A novel compound heterozygous mutation of the luteinizing hormone receptor –implications for fertility

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Abstract The luteinizing hormone/chorionic gonadotropin receptor (LHCGR) belongs to the family of G-protein coupled receptors and binds both luteinizing hormone (LH) and human chorionic gonadotropin (hCG). Ligand-receptor interaction mediates a downstream cascade of events which is essential for ovulation in women, and expression of the male phenotype in men. The human LHCGR gene consists of 11 exons and 10 introns. Homozygous and compound heterozygous mutations may inactivate the receptor by altering its structure and subsequent function. Herein we reported a novel, compound heterozygous inactivating LHCGR mutation in a woman who presented with secondary infertility, having previously carried to term a donor oocyte pregnancy. A 27 bp deletion was detected in exon I at amino acid number 12. This mutation involved the signal peptide region, which is important for protein targeting, maturation and cellular expression. Another mutation involving a 2 base pair (thymine and cytosine) deletion was detected in exon 11 at amino acid number 586. This deletion produced a frameshift resulting in a premature stop codon and a truncated protein. An XY sibling with the same mutations was phenotypically female and misdiagnosed as complete androgen insensitivity syndrome. Other unaffected family members were genetically tested and carried one of the two mutations.

Capsule A novel compound heterozygous mutation involving exon 1 and 10 resulting in LH/hCG resistance, infertility and female phenotype in XY and XX siblings.

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Introduction

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) secreted by gonadotrophs located in the anterior pituitary gland are essential for folliculogenesis and regulation of ovulation. In women, FSH is required for the monthly recruitment and growth of cohorts of developing follicles while LH activity mediates the final stages of follicle maturation and induces a cascade of events leading to ovulation and the formation and function of the corpus luteum [1]. During the late stages of follicular development, mural granulosa cells acquire LH/chorionic gonadotropin receptors (LHCGR) and become responsive to the presence of the ligand. Our understanding of the downstream events following LH binding is far from complete. A diverse group of signaling molecules functioning through various mechanisms has been implicated. LH binding stimulates increased cyclic AMP/protein kinase A production and activates the epidermal growth factor network (EGF) which subsequently increases prostaglandin formation, mevalonate kinase production and activates other cascades [2]. Extensive paracrine and autocrine communication between the ovum and surrounding cells mediated through distinct signaling pathways is important for folliculogenesis and may affect ovulation through cumulus cell apoptosis, cumulus expansion and mucification, as well as LH receptor expression [3]. Steroidogenesis is also dependent on LH activity for androgen production in the theca cells, a substrate for estrogen production, and for estrogen and progesterone production by the corpus luteum.

During the past two decades, a few reports were published describing activating and inactivating LHCGR mutations in

both sexes. The first to be described were males (46XY) with an ambiguous sexual phenotype. The first reports on women bearing inactivating LHCGR mutations were in sisters of affected males. Women (46XX karyotype) who carry inactivating mutations usually show normal external female genital and breast development but may have delayed or absent menarche. They are resistant to the action of LH and hence are infertile. They are prone to ovarian cyst formation and are usually diagnosed after discovering an affected 46XY sibling [4, 5]. LHCGR are present on the fetal testicular leydig cells and exert a prominent role in male development. During early pregnancy and embryogenesis, hCG binds to these receptors in utero producing testosterone and dihydrotestosterone, which are essential for male sexual differentiation. Pubertal maturation, male sexual function and fertility are also dependent on proper function of LH [4]. Individuals with the 46XY genotype carrying inactivating mutations present with leydig cell hypoplasia (LCH). These individuals can express either the partial or complete form depending on the severity of the mutation [6]. Complete inactivating mutations result in a complete female phenotype and male pseudohermaphroditism and may present with a blind vagina, amenorrhea and absent breast development. Partial LHCGR resistance leads to a milder phenotype with the presence of micropenis or hypospadias [7].

