[Frontiers in Bioscience 5, d343-352, March 1, 2000]

LUTEINIZING HORMONE RECEPTOR MUTATIONS IN DISORDERS OF SEXUAL DEVELOPMENT AND CANCER

Shao-Ming Wu [1],Ellen Werber Leschek [2],Owen M. Rennert [3],Wai-Yee Chan [1]

[1] Departments of Pediatrics, Cell Biology, Biochemistry and Molecular Biology, Georgetown University Children's Medical Center, Washington, DC [2] Developmental Endocrinology Branch, and [3] Center for Research for Mothers and Children, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD

TABLE OF CONTENTS

1. ABSTRACT

Human male sexual development is regulated by chorionic gonadotropin (CG) and luteinizing hormone (LH). Aberrant sexual development caused by both activating and inactivating mutations of the human luteinizing hormone receptor (LHR) have been described. All known activating mutations of the LHR are missense mutations caused by single base substitution. The most common activating mutation is the replacement of Asp-578 by Gly due to the substitution of A by G at nucleotide position 1733. All activating mutations are present in exon 11 which encodes the transmembrane domain of the receptor. Constitutive activity of the LHR causes LH releasing hormone-independent precocious puberty in boys and the autosomal dominant disorder familial male-limited precocious puberty (FMPP). Both germline and somatic activating mutations of the LHR have been found in patients with testicular tumors. Activating mutations have no effect on females. The molecular genetics of the inactivating mutations of the LHR are more variable and include single base substitution, partial gene deletion, and insertion. These mutations are not localized and are present in both the extracellular and transmembrane domain of the receptor. Inactivation of the LHR gives rise to the autosomal recessive disorder Leydig cell hypoplasia (LCH) and male hypogonadism or male pseudohermaphroditism. Severity of the clinical phenotype in LCH patients correlates with the amount of residual activity of the mutated receptor. Females are less affected by inactivating mutation of the LHR. Symptoms caused by homozygous inactivating mutation of the LHR include polycystic ovaries and primary amenorrhea.

2. INTRODUCTION

Chorionic gonadotropin (CG) and luteinizing hormone (LH) regulate human male sexual development at different stages of embryogenesis and growth. CG exerts its effect during early embryogenesis and induces the maturation of Leydig cells. LH promotes steroidogenesis by the Leydig cells, especially around the period of puberty. Both CG and LH exert their effects through interaction with the luteinizing hormone receptor (LHR) present on the surface of Leydig cells in the testis. Interaction of the hormones with the receptor initiates a sequence of membrane and intracellular events. The resulting gene activation leads to increase in testosterone production and male sexual development. Human female sexual differentiation and pubertal development do not depend on the action of LH. In females, LH is responsible for stimulating theca cells to produce androgen precursors for aromatization to estradiol by granulosa cells.

The LHR gene is located on human chromosome 2p21. It has 11 exons. The first 10 exons encode the extracellular domain, while the last exon encodes a small portion of the extracellular domain and the transmembrane

Table 1. Activating Mutations of the LHR in FMPP

Nucleotide Change	Amino Acid Change	Location	Case #	Genetics	Reference
C 1118 T	Ala 373 Val	TM I	3	Familial (2) Sporadic (1)	21, SM Wu & WY Chan, unpublished observations
T 1193 C	Met 398 Thr	TM II	8	Familial (4) Sporadic (4)	15,19,25
T 1370 G	Leu 457 Arg	TM III	1	Sporadic	23
A 1624 C	Ile 542 Leu	TM V	9	Familial (8) Sporadic (1)	15,25, SM Wu, EW Leschek & WY Chan, unpublished observations
A 1691 G	Asp 564 Gly	3rd cytoplasmic loop	3	Familial (1) Sporadic (2)	15,25, SM Wu, EW Leschek & WY Chan, unpublished observations
C 1703 T	Ala 568 Val	3rd cytoplasmic loop	4	Familial (1) Sporadic (3)	15,23,26
G 1713 A	Met 571 Ile	TM VI	2	Familial	15
C 1715 T	Ala 572 Val	TM VI	2	Familial (1) Sporadic (1)	15
A 1723 C	Ile 575 Leu	TM VI	3	Familial (2) Sporadic (1)	15,20,25
C 1730 T	Thr 577 Ile	TM VI	3	Familial	15,26
G 1732 T	Asp 578 Tyr	TM VI	4	Sporadic	15,22,25
A 1733 G	Asp 578 Gly	TM VI	54	Familial (46) Sporadic (8)	15, SM Wu, EW Leschek & WY Chan, unpublished observations
T 1734 A	Asp 578 Glu	TM VI	1	Familial	24
T 1741 C	Cys 581 Arg	TM VI	1	Familial	15

Note: Only references published after review 15 are listed. Please see bibliography of review 15 for earlier publications.

(TM) domain which is comprised of seven transmembrane helices (TM I to TM VII), the connecting extracellular and cytoplasmic loops, and the C-terminal cytoplasmic domain (1). The LHR belongs to the G protein-coupled receptor (GPCR) superfamily (2). LHR bears high homology at both the nucleotide and the amino acid level to the other two glycohormone receptors, the follicle stimulating hormone receptor (FSHR) and the thyroid stimulating hormone receptor (TSHR) (1,3). Binding of the hormone to the extracellular domain of the LHR activates the receptor leading to increased formation of cAMP and transcriptional activation of LH-responsive genes and steroidogenic changes (4,5).

Since the first description of a mutation of the LHR in boys with precocious puberty in 1993 (6), much progress has been made. Both activating and inactivating mutations of the LHR have been described. These mutations affect sexual development, especially in the male. A number of reviews on the gonadotropin receptor mutations have been published since 1996 (7-16). This article aims at reviewing the current status of work on the molecular genetics of the LHR mutations and their impact on human sexual development. The possibility of predisposition of FMPP patients to developing testicular tumor is also discussed.

3. ACTIVATING MUTATIONS

Activating mutations of the LHR affect primarily males. Constitutive activity of the LHR leads to stimulation of testicular Leydig cells in the fetal and prepubertal period in the absence of the hormone resulting in autonomous production of testosterone and pubertal development at a very young age. This autosomal dominant condition is termed familial male-limited precocious puberty (FMPP) (17) or testotoxicosis (18). Sporadic cases caused by new mutations of the LHR are called sporadic male-limited precocious puberty (SMPP).

3.1. Molecular genetics and biochemistry

Fourteen single base substitutions in exon 11 of the LHR gene have been identified among 98 kindreds with FMPP or SMPP (references in Table 1). These single base substitutions give rise to missense mutations in TM I, II, III, V, VI or the third cytoplasmic loop of the LHR (Figure 1). The majority of the mutations (8/14) are present in TM VI. With the exception of the replacement of Ile-542 by Leu (i.e. Ile542Leu) in TM V (Table 1), all mutations result in the substitution of amino acids which are conserved among the glycohormone receptors (3) suggesting that these amino acid residues are important for the integrity of the biological activity of these receptors. The most common mutation is Asp578Gly, which occurs in TM VI and represents about 54% of the mutations in all cases, and accounts for 62% of all FMPP and 29% of all SMPP with detectable mutation.

