**Gonadotropin receptor mutations**

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**Introduction**

The introduction of molecular biology techniques into the field of endocrinology has allowed the identification of single gene mutations as a cause for many endocrine hereditary syndromes. In many cases, and this certainly is true for the mutations in the receptors for luteinizing hormone (LH) and follicle-stimulating hormone (FSH) described herein, the studies have ascertained at the molecular level the function of these mutated genes, functions that previously had been shown in physiological studies. In this review mutations in gonadotropin receptors will be discussed in relation to the phenotype of the patients in which these mutations were demonstrated.

Among the gonadotropin receptor mutations many are in the LH receptor gene, and only two changes were found in the FSH receptor molecule. For this reason the major part of this review deals with mutations in the LH receptor gene and their effect on phenotype.

**Activating mutations**

**LH receptor** Activating mutations in the LH receptor gene were first identified in patients with familial male-limited precocious puberty (FMPP; for an overview of phenotypes see Table 1) (Kremer et al. 1993, Shenker et al. 1993). In boys, these mutations lead to precocious puberty at an early age (before the age of 4 years); in some cases an enlarged penis was noted even at birth (Gondos et al. 1985). This phenotype is the consequence of increased androgen production by testicular Leydig cells during the fetal and postnatal period irrespective of the presence of LH or human chorionic gonadotropin (hCG). The Leydig cells are stimulated continuously through the expression of the mutated LH receptor allele not affected by the expression of the other, wild type, allele.

Most amino acid changes that have been identified in the constitutively active LH receptor are located in the 6th transmembrane segment and in the C-terminal part of the flanking 3rd intracellular loop (Table 2). These findings are in agreement with the current model of the mechanism of signal transduction of the glycoprotein hormone receptors. The 3rd intracellular loop and its flanking 6th transmembrane segment are intimately involved in coupling to **G**α, the G protein that activates adenylyl cyclase. However, similar to the thyroid-stimulating hormone (TSH) receptor (Tonacchera et al. 1996), mutations in parts of the LH receptor outside the 6th transmembrane segment and the 3rd intracellular loop, such as **M**298**T** in the 2nd (Kraaij et al. 1995), and **1**422**L** in the 5th transmembrane segment (H Kremer, H G Brunner, A P N Themmen & J W M Martens, unpublished observations, Laue et al. 1995a) are also found. Activating amino acid changes may even be identified in the region of the receptor that connects the leucine-rich repeat segment of the large extracellular binding domain to the transmembrane domain (Bhowmick et al. 1996), as has been shown in the TSH receptor (G Vassart, personal communication).

What is the consequence of these LH receptor gene mutations for the carrier male or female? The hereditary pattern is autosomal dominant, but only males have precocious puberty. The dominance of the mutation is explained by the gain of function character of the mutants, but the limitation of the syndrome to male subjects is less easily understood (see below). Male sex differentiation depends on the presence of **SRY**, the testis determining region of the Y chromosome, that directs differentiation of the indifferent gonads into testes. Subsequently, androgen production by the testis induces male differentiation of the Wolffian ducts, urogenital sinus and growth and differentiation of the external sex organs. During the fetal period of testis differentiation, Leydig cell activity is dependent on the presence of maternal hCG, and the cells express the LH receptor gene. Although Leydig cells continue to express the LH receptor after birth, they are not activated because LH is present at very low levels until puberty. Puberty is induced when the hypothalamic-pituitary-testis axis is established, LH is secreted and the testicular Leydig cells start to produce androgens again. In contrast, an LH receptor gene that carries an activating mutation will not only stimulate Leydig cells during sex differentiation, but also continue to do so after birth, resulting in very early precocious puberty. The severity of the LH receptor...
### Table 1 Overview of phenotypes

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Defect</th>
<th>Phenotype</th>
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</thead>
<tbody>
<tr>
<td>LH</td>
<td>Activated</td>
<td>Familial male-limited precocious puberty</td>
</tr>
<tr>
<td></td>
<td>Inactivated (partial or complete)</td>
<td>Pseudohermaphroditism (partial or complete)</td>
</tr>
<tr>
<td>FSH</td>
<td>Activated</td>
<td>Rescue from hypophysectomy (see text)</td>
</tr>
<tr>
<td></td>
<td>Inactivated</td>
<td>Impaired spermatogenesis</td>
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</tbody>
</table>

* ?, No phenotype described.