The human LHCGR gene consists of 11 exons and 10 introns, is located on the short arm of chromosome 2 and encodes for a highly glycosylated mature glycoprotein receptor containing 699 amino acids with a molecular weight of 85–95KDa [8]. It belongs to the superfamily of G protein coupled receptors and is further categorized into group A [9]. Other members of this group include the rhodopsin receptor, β 2-adrenergic receptor, TSH and FSH receptors [9]. Both placental hCG and pituitary LH are natural ligands capable of binding to LHCGR. Structurally the receptor is composed of the N terminal exodomain and the C terminal endodomain. The exodomain is coded by exons 1–10 as well as a small portion of exon 11 and is involved in hormone binding. The endodomain which includes the transmembrane helix portion is important for signal transduction and coded mainly by exon 11. [10].

Case report

The patient was a 33 year old woman, referred to the Toronto Centre for Advanced Reproductive Technology (TCART) in 2013 after trying to conceive for 5 years. History revealed that her parents were not related. She was born following an uncomplicated gestation, unremarkable for any drug exposures. There were no pediatric medical problems and she underwent thelarche and pubarche before the age of 12 years, comparable to her peers. During her adolescent years, as part of the workup for primary amenorrhea, she was primed with

progesterone for several days and reportedly had a withdrawal bleed. The diagnosis of her condition was uncertain and she was maintained on monthly oral contraceptive pills from the age of 18 years. Hot flashes and absent menses were reported whenever the pill was discontinued necessitating consistent oral contraceptive use for several years. Subsequently, she stopped the pill, tried to conceive for 3 years and eventually sought infertility assessment in 2008. A large number of follicles was noted on her ovaries and she was presumed to have PCOS and underwent laparoscopic ovarian drilling. Subsequent infertility treatments included 2 intrauterine insemination cycles and 1 In-vitro fertilization cycle. The stimulation protocol followed during IVF was the long agonist protocol using GnRh agonist (Suprefact, 0.25 ml; Sanofi Aventis, Laval, Quebec) combined with daily doses of highly purified human urinary menopausal gonadotropins (Menopur, 150 IU daily; Ferring, Toronto) and recombinant FSH (Puregon 75 IU daily; Merck, Kirkland, Quebec) starting the third day of the cycle. Ovulation was triggered using 10,000 IU of purified urinary hCG (Pregnyl, Merck, Kirkland, Quebec). Despite having numerous follicles of mature size on both ovaries her estradiol levels were very low. At the time of retrieval no eggs were obtained. In 2010, a laparoscopic ovarian biopsy was done and the presence of primordial, primary and intermediary follicles was noted. However, secondary, preantral and antral follicles were not noted [11]. At that point she underwent a donor oocyte cycle and became pregnant using donor eggs, had an uncomplicated gestation and delivered a full-term baby girl in 2011.

Two years later in 2013, she desired to conceive using her own eggs and was referred to our clinic. On physical examination, she was found to be normotensive with a height 5 ft 4 in. and a weight of 135 lb. Examination revealed normal female breast development, external genitalia (Tanner stage 5) and pubic hair distribution (Tanner stage 4). Pelvic ultrasound showed a normal sized uterus and multifollicular ovaries (Table 1). She mentioned that she had an older sister, diagnosed 20 years ago with complete androgen insensitivity. However the genetic defect in the androgen receptor could not be detected upon testing. Her sister had inguinal gonads

Table 1 (Day 3 pelvic transvaginal ultrasound measurements). * Largest follicle

	Measurements (cm)
Cervical length	5.1 cm (external to internal os)
Uterus	6.4 cm×4.0 cm×3.2 cm
Endometrial thickness	0.5 cm
Right ovary	2.7 cm×1.7 cm×2.9 cm, 20 follicles noted, L*=1.1 cm
Left ovary	3.6 cm×1.5 cm×2.2 cm, 31follicles noted, L*=0.8 cm

which were surgically removed during adolescence and she was married and had adopted children.

Hormone testing

Baseline hormone measurements were obtained. Blood levels of FSH, LH, androstenedione, testosterone (free and total) and 17OH progesterone were all measured using immunoassay techniques. An hCG stimulation test was performed by administering 10,000 IU of hCG subcutaneously and the blood levels of total and free testosterone, androstenedione and 17-hydroxyprogesterone were measured 3 h and 18 h later respectively. The baseline blood levels of all of these hormones were within the normal range for our laboratory. Poor or no response to hCG stimulation testing was detected except for blunted 17OH progesterone levels which did rise significantly but remained at the lower levels of the normal range (Table 2).