The LHR is prone to mutation. Precocious puberty in a number of patients is the result of new mutation. Among the 26 cases of SMPP, 10 different mutations are found. On the other hand, 13 mutations account for 74 independent cases of FMPP. Eight of the mutations, namely, Ala373Val, Met398Thr, Ile542Leu, Asp564Gly, Ala568Val, Ala572Val, Ile575Leu, and Asp578Gly, are found in both FMPP and SMPP (Table 1). Rare mutations occur more often for patients of non-Caucasian ethnic background, including Hispanics, Asians, African Americans and African Brazilians (15,23,24,26). There are 11 putative FMPP kindreds in whom mutations

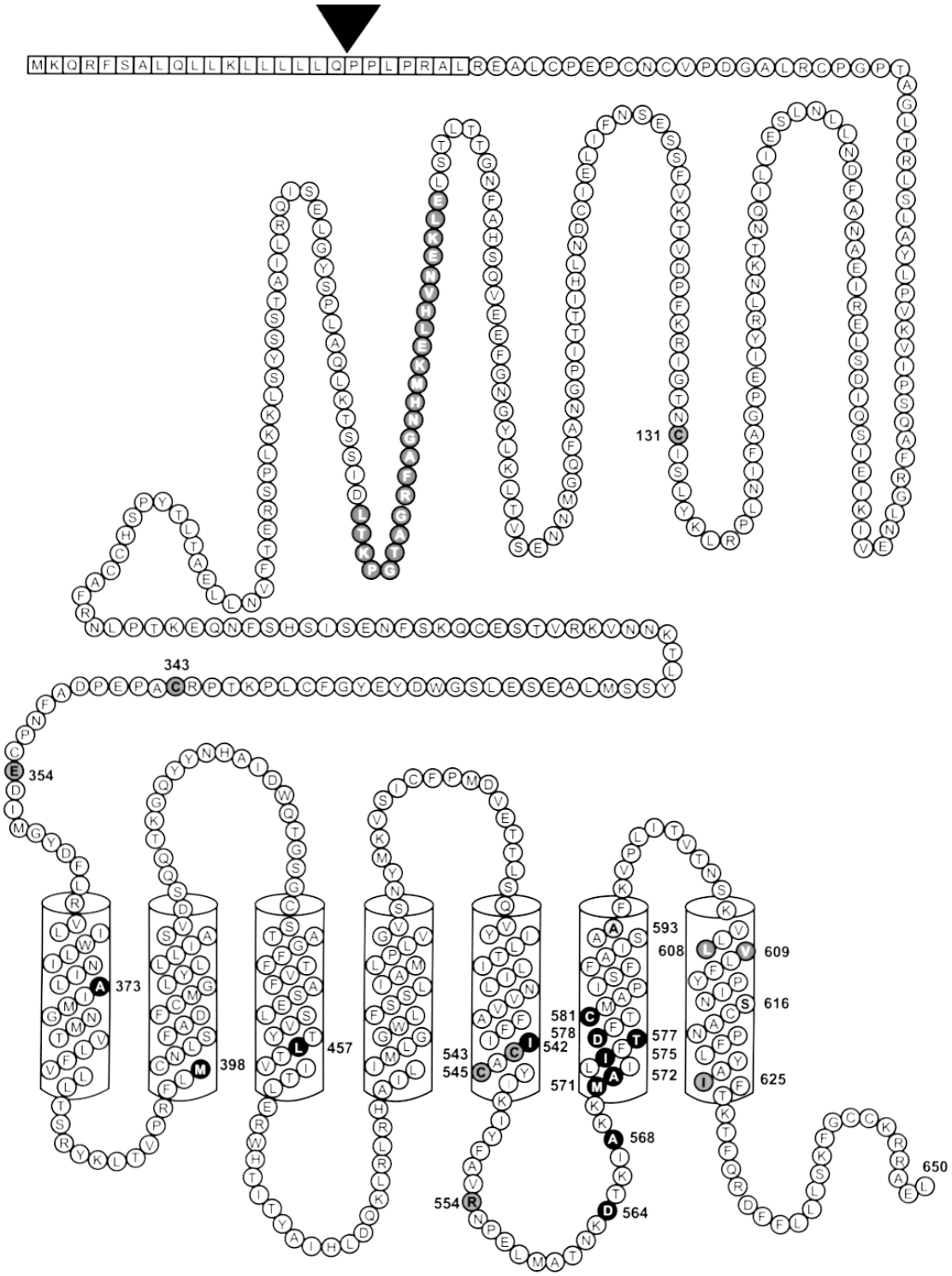

Figure 1. Activating and Inactivating Mutations of the Human LHR. Single letter code of amino acid residues are shown. Square represents amino acid residue in the putative signal peptide. Empty circle represents normal amino acid residue. Dark filled circle represents amino acid substituted in activating mutations in FMPP; grey shaded circle represents amino acid substituted in inactivating mutations in LCH; grey shaded circle with white letter represent amino acid deleted in inactivating mutation of the LHR. Insertion is represented by an inverted triangle. Position of the amino acid residue is indicated by its side. Vertical cylinder represents transmembrane α-helix.

of the LHR have not yet been identified. It has been suggested that FMPP of suspected patients who do not have LHR mutations are caused by other factors (25). So far no activating mutation in exons 1 to 10 of the LHR has been reported. However, this might be due to the fact that with the exception of a couple of patients (27) screening for mutation of the LHR in FMPP has been limited to only exon 11. In fact, the presence of activating mutations occurring in other part of the LHR gene cannot be completely ruled out. For example, constitutive activating mutations have been found in the extracellular domain of some GPCRs (28-30). Recent studies showed that amino acid residues in a region of the LHR extracellular domain near TM I are critical in ligand-mediated signaling (31,32). Thus, it is important to examine exons 1 to 10 of the LHR of patients in whom no mutation is found in exon 11.

The disease-causing mechanism of the activating mutations of the LHR is largely unknown. The majority of the activating mutations are found in TM VI of the LHR. TM VI has been shown to be involved in activating G_s during signal transduction (33,34). Studies also showed that the interhelical interactions of TM VI with TM V and TM VII are important in the ligand-independent activation of the LHR (35,36). Preliminary molecular modeling study and alignment of mutated amino acids on the same side of TM VI suggest that the amino acids affected by the disease-causing mutations of the LHR are in the interior of the helix bundle pointing towards the inside of the pocket formed by the seven TM helices (15). Thus mutation of these amino acids will alter the interhelical interaction and the conformation of the receptor leading to activation. This alteration may simply involve the opening of the pocket formed by the helices. Studies with natural mutations of Asp-578 in FMPP patients and site-directed mutagenesis showed that the degree of activation of the LHR by mutation of Asp-578 is largely determined by the size of the side-chain of the substituting amino acid and not by its charge (24,37). This led to the conclusion that the ability of Asp-578 to serve as a properly positioned hydrogen bond acceptor, rather than its negative charge, that is important for stabilizing the inactive state of the LHR (37). In fact the majority of the FMPP mutations involve substitution of one amino acid by another with the same charge but different size side-chain suggesting that constitutive activation of the receptor is likely to be the result of alteration in the distance among the TM helices. This agrees with the conclusions drawn from modeling studies with other GPCRs that receptor activation is accompanied by an "outward" movement of TM III and TM VI and that receptor conformation is the determinant of G-protein activation (38). Other type of interaction, such as electrostatic, has also been shown to be important in receptor activation. For example, it has been shown that the anionic charge of Asp-564 is important for maintaining the inactive conformation of the LHR (39). Replacement of Asp-564 by an amino acid with non-polar or cationic side-chain causes ligand-independent activation of the receptor in site-directed mutagenesis studies and in FMPP patients (15,25,39).