### Table 2 Overview of gonadotropin receptor mutations

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Phenotype</th>
<th>Amino acid change</th>
<th>In vitro activity</th>
<th>Location</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>δ</td>
<td>C&lt;sup&gt;133&lt;/sup&gt;R</td>
<td>+</td>
<td>EC</td>
<td>Misrahi et al. 1996</td>
</tr>
<tr>
<td></td>
<td>δ</td>
<td>M&lt;sup&gt;190&lt;/sup&gt;T</td>
<td>+</td>
<td>TM2</td>
<td>Kraaij et al. 1995, Laue et al. 1995a, Yano et al. 1996</td>
</tr>
<tr>
<td></td>
<td>δ</td>
<td>F&lt;sup&gt;542&lt;/sup&gt;L</td>
<td>+</td>
<td>TM5</td>
<td>Laue et al. 1995a</td>
</tr>
<tr>
<td></td>
<td>δ</td>
<td>C&lt;sup&gt;545&lt;/sup&gt;s</td>
<td>–</td>
<td>TM5</td>
<td>Laue et al. 1995c</td>
</tr>
<tr>
<td></td>
<td>δ/Φ</td>
<td>R&lt;sup&gt;554&lt;/sup&gt;s</td>
<td>Φ</td>
<td>IL3</td>
<td>Latronico et al. 1996</td>
</tr>
<tr>
<td></td>
<td>δ</td>
<td>D&lt;sup&gt;564&lt;/sup&gt;G</td>
<td>+</td>
<td>IL3</td>
<td>Laue et al. 1995a</td>
</tr>
<tr>
<td></td>
<td>δ</td>
<td>A&lt;sup&gt;568&lt;/sup&gt;V</td>
<td>+</td>
<td>IL3</td>
<td>Latronico et al. 1995</td>
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<tr>
<td></td>
<td>δ</td>
<td>M&lt;sup&gt;571&lt;/sup&gt;I</td>
<td>+</td>
<td>TM6</td>
<td>Kremer et al. 1993, Kraaij et al. 1995</td>
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<tr>
<td></td>
<td>δ</td>
<td>A&lt;sup&gt;572&lt;/sup&gt;V</td>
<td>+</td>
<td>TM6</td>
<td>Yano et al. 1995</td>
</tr>
<tr>
<td></td>
<td>δ</td>
<td>D&lt;sup&gt;577&lt;/sup&gt;I</td>
<td>+</td>
<td>TM6</td>
<td>H Kremer, HG Brunner, APN Themmen &amp; JWM Martens unpublished, Laue et al. 1995b</td>
</tr>
<tr>
<td></td>
<td>δ</td>
<td>D&lt;sup&gt;578&lt;/sup&gt;G</td>
<td>+</td>
<td>TM6</td>
<td>Kosugi et al. 1995</td>
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<tr>
<td></td>
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<td>D&lt;sup&gt;578&lt;/sup&gt;Y</td>
<td>+</td>
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<td>Kremer et al. 1993, Shenker et al. 1993</td>
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<td></td>
<td>δ</td>
<td>C&lt;sup&gt;581&lt;/sup&gt;R</td>
<td>+</td>
<td>TM6</td>
<td>Laue et al. 1995a</td>
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<tr>
<td></td>
<td>δ/Φ</td>
<td>A&lt;sup&gt;593&lt;/sup&gt;P</td>
<td>–</td>
<td>TM6</td>
<td>Laue et al. 1995a</td>
</tr>
<tr>
<td></td>
<td>δ</td>
<td>S&lt;sup&gt;616&lt;/sup&gt;Y</td>
<td>–</td>
<td>TM6</td>
<td>Kremer et al. 1995, Toledo et al. 1996</td>
</tr>
<tr>
<td></td>
<td>Φ</td>
<td>D&lt;sup&gt;627&lt;/sup&gt;G</td>
<td>+</td>
<td>TM6</td>
<td>Latronico et al. 1996</td>
</tr>
<tr>
<td></td>
<td>Φ</td>
<td>A&lt;sup&gt;698&lt;/sup&gt;V</td>
<td>–</td>
<td>EC</td>
<td>Aittomaki et al. 1995</td>
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</table>

