes, bilateral cryptorchid testicles of normal or slightly smaller size. Partial form (type 2 LCH) has variable ambiguous external genitalia, up to micropenis or hypospadias (due to the presence of LHR sensitivity to intrauterine hCG). Both forms have delayed puberty and hypergonadotropic hypogonadism (elevated LH by insufficient negative feedback upon the pituitary gland, normal FSH, low testosterone) (3).

Inactivating LHR mutations in women

They have a variable phenotype, with primary amenorrhea (rare), secondary amenorrhea or only oligomenorrhea, but with constant infertility (empty follicle syndrome = follicles without oocytes retrieved after ovulation induction for *in vitro* fertilization). Estradiol and progesterone levels usually remain in the early to mid-follicular phase levels, not reaching ovulatory or luteal phase levels, serum LH is elevated 10–38 IU/L, FSH is usually normal. The ovaries are normal or enlarged with cysts (differential diagnosis with PCOS).Usually they have normal breast development in puberty, but delayed menarche (3). Recently two novel homozygous inactivating LHCGR mutations, c.736 C>T (p.Q246*) and c.846dupT (p.R283*), have been described in two non-related women who had impaired E2 production during the ART process, and also male siblings with 46, XY disorder of sex development (46, XY DSD)(10). In 30-50% of cases with type 1 Leydig cell hypoplasia or in several women with features of LHR receptor resistance no LHR gene mutations were identified(3).

Activating FSHR mutations in women

There are a few known mutations, in all domains of FSHR, which produce a spontaneous ovarian hyperstimulation syndrome (with elevated E2, polycystic ovaries), mostly described during pregnancy. The mutated FSHR displays an increased responsiveness to the physiologically high serum level of hCG during the first trimester of pregnancy (11, 12). These mutations (eg FSHR D567N, T449A, T449I, S128T) induce an expansion of the receptor's specificity, named promiscuous activation, with hypersensitivity to elevated hCG and TSH levels (2). In some cases two FSHR mutations in the biallelic heterozygous form have been described (13). Depending on the severity of symptoms, these women may be managed conservatively or by induced abortion.

Activating FSHR mutations in men

They have been described in 2 cases, where the receptor had ligand - independent constitutive activity; one is a hypophysectomized man, with Asp567Gly mutation in the intracellular loop 3 of FSHR, who had hypogonadotropic hypogonadism (undetectable serum FSH and LH), but normal testicles and spermatogenesis; after testosterone therapy he had 3 children (14)(G; the second case is 1 man with normal spermatogenesis but suppressed FSH serum levels in whom the mutation (N431) induced apparent constitutive activity and impaired agonist-induced desensitization and internalization of the FSRH (15). Asp567Gly mutation with activating effect had been observed in TSH and LH receptor in some patients with thyroid adenoma or male precocious pseudopuberty (12).

Recent experimental studies in genetically modified mice have challenged the current knowledge in the hormonal regulation of male fertility (16). In LH receptor (R)-knockout mouse (LuRKO), it has been found that full spermatogenesis was induced by exogenous testosterone treatment in doses that did not restore the normal high intratesticular testosterone concentration. When hypogonadal LuRKO mouse was

crossed with a transgenic mouse expressing in a highly constitutively active mutated FSHR (FSHR-CAM) in his Sertoli cells, the double-mutant mice which had strong FSH signalling showed near-normal spermatogenesis in spite of a minimal testosterone production, even when this was blocked with an antiandrogen (16).

Inactivating FSHR mutations in women

Some 20 mutations (currently identified) express a variable phenotype. Mutations inducing complete resistance to FSH lead to hypoplastic or dysplastic ovaries, with blockade of the follicle maturation in initial stages; partial resistance was described in some cases having primary amenorrhea with normal puberty, elevated FSH and normal size ovaries, showing no response to FSH stimulation (resistant ovary syndrome)(3, 17, 18). These mutations demonstrated that FSH acts also in pre-antral or in early antral follicular growth. In cases with partial resistance, follicles grow up to the early antral stage, but a progressive increase of FSH seems necessary for the antral follicular growth before selection (19).

Inactivating FSHR mutations in men

Unclear phenotype (usually there is a minor impact on fertility).

Variable impairment of spermatogenesis (but no azoospermia), and small testicles have been described, with normal masculinization, normal testosterone, normal or slightly elevated LH, elevated FSH.

Two men had normal fertility (if androgens have normal levels, fertility may be possible in the absence of FSH action) (3, 12).

FSHR single nucleotide polymorphisms (SNPs)

SNPs falling within the FSHR gene may modulate receptor expression and signalling (2, 5) and have been associated with fertility and endocrine disorders, eg. polycystic ovary syndrome (20). They are studied as genetic markers for the prediction of the gonadal response at FSH stimulation in the treatment of infertility, in both sexes. The most studied FSHR polymorphisms, from more than 2000 described, are (5): SNP c.-29G > A falls within the gene promoter region and changes FSHR gene expression levels. p.Thr307Ala (c.919 A > G; rs6165) produces a change of the aminoacid threonine to alanine aminoacid at position 307, in the hinge region of the receptor. This SNP resides on exon 10 in linkage disequilibrium with p.Asn680Ser (c.2039A > G; rs6166), which results in the change of asparagine to serine within the intracellular domain of FSHR. The presence of the aminoacid serine induces a different kinetics of cAMP, pERK1/2 and pCREB activation (21).

Prediction of the ovarian response to FSH stimulation

Women harbouring the homozygous Ser-Ser genotype of FSHR p.Asn680Ser SNP had higher FSH serum levels, poorer ovarian response to FSH stimulation, needed a higher number of FSH ampoules than other genotypes in order to achieve a similar degree of ovarian stimulation and seemed more prone to a higher risk of iatrogenic ovarian hyperstimulation during assisted reproduction techniques (ART)(22, 23). This polymorphism was also associated with an increased time to obtain a spontaneous pregnancy in non-treated women (24).

However, women homozygous for Serine in both FSHR N680S and LHCGR N312S (rs2293275) polymorphisms had been shown to have a higher rate of pregnancies and of cumulative live birth during *in vitro* fertilization cycles (HR = 1.89, 95% CI [1.00, 3.57]) (25).

Polymorphisms of FSRH and LHR have been involved in women with PCOS: FSHR gene p. Thr307Ala or p. Asn680Ser are associated with an increased risk for PCOS, clomiphene resistance, but better response to rFSH (26); LH SNP (rs61996318) CA and AA genotype conferred a significant risk in the development of PCOS (OR 5.07, 95% CI [2.50-10.31](27).

FSHR polymorphisms may have an impact on reproductive function in men: FSHR 2039A>G, especially when associated with an SNP in the FSH β gene (c.-211G > T) was significantly associated with higher FSH, lower testicular volume and sperm count (28, 29). FSHR p.Asn680Ser has been associated to a reduced response to the empirical treatment with FSH in male idiopathic oligozoospermia and infertility(30)

It has been proposed that a multigenic, pharmacogenetic-based patient selection could improve assisted reproduction techniques, in both sexes (5).

In conclusion, FSHR and LHR mutations are rarely diagnosed, due to the lack of specific clinical features in most of inactivating mutations and loss of transmission due to infertility. The clinical picture depends on the mutation's severity. Human described cases contributed to better identification of FSHR and LHR role in human physiology. Further studies are necessary in order to clarify the impact of individual pharmacogenetic evaluation in the treatment and prognosis of infertile patients.

Conflict of interest

The authors declare that they have no conflict of interest.

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