All FMPP mutations have been shown to confer constitutive activity to the mutated LHR by *in vitro* expression studies in COS-7 or HEK 293 cells (reviewed in 15). In spite of elevated basal level of intracellular cAMP, cAMP production of cells transfected with the mutated LHR still responds to hCG stimulation except in cells expressing LHR with the Leu457Arg (23), Ile542Leu, or the Cys581Arg mutation (15). Agonist affinity of the mutated receptors is largely unchanged while cell surface expression is either the same or reduced when compared to the wild-type receptor. The FMPP mutations have no or only slight effect on the inositol phosphate signaling pathway (40-42).

3.2. Effects on the male

Puberty normally occurs after the age of 9 in a boy. Normal sexual development at puberty is dependent on luteinizing hormone releasing hormone (LHRH), which regulates the secretion of LH and, in turn, the production of testosterone. Increased amounts of circulating testosterone lead to increase in height, development of secondary sexual characteristics, skeletal maturation, and the fusion of the epiphyses. Mutations resulting in constitutive activation of the LHR cause LHRH-independent isosexual precocious puberty in boys. Signs of puberty usually appear by 3-4 years of age in boys with FMPP (43). Histologic examination of testicular biopsy shows hyperplasia of Leydig cells (17). Affected boys have secondary sexual development with penile growth and bilateral enlargement of testes and pubic hair development indistinguishable from true puberty (44). Diagnosis is made by the findings of pubertal to adult levels of testosterone with normal clearance while the basal and LHRH-stimulated level of gonadotropins is appropriate for the age, i.e. prepubertal. There is also the lack of a pubertal pattern of LH pulsatility (45).

Combined treatment with an antiandrogen (spironolactone) and an inhibitor of aromatase activity (testolactone) has been shown to be effective in restoring both growth and bone maturation of FMPP patients to normal prepubertal rates (46). A recent study showed that upon the inception of true puberty at 11 year of age in a patient carrying the Asp578Gly mutation the gonadotropin responses normalized for the state of development. This observation suggests that the prepubertal activation of Leydig cells by the activating mutation of the LHR was incomplete (47). Post-pubertal development of FMPP patients is not affected. A normal adult pattern of LH secretion and normal gonadotropin response to LHRH is achieved in adulthood. Fertility is also largely unaffected in FMPP patients (48,49).

3.3. Effects on the female

Activating mutation of the LHR has no apparent effect on heterozygous female carriers. Hyperandrogenism, the presumed cause of polycystic ovarian disease, has been attributed to excess LH (50). Targeted over-expression of LH in transgenic mice causing ovarian hyperandrogenism has been shown to lead to infertility and the development of polycystic ovaries and ovarian tumors (51). In spite of such observations, similar condition has not been described in females carrying activating mutation of the LHR. Recent study in a female with two sons with FMPP also showed no developmental or reproductive abnormality (47). The authors of the study concluded that the mutation

may not have activated the LHR function beyond a pubertal level and thus is not sufficient to result in overproduction of androgens by theca cells. The observation that prolonged stimulation by FSH, which is not present in females carrying constitutively active LHR mutation, is required for the induction of ovarian cysts by CG (52) might also explain these phenomena.

3.4. Germline and somatic activating mutations in cancer

A number of studies have shown that constitutive activation of the signal transduction pathway of GPCRs as a result of mutations of either the receptor or the G protein may lead to neoplasia. Mutations of $G_1\alpha1$ and $G_1\alpha2$ have been described in functional sporadic thyroid adenomas and adrenal cortical tumors (53,54). Both germline and somatic activating mutations of the TSHR have been shown to be a cause of toxic thyroid adenoma (55). Sex hormones are known to stimulate the growth of cancer cells (56). Perinatal exposure to sex hormones has been proposed as one factor influencing the risk of malignant changes (57-59). Early exposure to prolonged elevated concentrations of testosterone occurs in FMPP patients. Thus these patients are potentially predisposed to the development of testicular tumor. However, due to the lack of long term follow-up of FMPP patients past puberty, it is not until recently that testicular tumor development in FMPP patients was reported.

In 1981, isosexual precocious puberty in a 9-year-old boy with nodular interstitial cell hyperplasia was described (60). Precocious puberty in the boy was diagnosed at 8 months of age basing on physical examination, and at 5 years of age, on bone age measurement and the presence of adult level of plasma testosterone. The diagnosis of a testicular interstitial cell tumor was made at 9 years of age. Histological examination showed the evidence of nodular hyperplasia in the testicular tissue surrounding the main tumor nodule. Since molecular analysis of the LHR was not available then, it is not known whether the testicular tumor in this boy is the result of FMPP due to constitutive activation of the LHR. The first case of a testicular germ cell tumor in a FMPP patient with the activating mutation Asp578Gly was published in 1998 (61). The patient was diagnosed to have FMPP at the age of 27 months and was found to have a testicular seminoma at 35 years of age. More recently, another FMPP patient with the activating mutation Asp564Gly developing two asymptomatic Leydig cell nodules was identified (EW Leschek, unpublished observations). Besides germline mutations as those found in the two FMPP patients, somatic activating mutation of the LHR has also been found in Leydig cell adenoma. Liu *et al.* (62) reported the presence of an activating mutation Asp578His in the LHR gene in Leydig cell adenoma tissue of three boys. This mutation was not present in adjacent normal tissue or blood cells. Thus, prolonged elevated levels of testosterone with onset in infancy appear to predispose the patient to the development of testicular tumor. This suggests that even though FMPP patients have no developmental problem after puberty, long term follow-up is warranted.

3.5. Genotype-Phenotype Correlation

There is no consensus on the phenotype-genotype correlation in FMPP (63,64). A European multicenter study showed much variability in the age of onset between cases with the same mutation as between cases with different mutation (25). While the mutated LHRs are present on the Leydig cells from birth, the age at which signs of puberty develop in boys with FMPP is around 3 years. This delay in phenotypic expression may depend on the relative expression of other genes critical for Leydig cell maturation as well as the extent to which the mutant allele is expressed as protein. The increase in plasma testosterone levels in response to exogenous hCG stimulation is similar in normal boys and in boys with FMPP (45,65). Therefore, in spite of its constitutive activity, the mutated LHRs would be expected to respond to high concentrations of hormone and produce a similar response as the wild-type receptor. Indeed, the majority of the mutated LHRs were found to be responsive to hCG stimulation when expressed in culture cells (reviewed in 15). However, there are two activating mutations, i.e. Ile542Leu and Cys581Arg, that render the mutated receptor unresponsive to ligand stimulation (63). Whether this difference in ligand responsiveness between the mutated LHRs causes any difference in clinical presentation of the disorder is unclear at the present time. Even though agonist affinity appears to be unaltered, cell surface expression of a number of mutated LHRs is reduced when compared to the wild-type (reviewed in 15). The cause of this reduced surface expression and its effect on disease presentation is also unclear.