<sup>a</sup>Wild type amino acid, followed by the position according to the numbering of Minegishi et al. (1990) and the mutant amino acid; * , nonsense mutation of that codon to a stop; Φ, in vitro activity not tested; +, constitutively active; –, partially or completely inactivated.

Mutation determines the age at which pubertal development becomes obvious. Laue et al. (1995a) reported that the LH receptor with the D<sup>578</sup>Y mutation showed a higher activity in the absence of hCG than the D<sup>578</sup>G mutant. They correlated this difference to the clinical phenotype of the carrier of the D<sup>578</sup>Y mutation, a boy who already showed signs of puberty at the age of one year (Babovic-Vuksanovic et al. 1994). We have made similar observations (H Kremer, HG Brunner, APN Themmen & J W M Martens, unpublished observations).

Although the constitutively active LH receptors show elevated basal activity, this activity will still increase upon stimulation with endogenous LH when true puberty commences. We have mimicked the heterozygous situation in FMPP patients by transfecting constitutively active LH receptor mutants into mouse MA10 tumour Leydig cells that express the endogenous mouse LH receptor gene (Themmen & Brunner 1996). These experiments showed that the increased basal cAMP levels caused increased steroid hormone production by Leydig cells. In addition, the cells were still responsive to higher concentrations of LH or hCG. The mutation causes only moderate activation of the receptor and modulation of receptor activity by endogenous LH is still possible. Thus, the gonadotropin-releasing hormone (GnRH)-LH-testosterone negative feedback is still intact in these males, and clinical signs of continuous maximal androgen production are not found.

Female sex differentiation during fetal life does not depend on LH or hCG, and LH receptors are not expressed. Also the onset of puberty is more dependent on FSH rather than on LH, since LH receptor expression in the ovarian granulosa cells only occurs in the presence of FSH. Therefore, activating mutations in the LH receptor gene do not affect female development.

Adult females carrying a constitutively active LH receptor gene do not present with a clear phenotype. This
finding is unexpected and not completely understood. The timing of ovulation which is regulated by LH does not appear to be affected, since no fertility problems in female carriers of an activating LH receptor mutation have been reported. Possibly at the time of ovulation, the follicular LH receptors have to be maximally stimulated by LH to induce ovulation. Although constitutively active LH receptors cause a high basal cAMP production when expressed in vitro, addition of large amounts of hCG or LH still leads to further enhancement of cAMP production for a normal ovariolytique LH peak. Recently, a clinical study of a female carrier of the D578G mutation was published (Rosenthal et al. 1996). The hormonal responses to a GnRH agonist challenge were found to be normal, and the authors suggested that the mutation did not activate LH receptor function beyond the pubertal level, and that the negative feedback systems that regulate ovarian function are intact.

FSH receptor To date only one patient carrying an activating FSH receptor mutation has been reported (Gromoll et al. 1996). This male patient was identified in an unexpected way. After diagnosis of a pituitary adenoma, the patient was hypophysectomized and substituted with glucocorticoids, T4 and androgens. Surprisingly, the patient proved to be fertile notwithstanding the complete absence of pituitary FSH. Because it is thought that both FSH and androgens are necessary for correct and complete spermatogenesis in the human, Gromoll et al. (1996) hypothesized that a constitutively active FSH receptor might be the cause of the intact spermatogenesis in this hypophysectomized, androgen–treated man. Indeed an FSH receptor gene alteration was found, and expression of this mutant receptor in COS cells resulted in the production of approximately 2-5-fold more cAMP than in control cells transfected with the wild type FSH receptor. The increases in cAMP may be slight, but their effects on Sertoli cell function may be strong (Fritz et al. 1978). The unexpected fertility in this hypophysectomized man strongly suggests that the identified amino acid change is the cause of the active spermatogenesis. In the absence of information about other cases and details such as testis histology or abnormalities during puberty, it remains unclear whether an FSH receptor mutation with such a mild in vitro effect will alter sex differentiation or male fertility under normal conditions.