Stimulation protocol and ICSI

One IVF-ICSI cycle was performed at TCART in April 2013 using an antagonist protocol. Daily subcutaneous injections of 200 IU of recombinant FSH (Puregon; Merck, Kirkland, Quebec) were administered starting on day 3 of the cycle. A GnRh antagonist (Cetrotide 0.125 mg; EMD Serono, Mississauga) was administered daily subcutaneously starting on day 7 of the cycle. After 9 days of stimulation, ultrasound measurement of both ovaries revealed a total of 13 follicles within the 1.8 cm–2.5 cm range. The peak estradiol level was 1,267 pmol/l and did not correlate with the number of follicles. The endometrial thickness was 0.9 cm. She received 1 mg of the GnRh agonist buserelin acetate (Suprefact; Sanofi-Aventis, Laval, Quebec) to trigger ovulation and two doses of intravaginal PGE2 (Cervidil; Ferring Incorporated, Toronto) were administered every 18 h starting on the day of the trigger shot. Oocyte retrieval was done 36 h later and both large and small follicles were drained and curetted. A two-stage oocyte retrieval was performed. In the first retrieval no oocytes were recovered from the large preovulatory sized follicles from the

first ovary and only 3 immature (GV oocytes) were retrieved after curetting small antral follicles with the beveled needle tip. The patient was then given misoprostol 200 µg (AA Pharma Incorporated, Vaughan, Ontario) vaginally every 8 h in the hope of mucifying the cumulus oocyte complex (COC) and releasing the COCs from the follicle wall. A repeat retrieval was performed the next day and again no oocytes were retrieved from the large follicles but another 4 oocytes were obtained by curetting the small follicles. A total of 7 oocytes was retrieved one of which was mature (MII) and 6 were germinal vesicle (GV) stage. In vitro maturation was performed with the EGF analogue amphiregulin added to the culture media in a concentration of 100 ng/ml [12–14] and another two oocytes reached the MII stage. ICSI was performed, 3 embryos developed and were successfully cultured up to day 5 but arrested before reaching the blastocyst stage and embryo transfer was not performed.

Genetic sequencing

Buccal swab collection kits and instruction guides were provided to all four members of the family, i.e. the patient, her sister and both parents. Samples were self-collected, stored in special envelopes and sent to the Clinical Genomics Centre in Mount Sinai Hospital, Toronto, Ontario, Canada. Using the 3730xl DNA Analyzer and Sequencing Analysis 5.3 software (Applied Biosystems Inc.) the entire LHCGR gene was sequenced in both forward and reverse directions for all of the samples. This revealed a 27 bp deletion in exon 1 involving one allele at mRNA position 34–60 and amino acid number 12 in both sisters (Fig. 1). This deletion was also noted in the maternal genome on one of the alleles and likely inherited by both offspring. Another heterozygous 2 base pair TC (Thymine, Cytosine) deletion in exon 11 at amino acid position number 586 was detected in both sisters, resulting in a frameshift mutation (Fig. 2). This mutation was noted in the paternal gene involving one allele and likely inherited by the offspring. A missense mutation substituting serine for asparagine on exon 10 at position 312 was also identified in both sisters as well as in their father's genetic sequence.

Table 2 (Hormonal values at baseline and after HCG stimulation)

	Baseline	3 h after HCG stimulation	18 h after HCG stimulation
Androstenedione	2.9 nmol/L (1.5–11.5 nmol/L)	3.8 nmol/L	2.1 nmol/L
17 OH progesterone	0.6 nmol/L (0.6–5.5 nmol/L)	1.8 nmol/L	N/A*
Testosterone (free)	Less than 0.5 pmol/L (<8.9 pmol/L)	Less than 0.5 pmol/L	Less than 0.5 pmol/L
FSH (day 3)	4.3 IU/ml (4–13 IU/ml)		
LH (day 3)	5.4 IU/ml (2–13 IU/ml)

Fig. 1 Portion of the amino acid sequence of exon 1 including the signal peptide (first 24 amino acids). Red areas (italic font)- Deleted segment of exon 1 lying within the signal peptide. Green areas (underlined)-