It was observed that the patient with the Asp578Tyr mutation which led to the production of the highest basal level of cAMP in transfected cells presented at 1 year of age with signs of pubertal development (63,66). Similar observations were also reported by others (22,25). Thus, classes of activating mutations of the hLHR gene may exist for which genotype correlates with the phenotype of an unusually early clinical expression.

4. INACTIVATING MUTATIONS

Contrary to the gain-of-function activating mutations, inactivating mutations of the LHR are recessive in nature (67). In the male, inactivation of the LHR causes resistance to LH stimulation resulting in the failure of testicular Leydig cell differentiation. This gives rise to the disorder Leydig cell hypoplasia (LCH). Clinical presentation of LCH varies from male hypogonadism to a form of male pseudohermaphroditism with female external genitalia. In the female, inactivation of the LHR causes hypergonadotropic hypogonadism and primary amenorrhea.

4.1. Molecular genetics and biochemistry

Since the description of the first inactivating mutation of the LH receptor in a LCH patient in 1994 (68), twelve different mutations of the LHR which inactivate the LHR have been described. These mutations include single base substitutions, deletions, and in-frame insertion. The amino acid residues affected by the inactivating mutations are shown in Figure 1.

Table 2. Inactivating Mutations of Human LHR

Nucleotide Change	Amino Acid Change	Location	Case #	Genetics	Reference
33 bp insertion after nt[1] 54	11 a.a.[2] insertion	Extracellular	1	Heterozygous	71
81 bp deletion nt 606-680	27 a.a. deletion	Extracellular	1	Heterozygous	15
T 391 C	Cys 131 Arg	Extracellular	1	Homozygous	15
T1027 A	Cys343 Ser	Extracellular	1	Heterozygous	70
G 1060 A	Glu 354 Lys	Extracellular	1	Homozygous	80
T 1627 C	Cys 543 Arg	TM V	1	Heterozygous	70
C 1635 A	Cys 545 Stop	TM V	1	Heterozygous	15
C 1660 T	Arg 554 Stop	3rd cytoplasmic loop	3	Homozygous (2) Heterozygous (1)	15,72
G 1777 C	Ala 593 Pro	TM VI	1	Homozygous	15
Deletion of nt 1822-1827	- L608, V609	TM VII	1	Homozygous	77
C 1847 A	Ser 616 Tyr	TM VII	2	Homozygous (1) Heterozygous (1)	15
T 1874 A	Ile 625 Lys	TM VII	1	Homogenous	69

Note: Only references published after review 15 are listed. Please see bibliography of review 15 for earlier publications. nt [1] : Nucleotide number, a.a.[2] : Amino acid

Nine single base substitutions leading to either missense mutations (Cys131Arg, Cys343Ser, Glu354Lys, Cys543Arg, Ala593Pro, Ile625Lys, and Ser616Tyr) or non-sense mutations (Cys545Stop and Arg554Stop) have been identified in ten LCH kindreds (references in Table 2). The Arg554Stop mutation was also identified in one LHR allele of a Brazilian family (72). Patients in seven of the LCH kindreds have homozygous mutations while those in three LCH kindreds have compound heterozygous mutations. All single base substitutions affect amino acids, which are conserved among the LHR of different species (73) as well as among the three glycohormone receptors (3).

Inactivation of the LHR due to partial gene deletion has been demonstrated in two cases. In one kindred with compound heterozygous mutations of the LHR, one allele carries the Ser616Tyr single base substitution, while exon 8 of the other LHR allele is deleted (74). Exon 8 of the human LHR encodes part of the Leucine-rich repeats in the extracellular domain which are responsible for specific high-affinity binding of the hormone (75,76). A 46 XY pseudohermaphrodite and his 46 XX sister inherited a homozygous deletion of nucleotides 1822-1827 from their consanguineous parent (77). This causes the deletion of the highly conserved amino acid residues Leu-608 and Val-609 within TM VII of the LHR (3,73). Insertion can also inactivate the LHR. A 33-bp in-frame insertion has been found in the maternal LHR allele in a kindred with two 46 XY male pseudohermaphrodites (71). The paternal LHR allele of the patients carries the Cys545Stop nonsense mutation (78). The insertion occurs between amino acid residues 18 and 19, immediately upstream of the signal peptide cleavage site (75).

The genetic cause and location of the inactivating mutations are more variable than the activating mutations. There is also no predominant form of inactivating mutation. There are a number of LCH kindreds in which mutations of the LHR have not been identified (SM Wu & WY Chan, unpublished observations) indicating that inactivating mutations are very heterogeneous or that LCH is caused by mutation of the LHR as well as other gene(s).

The effect of most of these inactivating mutations on the signal transduction activity of the LHR has been demonstrated by *in vitro* expression studies in either HEK 293 or COS-7 cells. Mutations in the extracellular domain that affect hormone binding often lead to diminution but not absence of signal transduction (74,79,80). This is probably due to the presence of low affinity binding sites for the hormone in the transmembrane domain of the receptor (1). Thus mutations in the extracellular domain such as Cys131Arg (79), Glu354Lys (80), and exon 8 deletion (74) led to impaired cAMP response of cells expressing LHR with such mutations. On the other hand, the effect of mutations in the transmembrane domain on the signal transduction activity of the receptor is more variable largely depending on the role of the affected amino acid residue in processes such as trafficking, coupling efficiency, etc. (1,33,81,82). For example, cell surface expression of the LHR appears to be affected by the presence of the Ser616Tyr mutation (74) and the coupling efficiency appears to be affected by mutations such as Ala593Pro (83) and Ile625Lys (69). Deletion of a couple of amino acids in this region (77) or nonsense mutations that cause premature truncation of the receptor (72,78,84) are likely to be more disruptive causing abolition of signal transduction. For example Cys545Stop mutation (78) causes truncation of the receptor and the loss of transmembrane helix (TM) VI, which is known to be critical for signal transduction (33), and residues in the cytoplasmic C-domain, which have been shown to be critical for cell surface expression of the receptor (81,82). Cells expressing LHR with the Cys545Stop mutation did not respond to hCG stimulation (78).

4.2. Effect on the male

A number of features distinguish LCH from the other forms of male pseudohermaphroditism (85). LCH is

an autosomal recessive disorder. Patients are genetic males with a 46 XY karyotype (67). The hormonal profile of LCH patients shows elevated serum level of LH, normal to elevated level of FSH, and low level of testosterone, which is unresponsiveness to CG stimulation. Clinical presentation of LCH is variable. In its severe form, the patient has normal-appearing female external genitalia often leading to female sex assignment. In its mild form the patient has hypergonadotropic hypogonadism with microphallus and hypoplastic male external genitalia (86). In between are patients with variable degree of masculinization of the external genitalia. Müllerian derivatives are not present in LCH patients and the testes are cryptorchid. Histological examination of the testis often shows relatively well preserved seminiferous tubules and absence of mature Leydig cells. In some cases with partial or transient fetal Leydig cell function, there may be posterior labial fusion associated with the presence of the vas deferens and epididymis (reviewed in 8 and 15). LCH patients show no development of either male or female secondary sexual characteristics at puberty.