No female cases carrying a constitutively active FSH receptor molecule have been reported. It is difficult to envisage what the phenotype of such a case would be. One could argue that the higher FSH receptor activity might force more follicles to grow leading to an increase in pre-ovulatory follicles and a higher incidence of dizygotic twinning. Correlations between FSH levels or action and dizygotic twinning have been indicated (Martin et al. 1984, 1991), but in a recent study no linkage disequilibrium with respect to genes for FSHβ, CGβ, inhibinβb and GnRH could be found in mothers of dizygotic twins (Chenevix-Trench et al. 1993). It would seem logical to extend these studies to include the FSH receptor. However, to date no FSH receptor studies in women with multiple dizygotic twinning have been reported. An attenuating effect on fertility might occur when a mutation in the FSH receptor causing hypersensitivity to FSH has occurred. Such a receptor might stimulate the growth of small follicles, and lead to follicle maturation before the LH peak occurs. Finally, subtle effects of aberrantly activated FSH receptors might adversely affect follicle selection or timing of ovulation. A minimum concentration of serum FSH, the so-called FSH threshold, appears to be essential for follicle selection (Fauser et al. 1993, van Santbrink et al. 1995). A mutant FSH receptor that does not respond to slight changes in FSH might influence this delicate regulation, and affect female fertility.

Inactivating mutations

LH receptor The first described cases with inactivating LH receptor mutations were 46,XY male pseudohermaphrodites that suffered from a severe form of Leydig cell hypoplasia (type 1 (Toledo et al. 1985)) characterized by a female external phenotype, a small blind-ending vagina, inguinal testes and absent glandular breast tissue. A homozygous DNA mutation leading to a A593P amino acid change was identified in these cases (Kremer et al. 1995). Upon expression in vitro the mutant LH receptor was found to be completely unable to transduce the LH/hCG signal to the Gs protein and adenylyl cyclase, although LH/hCG binding was relatively normal (Kremer et al. 1995), suggesting that the homozygous LH receptor gene mutation caused the Leydig cell hypoplasia phenotype. In another pedigree a homozygous nonsense mutation was found that results in truncation of the LH receptor (Latronico et al. 1996). Although this mutation was not functionally evaluated, one may expect that the truncation in the 3rd intracellular loop (R545Stop) completely abolishes signal transduction, because the domains that confer G protein coupling are not present. Until now affected patients are found in inbred families, and are homozygous for the LH mutations. Only one compound heterozygote has been described (see below). In these males the phenotype, complete pseudohermaphroditism, is consistent with complete absence of testosterone–mediated genital development. This underscores the crucial role of the hCG/LH receptor in male sex differentiation.

A second variant of Leydig cell hypoplasia (LCH type 2) was described by Toledo (1992). This milder LCH type 2 phenotype is characterized by undervirilization and a micropenis and is expected to be the result of a low but not absent androgen production during sex differentiation, caused by LH receptor mutations that compromise, but do not completely inhibit hCG binding and signal transduction. Recently, a homozygous S616Y amino acid change in the 7th transmembrane segment of the LH receptor was identified in a patient with no other clinical manifestations.
of subvirilization but a micropenis (Latronico et al. 1996). The same S^616Y amino acid change was found in a compound heterozygous Leydig cell hypoplasia patient (Laue et al. 1996). The other allele in this patient encoded a completely inactive LH receptor with missing exon 8 (no ligand binding). Unexpectedly, in the former report (Latronico et al. 1996), the S^616Y mutant receptor when tested in vitro showed complete inability to bind LH or stimulate cAMP production, possibly caused by trapping of the protein within the intracellular organelles, while Laue et al. (1996) observed residual activity better fitting the mild phenotype. A mutation in the leucine-rich repeat segment of the LH receptor (C^133R) in an LCH type 2 patient has been described that may interfere with high affinity LH/hCG binding (Misrahi et al. 1996).