Homozygous mutations of any of these amino acids abolishes receptor activity Blue areas (bold font)- Crucial for receptor targeting

Discussion

A number of inactivating LH receptor mutations has been identified to date [8]. These mutations have been localized to various segments of the gene and they follow a recessive pattern of inheritance. This family demonstrates novel combined heterozygous mutations forming a “compound heterozygote” genotype. The mutations were inherited separately from each parent, neither of whom displayed any phenotypic abnormalities themselves. The combination of these two mutations on separate alleles most likely resulted in aberrant receptor activity in the offspring. The older sister (46XY karyotype) displayed a phenotype compatible with complete leydig cell hypoplasia that was misdiagnosed at the time as complete androgen insensitivity, even though an androgen receptor mutation could not be found. Therefore, we can infer that these mutations completely abolished LHCGR activity. Exon 1 codes for the signal peptide, part of the leucine rich repeats and the N terminal cysteine rich regions. The first 24 amino acid residues of the human LHCGR constitute the

signal peptide [4]. This segment is essential for targeting and translocating newly synthesized precursor proteins from the ribosomes into the endoplasmic reticulum [15]. This is a coupled process that needs to be precisely timed and coordinated for the successful completion of the maturation processes that includes glycosylation, disulfide bond formation and chaperone mediated folding of the glycoprotein [15]. The signal peptide consists of a central hydrophobic region (h-region) with flanking N-terminal and C-terminal polar regions. The central region is the portion most important for protein translocation and glycosylation. The C-terminal portion contains break point polar amino acids such as glycine or proline and is required for molecule cleavage [15]. The 27 bp deletion identified in exon 1 lies entirely within the signal peptide and involves the segment starting from Lys¹² (from methionine at the N- terminus) upstream until Pro²⁰. This deletion occupies almost half of the entire signal peptide sequence and it lies within the central portion spanning the hydrophobic leucine rich area and part of the C- flank. Consequently, nascent protein targeting, expression and

Fig. 2 Amino acid sequence within a portion of exon 11. **(a)** Normal sequence of amino acids. Underlined nucleotides were deleted resulting in a frameshift mutation. TMH 6- Transmembrane helix 6; ECL 3-

Extracellular loop 3; TMH 7- Transmembrane helix 7 **(b)** Mutation altering the sequence of amino acids ultimately terminating in a stop codon. Red areas (italic font) represent the altered amino acids

maturation of glycosylated receptor are disrupted and LHCGR function is abolished. Furthermore since the deletion occupies a portion of the C- flank, this could prevent signal peptide cleavage resulting in LHCGR ‘trapping’ within the endoplasmic reticulum and production of unstable unprocessed receptor incapable of hormone binding [16]. The receptor would not be expressed on the surface membrane and ligand would be unable to bind. Base pair substitutions and insertions within the signal peptide have been previously reported. To our knowledge, this is the first deletion reported involving the signal peptide. However, the mother carried this deletion only on one allele, and yet was phenotypically normal and able to conceive spontaneously. This observation suggests there must be a requirement for a homozygous deletion of the 27 BP deletions or a combination of heterozygous mutations to inactivate the LHGCR as the presence of one normal allele could result in synthesis of normal protein.

Downstream and in proximity to this deletion, a 13 amino acid segment containing cysteine, glycine, aspartate and leucine residues was noted (Fig. 1). This area is separate from the signal peptide deletion and spans Cys³⁴ until Gly⁴⁶ in the human LHCGR which is almost completely synonymous to the residues spanning Cys¹² until Gly²⁴ in the rat LHR [4]. Previous studies have demonstrated that alanine substitution, photoaffinity labeling and progressive receptor truncation of some of the amino acids in this area altered receptor structural formation and binding [17]. Therefore these may be sites of direct hormone binding [17]. Homozygous mutations of cysteine residues located in this region have been noted to abolish receptor activity [18]. Furthermore, glycine and aspartate residues located in the first portion of this segment, corresponding to Gly¹⁷ and Asp¹⁸ in the rat, are essential for receptor targeting [17]. It is not known whether proximity of all of these significant amino acids to the underlying deletion in exon 1 could affect receptor function. However direct involvement and absence of a large portion of the signal peptide seems to be the primary problem.