4.3. Effects on the female

In the female, inactivation of the LHR resulting in abnormal LH action causes subnormal follicular development and ovulation, amenorrhea, and infertility. Due to the later onset time and milder disease phenotype, 46 XX females with homozygous or compound heterozygous inactivating mutations of the LHR are more difficult to identify unless an LCH male sibling is present. Studies of several 46 XX females with homozygous or compound heterozygous inactivating mutations of the LHR showed them to have normal breast and pubic hair development at puberty, develop hypergonadotrophic hypogonadism, have amenorrhea or oligoamenorrhea and enlarged cystic ovaries, and infertility (77,80,84,87,88). In spite of the fact that they manifest ovarian resistance to LH follicular development is not compromised (72).

4.4. Genotype-phenotype correlation

Different from FMPP, clinical presentation of LCH patients can be correlated with the amount of residual activity of the mutated LHR. Patients with the severe phenotype, i.e. male pseudohermaphroditism, have mutated LHRs that either fail to be expressed on the cell surface or are unable to transduce the signal of hormone binding. Thus, mutations such as homozygous Glu354Lys (80), Ala593Pro (83), and Leu-608-Val-609 deletion (77), and compound heterozygous Cys545Stop/insertion mutations (71,78) are found in male pseudohermaphrodites with female external genitalia and undescended testis. On the other hand, patients with male hypogonadism have mutated LHRs which have reduced, but not absent, cell surface hCG binding and signal transduction. Thus, homozygous Cys131Arg (79) and Ile625Lys (69), or compound heterozygous Ser616Tyr/exon 8 deletion (74) are found in patients with micropenis and hypospadias.

5. PERSPECTIVE

The occurrence of natural mutations that inactivate the LHR or activate it constitutively offers a unique opportunity to study the mechanism of signal transduction by this receptor, and because of high homology, the other glycohormone receptors. Both FMPP and LCH mutations give clearly identifiable phenotype and thus offer much advantage over artificial mutations generated by site-directed mutagenesis. Identification of new mutations in the other parts of the receptor will further our understanding of the interaction among different regions of the receptor molecule during signal transduction. Future studies on the biochemical and biophysical effects of the mutations identified in FMPP and LCH patients are expected to provide better understanding of the mechanism of activation of the glycohormone receptors.

Clinically, it is important to identify as many mutations as possible so that more accurate diagnosis can be achieved. Since all known activating mutations of the LHR are clustered in the transmembrane domain, molecular diagnosis of this disorder can be accomplished relatively easily. However, searching the other regions of the LHR gene for mutations in FMPP patients in whom no mutation can be found in exon 11 will enhance the accuracy of the molecular diagnosis. The observation of the potential predisposition of FMPP patients to the development of testicular tumor makes accurate diagnosis of this condition even more important. Molecular diagnosis for LCH is less established. Until we know more about the molecular genetics of this disorder, molecular diagnosis of LCH is rather difficult. Accurate diagnosis is important for LCH since there are many causes of male pseudohermaphroditism. Identification of the correct molecular genetic defect will enhance prognosis and the design of the most appropriate treatment modality. Finally, an in-depth understanding of the molecular genetics of mutation causing these diseases is the prerequisite for their ultimate treatment by gene therapy.

6. ACKNOWLEDGMENTS

We are indebted to Drs. Gordon B. Cutler, Jr and Aaron J.W. Hsueh for their invaluable help. We also like to thank all colleagues who referred their patients to us for our study.

7. REFERENCES

1. ML Dufau: The luteinizing hormone receptor. In: The Leydig Cell. Eds: Payne AH, Hardy MP, Russell LD. Cache River Press, IL 333-350 (1996)
2. TP Iismaa, TJ Biden & J Shine: G Protein-Coupled Receptors. R.G. Landes Co., TX (1995)
3. W. C. Probost, L. A. Snyder, D. I. Schuster, J. Brosius & S. C. Sealfon: Sequence alignment of the G-protein coupled receptor superfamily. *DNA Cell Biol* 11, 1-20 (1992)
4. BA Cooke: Transduction of the luteinizing hormone signal within the Leydig cell. In: The Leydig Cell. Eds: Payne AH, Hardy MP, Russell LD. Cache River Press, IL 351-363 (1996)
5. MR Waterman: Production of sex steroids. In: Sex Differentiation: Clinical and Biological Aspects. Ed: Hughes IA. Frontiers in Endocrinology, Vol. 20. Serono Symposia, Rome, Italy 61-69 (1996)
6. A. Shenker, L. Laue, S. Kosugi, J. J. Merendino Jr, T. Minegishi & G. B. Cutler Jr: A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. *Nature* 365, 652-654 (1993)