Recently, the female counterpart of LCH type 1 has been reported (Toledo et al. 1996). In this 46,XX woman a homozygous inactivating LH receptor mutation was found, but she presented with a relatively mild phenotype: primary amenorrhea with normally developed primary and secondary sex characteristics. The patient is a sister of the two 46,XY pseudohermaphrodites described by Kremer et al. (1995), and carries the same homozygous mutation in the LH receptor gene rendering the LH receptor protein completely inactive. An hCG challenge revealed complete resistance to hCG/LH. Clinical examination revealed a small uterus, normal-sized vagina with thin walls and hyposecretory function and some signs of decreased bone mass. All these observations are indicative of low estrogen levels. As was expected, serum estrogen levels were found to be always in the low-normal range for the follicular part of the menstrual cycle. No pre-ovulatory follicles or corpora lutea were observed in the ovary. Some collapsed antral follicles with theca cells that had a luteinized appearance, as well as clusters of primordial follicles were noted. These observations are all consistent with absence of LH receptor function leading to amenorrhea, and very low estrogen production. In another pedigree a similar female patient was described with an inactivating mutation in the LH receptor gene that leads to a truncation of the LH receptor protein (Latronico et al. 1996).

These first results of the investigation of mutations in the LH receptor gene indicate that the phenotypic effect of a mutation that completely inactivates the LH receptor is much more severe in males than in females. This difference between sexes is caused by the dependence of male sex differentiation on fetal testosterone production by fetal Leydig cells under the control of maternal hCG.

**FSH receptor** In females, inactivating FSH receptor gene mutations appear to have a much more severe effect on phenotype than the inactivation of the LH receptor molecule. Patients carrying an inactivating mutation in the FSH receptor gene have unresponsive ovarian cells, almost absent female sex steroid production resulting in underdeveloped secondary sex characteristics (Aittomaki et al. 1994, Aittomaki et al. 1995). In our view, the nomenclature of this syndrome needs clarification. Ovarian dysgenesis has been used, but this term suggests that the ovary has never formed as the result of a defect during fetal development. However in these patients the dysfunctioning ovary is the result of unresponsiveness to FSH. In analogy to the use of androgen insensitivity syndrome for the now defunct term testicular feminization, we propose to use the term FSH insensitivity syndrome instead of ovarian dysgenesis for these patients.

The observations in the patients with an FSH receptor mutation (Aittomaki et al. 1995) fit the model of endocrine regulation of ovarian function: FSH stimulates growth of the follicles, and subsequent induction of LH receptors. Consequently, the lack of an FSH signal results in the complete absence of growing follicles and concomitantly an absence of estrogens. In contrast, absence of signaling through the LH receptor allows for follicular growth and, albeit limited, estrogens being produced.

A controversial situation exists in the male. Homozygous carriers of the same FSH receptor mutation as the female patients appear to have small testes indicating absent or poor spermatogenesis (I Huhtaniemi, personal communication, Aittomaki et al. 1995). In light of the effect of the activating FSH receptor gene mutation in the fertile hypophysectomized man described above, this is a puzzling finding. On the one hand a minimally active FSH receptor rescues fertility in the absence of endogenous FSH, while on the other hand a compromised and minimally active FSH receptor causes infertility in the presence of high levels of FSH (both findings in the presence of androgens, either administered or endogenous). Furthermore, the FMPP patients that go into puberty because of their constitutively active LH receptor, do show onset of spermatogenesis in the absence of FSH. The results of investigation of the males carrying an inactive FSH receptor gene are eagerly awaited, and certainly more male patients need to be screened before more definite conclusions can be drawn.

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