Exon 11 codes for an extensive portion of the LHCGR, which includes the entire transmembrane helix and the carboxyl terminus. The transmembrane helices are largely involved in signal transduction and several models have been described [9]. The active and inactive conformations of the receptor are stabilized by hydrogen bonds and electrostatic interactions between transmembrane loops 1, 2,3,6,7 and especially between TMH6 and 3 [10, 19]. Dynamic processes involving spatial rearrangements of the helices have also been described, particularly involving TMH6 which seems to be imperative to this process [20]. Disruption of the interactions between the helices may impair receptor function by stabilizing a specific conformation. The majority of activating mutations occur in TMH6 highlighting its significant role in stabilizing the receptor in an inactive state and in mediating G protein activation. However, it is interesting to note that activating mutations usually occur between positions 564 to 581 which lie

downstream from the mutation we have reported. Substitutions for Asp 578 in TMH6 are the most common activating LHR mutations [21]. In contrast mutations in the upper portions of TMH6 and involving TMH7 cause loss of function [4, 8].

The absence of two base pairs (TC) in the highly conserved T- rich region at position 586 was detected in the second allele in both sisters. This produced a frame-shift mutation in transmembrane helix 6 (TMH6) involving the third intracellular loop which resulted in an alteration of the sequence of successive amino acids and placement of a premature stop codon (GTA) at position 604. Consequently translation of this genetic sequence would produce a truncated LH receptor protein with an alteration in the terminal amino acids spanning TMH6 and complete absence of TMH7 as well as the carboxyl terminal chain portions. This would render it unresponsive to LH/HCG binding and nonfunctional (Fig. 2) [22, 23]. Furthermore, the receptor alteration and truncation may result in abnormal folding and posttranslational modifications. Quality control check at the level of the endoplasmic reticulum would therefore prevent plasma membrane receptor expression leading to ‘receptor trapping’ similar to the consequences of the mutation inherited in exon 1 [24]. Interestingly, the successive sequence of amino acids starting at amino acid position 589 is identical to those seen in a previously reported mutation involving a 46XY individual with Leydig cell hypoplasia [23]. A thymine base pair inserted at amino acid 589 altered the sequence of amino acids ultimately producing a stop codon at position 605 and entirely abolished LH activity [23]. Another homozygous deletion of 2 base pairs at amino acid 608 and 609 also resulted in leydig cell hypoplasia ⁽⁷⁾. Analysis of three mutations starting at position 591 and going downstream have shown that receptor activity declines according to the level of the mutation [25]. More severe phenotypes occur when the mutation is upstream with a higher incidence of protein truncation and this is consistent with the findings in this family. In vitro studies describe the significance of amino acids in TMH7 in activating the receptor. Substitution of Lys⁵⁸³ in the rat which corresponds to Lys⁶⁰⁵ in human LHCGR abolishes receptor function and could be indispensable for cAMP formation [18]. These studies highlight the significance of this area of the glycoprotein which is completely eliminated in one allele as a result of the stop codon at position 604.

In essence, each of the two alleles was defective and consequently disrupted normal LHCGR expression or activity (Fig. 3). Compound heterozygous mutations resulting in leydig cell hypoplasia have been reported previously [26]. These mutations could potentially synergize with each other as well. Initial exodomain binding may allow receptor conformational change exposing secondary sites present on the extracellular loops for additional binding [9]. Conversely, the structure of the endodomain may modulate binding that occurs in the exodomain [27]. Receptors may also trans-

a

Exon 1	ID	Function	Chromosome Position	mRNA P	Allele change	Amino Acid Position	Residue change
A	c.34_60del27bp(Heterozygous)	Deletion	Chr.2, 48982751-48982777		Deletion(27bp)	12-20	Deletion(K-L-L-L-L-L-Q-P-P)
	GCAAGCCGAGAAAGCCAGTTCCGCGCCATGAAAGCAGCGGTTCTCGGCGCTGCAAGCTG CTGAAAGCTGCTGCTGCTGCTGCAAGCCGCGCTGCGACGAGCGCTGCGGAGGCGCTGCTG						

b

c.1756_1757del	Frame shift	Chr.2, 48915179:48	1756-1757	Frame shift	586	Frame shift
-/c.1779_1780delTTC						
TCACCTGCATGGCACTA TCTCT TTTT TTTT GCCATCTCAGCTG/TCACCTGCATGGCACTA TC TTTT TTTT GCCATCT						