7. P. L. M. Dahia & S. P. A. Toledo: Peptide hormone receptor defects leading to abnormal phenotypes. *Curr Opin Endocrinol Diab* 3, 132-137 (1996)
8. G. S. Conway: Clinical manifestations of genetic defects affecting gonadotrophins and their receptors. *Clin Endocrinol* 45, 657-663 (1996)
9. A. P. N. Themmen & H. G. Brunner: Luteinizing hormone receptor mutations and sex differentiation. *Eur J Endocrinol* 134, 533-540 (1996)
10. A. P. N. Themmen, J. W. M. Martens & H. G. Brunner: Gonadotropin receptor mutations. *J Endocrinol* 153, 179-183 (1997)
11. L. A. DiMeglio & O. H. Pescovitz: Disorder of puberty: Inactivating and activating molecular mutations. *J Pediatr* 131, S8-12 (1997)
12. W. Y. Chan & G. B. Cutler, Jr: Mutations of gonadotrophin receptors leading to loss and gain of function. *Top Endocrinol* 8**,** 9-12 (1998)
13. A Shenker: Disorders caused by mutations of the lutropin/choriogonadotropin receptor gene. In: Contemporary Endocrinology: G Proteins, Receptors, and Disease. Ed: Spiegel AM. Humana Press Inc., NJ 139-152 (1998)
14. W. Y. Chan & G. B. Cutler, Jr: Molecular aspects of precocious puberty. *Adv. Mol. Cell. Endocrinol* 2, 121-141 (1998)
15. W. Y. Chan: Molecular genetics, biochemical and clinical implications of gonadotropin receptor mutations. *Mol Genet Metab* 63, 75-84 (1998)
16. A. C. Latronico & D. L. Segaloff: Naturally occurring mutations of the luteinizing-hormone receptor: Lessons learned about reproductive physiology and G protein-coupled receptors. *Am J Hum Genet* 65, 949-958 (1999)
17. H.K. Schedewie, E. O. Reiter, I. Z. Beitins, S. Seyed, V. D. Wooten, J. F. Jimenez, E. Aiman, G. W. de Vane, J. F. Redman & J. M. Elders: Testicular Leydig cell hyperplasia as a cause of familial sexual precocity. *J Clin Endocrinol Metab* 52, 271-278 (1981)
18. S. M. Rosenthal, M. .M. Grumbach & S. L. Kaplan: Gonadotropin-independent familial sexual precocity with premature Leydig and germinal cell maturation (familial testotoxicosis): Effects of a potent luteinizing hormone-releasing factor agonist and medroxyprogesterone acetate therapy in four cases. *J Clin Endocrinol Metab* 57, 571-579 (1983)
19. S. Bertelloni, G. I. Baroncelli, R. Lala, M. Cappa, P. Matarazzo, C. de Sanctis & G. Saggese: Long-term outcome of male-limited gonadotropin-independent precocious puberty. *Horm Res* 48, 235-239 (1998)
20. G. Praksasam, M. Castro-Magana, R. Arguello, M. Angulo, A. Canas, R. Kam, P. Vitollo & W. Y. Chan: Hormonal profile during treatment in familial male-limited precocious puberty due to a novel heteroxygous activating mutation of the LH receptor. *Program and Abstract. The 80th Annual Meeting of the Endocrine Society* p. 283 (1998)
21. J. Gromoll, C.-J. Partsch, M. Simoni, V. Nordhoff , W. G. Sippell, E. Nieschlag & B. B. Saxena: A mutation in the first transmembrane domain of the lutropin receptor causes male precocious puberty. *J Clin Endocrinol Metab* 83, 476-480 (1998)
22. J. Müller, B. Gondos, S. Kosugi, T. Mori & A. Shenker: Severe testotoxicosis phenotype associated with Asp578–Tyr mutation of the lutrophin/ choriogonadotrophin receptor gene. *J Med Genet* 35, 340-341 (1998)
23. A. C. Latronico, A. N. Abell, I. J. P. Arnhold, X. Liu, T. S. S. Lins, V. N. Brito, A. E. Billerbeck, D. L. Segaloff & B. B. Mendonca: A unique constitutively activating mutation in third transmembrane helix of the luteinizing hormone receptor causes sporadic male gonadotropin-independent precocious puberty. *J Clin Endocrinol Metab* 83, 2435-2440 (1998)
24. S. M. Wu, E. W. Leschek, C. Brain & W. Y. Chan: A novel activating mutation of the luteinizing hormone/chorionic gonadotropin receptor in a patient with FMPP - Effects of size versus charge. *Mol Genet Metab* 66, 68-73 (1999)
25. H. Kremer, J. W. M. Martens, M. van Reen, M. Verhoef-Post, J. M. Wit, B. J. Otten, S. L. S. Drop, H. A. Delemarre-van de Waal, M. Pombo-Arias, F. De Luca, N. Potau, J. M. H. Buckler, M. Jansen, J. S. Parks, H. A. Latif, G. W. Moll, W. Epping, G. Saggese, E. C. M. Mariman, A. P. N. Themmen & H. G. Brunner: A limited repertorie of mutations of the luteinizing hormone (LH) receptor gene in familial and sporadic patients with male-limited precocious puberty. *J Clin Endocrinol Metab* 84, 1136-1140 (1999)
26. A. C. Latronico, V. N. Brito, T. S. Lins, I. J. P. Arnhold & B. B. Mendonca: Effects of three distinct activating mutations of the luteinizing hormone receptor (LHR) on pituitary-gonadal axis in both sexes. *Program and Abstracts. The 81th Annual Meeting of the Endocrine Society* p. 310 (1999)
27. L. Laue, S. M. Wu, M. Kudo, A. J. W. Hsueh, G. B. Cutler, Jr, D. H. Jelly, F. B. Diamond & W. Y. Chan: Heterogeneity of activating mutations of the human luteinizing hormone receptor in male-limited precocious puberty. *Biochem Mol Med* 58, 192-198 (1996)
28. M. R. Pollack, E. M. Brown, H. L. Estep, P. N. McLaine, O. Kifor, J. Park, S. C. Hebert, C. E. Seidman & J. G. Seidman: Autosomal dominant hypocalcaemia caused by a Ca^{2+}-sensing receptor gene mutation. *Nat Genet* 8, 303- 307 (1994)
29. J. Baron, K. K. Winer, J. A. Yanovski, A. W. Cunningham, L. Laue, D Zimmerman & G. B. Cutler, Jr: Mutations in the Ca^{2+}-sensing receptor gene cause autosomal dominant and sporadic hypoparathyroidism. *Hum Mol Genet* 5, 601-606 (1996)
30. L. Duprez, J. Parma, S. Costagliola, J. Hermans, J. van Sande, J. E. Dumont & G. Vassart: Constitutive activation of the TSH receptor by spontaneous mutations affecting the N-terminal extracellular domain. *FEBS Lett*. 409, 469-474 (1997)
31. J. Huang & D. Puett: Identification of two amino acid residues on the extracellular domain of the lutropin/choriogonadotropin receptor important in signaling. *J Biol Chem* 270, 30023-30028 (1995)
32. C. A. Alvarez, P. Narayan, J. Huang & D. Puett: Characterization of a region of the lutropin receptor extracellular domain near transmembrane helix I that is important in ligand-mediated signaling. *Endocrinology* 140, 1775-1782 (1999)
33. A. N. Abell & D. L. Segaloff: Evidence for the direct involvement of transmembrane region 6 of the lutropin/choriogonadotropin receptor in activating Gs. *J Biol Chem* 272, 14586-14591 (1997)
34. A. N. Abell, D. J. McCormick & D. L. Segaloff: Certain activating mutations within helix 6 of the human luteinizing hormone receptor may be explained by alterations that allow

transmembrane regions to activate Gs. *Mol Endocrinol* 12, 1857-1869 (1998)
35. M. K.udo, Y. Osuga, B. K. Kobilka & A. J. W. Hsueh: Transmembrane regions V and VI of the human luteinizing hormone receptor are required for constitutive activation by a mutation in the third intracellular loop. *J Biol Chem* 271, 22470-22478 (1996)
36. S. Kosugi, Z. Lin, R. Pearlstein, T. Mori & A. Shenker: Evidence that interhelical hydrogen bonding between Asp-578 and Asn 619 helps maintain the inactive conformation of the human lutropin receptor (LHR). *Program and Abstracts. The 79th Annual Meeting of the Endocrine Society* p.171 (1997)
37. S. Kosugi, T. Mori & A. Shenker: The role of Asp^{578} in maintaining the inactive confromation of the human lutropin/choriogonadotropin receptor. *J Biol Chem* 271, 31813-31817 (1996)
38. J. Wess: G-protein-coupled receptors: Molecular mechanisms involved in receptor activation and selectivity of G-protein recognition. *FASEB J* 11,346-354 (1997)
39. S. Kosugi, T. Mori & A. Shenker: An anionic residue at position 564 is important for maintaining the inactive conformation of the human lutropin/choriogonadotropin receptor. *Mol Pharmacol* 53, 894-901 (1998)
40. Y. Yano, M. Saji, A. Hidaka, N. Moriya, A. Okuno, L. D. Kohn & G. B. Cutler Jr: A new constitutively activating point mutation in the luteinizing hormone/chorionic gonadotropin receptor gene in cases of male-limited precocious puberty. *J Clin Endocrinol Metab* 80, 1162-1168 (1995)
41. S. Kosugi, C. Van Dop, M. E. Geffner, W. Rabl, J. C. Carel, J. L. Chaussain, T. Mori, J. J. Merendino & A. Shenker: Characterization of heterogeneous mutations causing constitutive activation of the luteinizing hormone receptor in familial male precocious puberty. *Hum Mol Genet* 4:183-188, (1995)
42. Y. Yano, A. Hidaka, M. Saji, A. Polymeropoulos, A. Okuno, L. D. Kohn & G. B. CutlerJr. A sporadic case of male-limited precocious puberty has the same constitutively activating point mutation in luteinizing hormone/choriogonadotropin receptor as familial cases. *J Clin Endocrinol Metab* 79, 1818-1823 (1994)
43. GB Cutler Jr: Overview of premature sexual development. In: Sexual Precocity: Etiology, Diagnosis, and Management. Eds. Grave GD, Cutler GB Jr. Raven Press, NY 1-10 (1993)
44. S. Berttelloni, G. I. Baroncelli, R. Lala,. M. Cappa, P. Matarazzo, C. de Samctos & G. Saggese: Long-term outcome of male-limited gonadotropin-independent precocious puberty. *Horm Res* 48, 235-239 (1997)
45. F. J. Holland: Gonadotropin-independent precocious puberty. *Endocrinol. Metab. Clin. North Amer* 20, 191–210 (1991)
46. L. Laue & G. B. Cutler Jr: Current treatment of familial male precocious puberty. In: Sexual Precocity: Etiology, Diagnosis, and Management. Eds. Grave GD, Cutler GB Jr. Raven Press, NY 193-205 (1993)
47. I. M. Rosenthal, S. Refetoff, B. Rich, R. B. Barnes, T. Sunthornthepvarakul, J. Parma & Rosenfield R. L.: Response to change with gonadotropin-releasing hormone agonist in a mother and her two sons with a constitutively activating mutation of the luteinizing hormone receptor - a clinical research center study. *J Clin Endocrinol Metab*. 81, 3802-3806 (1996)
48. MM Grumbach & DM Styne: Puberty: Ontogeny, neuroendocrinology, physiology, and disorders. In: Williams Textbook of Endocrinology. Eds: Wilson JD, Foster DW, Kronenberg HM, Larsen PR. 9th Edition. WD Saunders, NY 1509-1625 (1998)
49. G. B. Kletter & R. P. Kelch: Disorders of puberty in boys. *Endocrinol Metab Clin North Amer* 22, 455-477 (1993)
50. G. W. De Vane, N. M. Czekala, H. L. Judd & S. S. C. Yen : Circulating gonadotropins, estrogens, and androgens in polycystic ovarian disease. *Am J Obstet Gynecol* 121, 496-500 (1975)
51. K. A. Risma, C. M. Clay, T. M. Nett, T. Wagner, J. Yun & J. H. Nilson: Targeted overexpression of luteinizing hormone in transgenic mice leads to infertility, polycystic ovaries and ovarian tumors. *Proc Natl Acad Sci, USA*. 92, 1322-1326 (1995)
52. K. Bogovich: Prolonged stimulation of follicle-stimulating hormone is required for the induction of ovarian follicular cysts by human chorionic gonadotropin in hypophysectomized rats. *Endocrine* 4, 107-114 (1997)
53. L. Laue: Ligand-independent hormone secretion. *Curr Opin Pediatr* 7, 434-439 (1995)
54. L. S. Weinstein & A. Shenker: G protein mutations in human disease. *Clin Biochem* 26, 333-338 (1993)
55. J. van Sande, J. Parma, M. Tonacchera, S. Swillens, J. Dumont & G. Vassart: Genetic basis of endocrine disease. Somatic and germline mutations of the TSH receptor gene in thyroid disease. *J Clin Endocrinol Metab* 80, 2577-2585 (1995)
56. E. Venturelli, D. Coradini, D. Gornati & G. Secreto: Growth stimulatory effect and metabolism of testosterone in MCF-7 human breast cancer cells. *Internat J Oncol* 8, 687-692 (1996)
57. E. Rajpert-De Meyts & N. E. Skakkabaek: The possible role of sex hormones in the development of testicular cancer. *Euro Urol* 23, 54-59 (1993)
58. E. Rajpert-De Meyts, N. Jorgensen, J. Muller, A. Giwercman & N. E. Skakkabaek: Origin of germ cell tumors. In: Sex Differentiation: Clinical and Biological Aspects. Frontiers in Endocrinology 20. Ed. Hughes IA. Serono Symposia, Rome, Italy. 45-54 (1996)
59. O. Akre, A. Ekbom, C. C. Hsieh, D. Trichopoulos & H. O. Adami: Testicular nonseminoma and seminoma in relation to perinatal characteristics. *J Nat Canc Inst* 88, 883-889 (1996)
60. J. Rottger, D. R. Hadden, E. Morrison & F. McKeown: Isosexual precocious puberty in a 9-year-old boy: nodular interstitial cell hyperplasia. *J Royal Soc Med* 74, 66-68 (1981)
61. M. M. Martin, S. M. Wu, A. L. A. Martin, O. M. Rennert & W. Y. Chan: Testicular seminoma in a patient with a constitutively activating mutation of the luteinizing hormone/chorionic gonadotropin receptor. *Euro J Endocrinol* 139, 101-106 (1998)
62. G. Liu, L. Duranteau, J. Monroe, D. A. Doyle, J. C. Carel & A. Shenker: A novel somatic mutation of the lutropin receptor (LHR) gene in Leydig cell adenoma. *Program and Abstracts. The 80th Annual Meeting of the Endocrine Society* p. 62 (1998)
63. L. Laue, W. Y. Chan, A. J. W. Hsueh, M. Kudo, S. Y. Hsu, S. M. Wu, L. A. Blomberg & G. B. Cutler Jr: Genetic heterogeneity of constitutively activating mutations of the human luteinizing hormone receptor in familial male precocious puberty. *Proc Natl Acad Sci USA*. 92, 1906-1910 (1995)
64. B. A. J. Evans, D. J. Bowen, P. J. Smith, P. E. Clayton & J. W. Gregory: A new point mutation in the luteinizing hormone receptor gene in familial and sporadic male-limited precocious puberty: Genotype does not always correlate with phenotype. *J Med Genet* 33, 143-147 (1996)
65. K. E. Oerter, M. Uriarte, S. R. Rose, K. M. Barnes & G. B. Cutler, Jr: Gonadotropin secretory dynamics during puberty in

normal girls and boys. *J Clin Endocrinol Metab* 71, 1251-1258 (1990)
66. P. A. Clark & W. L. Clarke: Testotoxicosis: An unusual presentation and novel gene mutation. *Clin Pediat* 34,271-274 (1995)
67. P. H. Saldanha, I. J. P. Arnhold, B. B. Mendonça, W. Bloise & S. P. A. Toledo: A clinico-genetic investigation of Leydig cell hypoplasia. *Am J Med Genet* 26, 337-344 (1987)
68. L. Laue, S. M. Wu, M. Kudo, A. J. W. Hsueh, J. E. Griffin, J. D. Wilson, D. B. Grant, A. C. Berry, G. B. Cutler, Jr. & W. Y. Chan: The Gene defect that causes genetically XY males to develop as apparent females (Leydig cell hypoplasia). In: Selected Biomedical Research Summaries, 1994 ASCB Annual Meeting. Third Annual Press Book, American Society for Cell Biology, MD, p.1 (1994)
69. J. W. M. Martens, M. Verhoef-Post, N. Abelin, M. Ezabella, S. P. A. Toledo, H. G. Brunner & A. P. N. Themmen: A homozygous mutation in the luteinizing hormone receptor causes partial Leydig cell hypoplasia: correlation between receptor activity and phenotype. *Mol Endocrinol.* 12, 775-784 (1998)
70. S. Lumbroso, M. Szarras-Czapnik, C. Caubel, I. Ringeard, T. E. Romer & C. Sultan: Novel compound heterozygous mutations of luteinizing hormone receptor gene in a patient with male pseudohermaphroditism due to Leydig cell hypoplasia. *Program and Abstracts. The 80th Annual Meeting of the Endocrine Society* p. 283 (1998)
71. S. M. Wu, K. Hallermeier, L. Laue, C. Brain, E. C. Berry, D. B. Grant, J. E. Griffin, J. D. Wilson, G. B. Cutler, Jr. & W. Y. Chan: Inactivation of the luteinizing hormone/chorionic gonadotropin receptor by an insertional mutation in Leydig Cell Hypoplasia. *Mol Endocrinol* 12, 1651-1660 (1998)
72. I. J. P. Arnhold, A. C. Latronico, M. C. Batista & B. B. Mendonca: Menstrual disorders and infertility caused by inactivating mutations of the luteinizing hormone receptor gene. *Fertil. Steril.* 71,597-601 (1999)
73. X. C. Jia, M. Oikawa, M. Bo, T. Tanaka, T. Ny, I. Boime & A. J. W. Hsueh: Expression of human luteinizing hormone (LH) receptor: Interaction with LH and chorionic gonadotropin from human but not equine, rat, and ovine species. *Mol Endocrinol* 5, 759-768 (1991)
74. L. Laue, S. M. Wu, M. Kudo, C. J. Bourdony, G. B. Cutler, Jr., A. J. W. Hsueh & W. Y. Chan: Compound heterozygous mutations of the luteinizing hormone receptor gene in Leydig cell hypoplasia. *Mol Endocrinol* 10, 987-997 (1996)
75. K. C. McFarland, R. Sprengel, H. S. Phillips, M. Kohler, N. Rosemblit, K. Nikolics, D. L. Segaloff & P. H. Seeburg: Lutropin-chorionadotropin receptor: An unusal member of the G protein-coupled receptor family. *Science* 245, 494-499 (1989)
76. D. Thomas, T. G. Rozell, X. Liu & D. L. Segaloff: Mutational analysis of the extracellular domain of the full-length lutropin/choriogonadotropin receptor suggest leucine-rich repeats 1-6 are involved in hormone binding. *Mol Endocrinol* 10, 760-768 (1996)
77. A. C. Latronico, Y. Chai, I. J. P. Arnhold, X. Liu, B. B. Mendonca & D. L. Degaloff: A homozygous microdeletion in helix 7 of the luteinizing hormone receptor associated with familial testicular and ovarian resistance is due to both decreased cell surface expression and impaired effector activation by the cell surface receptor. *Mol Endocrinol 12*, 442-450 (1998)
78. L. Laue, S. M. Wu, M. Kudo, A. J. W. Hsueh, G. B. Cutler, Jr., J. E. Griffin, J. D. Wilson, C. Brain, A. C. Berry, D. B. Grant & W. Y. Chan: A nonsense mutation of the human luteinizing hormone receptor gene in Leydig cell hypoplasia. *Hum Mol Genet* 8, 1429-1433 (1995)
79. M. Misrahi, G. Meduri, S. Pissard, C. Bouvattier, I. Beau, H. Loosfelt, A. Jolivet, R. Rappaport, E. Milgrom & P. Bougneres: Comparison of immunocytochemical and molecular features with the phenotype in a case of incomplete male pseudohermaphroditism associated with a mutation of the luteinizing hormone receptor. *J Clin Endocrinol Metab* 82, 2159-2165 (1997)
80. S. S. Stavrou, Y. S. Zhu, L. Q. Cai, M. D. Katz, C. Herrera, M. DeFillo-Ricart & J. Imperato-McGinley: A novel mutation of the human luteinizing hormone receptor in 46XY and 46XX sisters. *J Clin Endocrinol Metab* 83, 2091-2098 (1998)
81. N. Kawata & K. M. J. Menon: Palmitoylation of luteinizing hormone/human choriogonadotropin receptors in transfected cells. *J Biol Chem* 269, 30651-30658 (1994).
82. M. C. Rodriguez, Y. B. Xie, H. Wang, K. Collison & D. L. Segaloff: Effects of truncations of cytoplasmic tail of the LH/CG receptor on receptor-mediated hormone internalization. *Mol Endocrinol* 6, 327-336 (1992)
83. H. Kremer, R. Kraaij, S. P. A. Toledo, M. Post, J. B. Fridman, C. Y. Hayashida, M. van Reen, E. Milgrom, H. H. Ropers, E. Mariman, A. P. N. Themmen & H. G. Brunner: Male pseudohermaphroditism due to a homozygous missense mutation of the luteinizing hormone receptor gene. *Nat Genet* 9, 160-164 (1995).
84. A. C. Latronico, J. Anasti, I. J. P. Arnhold, R. Rapaport, B. B. Mendonca, W. Bloise, M. Castro, C. Tsigos & G. P. Chrousos: Brief Report: Testicular and Ovarian resistance to luteinizing hormone caused by inactivating mutations of the luteinizing hormone-receptor gene. *New Engl J Med* 334, 507-512 (1996)
85. J. D. Zajac & G. L. Warne: Disorders of sexual development. *Bailliere's Clin Endocrinol Metab* 9, 555-579 (1995)
86. S. P. A. Toledo: Leydig cell hypoplasia leading to 2 different phenotypes: Male pseudohermaphroditism and primary hypogonadism not associated with this. *Clin Endocrinol*. 36, 521-522 (1992)
87. S. P. A. Toledo, H. G. Brunner, R. Kraaij, M. Post, P. L. M. Dahia, C. Y. Hayashida, H. Kremer, & A. P. N. Themmen: An inactivating mutation of the luteinizing hormone receptor causes amenorrhea in a 46,XX female. *J Clin Endocrinol Metab.* 81, 3850-3864 (1996)
88. S. P. A. Toledo: Inactivating mutations of the LH receptor gene: more than two different phenotypes. *Eur J Endocrinol* 140, 186 (1999)

Key Words: Molecular Endocrinology, Luteinizing Hormone Receptor, Activating, Familial Male-Limited Precocious Puberty, Inactivating, Leydig Cell Hypoplasia, Male Pseudohermaphroditism, Amenorrhea, Review

Send correspondence to: Dr Wai-Yee Chan, Department of Pediatrics, Georgetown University Children's Medical Center, 3800 Reservoir Road, NW, Washington, DC 20007, Tel:202-687-7068, Fax:202-687-1629, E-mail: chanw@gunet.georgetown.edu

This manuscript is available in full text on line at:

http://www.bioscience.org/2000/v5/d/wu/fulltext.htm