Fig. 3 **a** Original sequence data illustrating maternally inherited mutation in exon 1. Red: Deleted nucleotides **b** Original sequence data illustrating paternally inherited mutation in exon 11. Red: area where thymine and

cytosine nucleotide residues were deleted. Both of these mutations were detected in the patient and her sister

activate other receptors and combined mutations could affect this process adding to the overall complexity of this mechanism [28]. This association between these two portions of the receptor not only contributes to the complex structure function relationship but also to the impact a mutation in each segment may have on the overall receptor function [29, 30].

Construction of a molecular model using computational analysis of the receptor in 3 dimensions may provide useful information about the tertiary structure and receptor configurations [6, 9]. It is interesting to note the polymorphism at Ser³¹² does not affect signal binding or transduction and has no functional clinical significance [31]. This polymorphism might be associated with lower susceptibility towards developing breast cancer when compared to Asn³¹² [31]. Combined with the reduced receptor activity and lower estrogen production, this would probably diminish the overall risks of breast cancer.

This case report provides insight into other interesting aspects of the LHCGR, which is also detected in the uterus, fallopian tubes, uterine arteries, fetal membranes, placenta and umbilical cord in humans [32]. LHCGR are localized to stromal and epithelial uterine cells and mostly expressed during the luteal phase [33]. It has been suggested that LHCGR affects implantation by mediating embryo endometrial interaction, providing a relaxing effect on the uterus and improving uterine blood flow [34, 35]. The significance of extragonadal LHCGR function during the process of implantation is unclear. Increased endometrial LHCGR expression may occur during implantation, and coincide with LH signals released from the developing blastocyst [36]. Embryonic development to the blastocyst stage may be enhanced by embryo coculture using LH or hCG [37]. Murine LHCGR knockout models (LuRKO) have been developed. LuRKO mice had thinner uterine layers and altered endometrial receptor and gene expression [38]. Estrogen and progesterone administration only normalized some of these changes [38]. Pregnancy was impaired in null mice following donor embryo transfer, even after estrogen and progesterone hormonal pretreatment and priming [39]. A more recent study however, showed that LuRKO mice recovered most of their fertility potential and

achieved pregnancy after orthotopic ovarian tissue transplantation using wild type ovaries [40]. Our patient had a successful pregnancy after using donor eggs supporting the findings reported by Pakarainen et al. [40] that functional extragonadal LHCGR may not be indispensable for pregnancy. Whether the murine model is different from the human model and whether progesterone replacement in humans can compensate for the defective uterine LHCGR is unknown. Activating mutations of the LHCGR do not seem to alter fertility and it is likely that the presence of LHCGR within the female reproductive tract may be redundant and less significant than was previously thought.

Various signaling cascades are involved in the ovulatory process. Two pathways downstream from the LHCGR are particularly important in relaying the paracrine and autocrine signals implicated in ovulation, cumulus mucification and expansion. These include the prostaglandins, and the EGF-like growth factors amphiregulin, epiregulin and betacellulin. Prostaglandin E2 might positively regulate the effects of EGF-like factors released from mural granulosa cells acting on the cumulus cells ultimately leading to COC expansion. We had hoped that treatment with PGE2 prior to oocyte retrieval might result in mucification of the cumulus cells with release of the COC from the follicle wall. This did not happen in the present case since no oocytes were obtained from the large preovulatory follicles. All oocytes retrieved came from small follicles less than 6 mm in diameter in which the bevel of the retrieval needle was inserted and twirled to literally curette the COC from the follicle wall. In terms of oocyte maturation in vitro, several different concentrations of EGF-like factors have been studied but both the timing and optimal concentration for oocyte maturation remains to be determined [12–14]. In the present case report, amphiregulin was added to the medium with the aim of mimicking activation of EGF-like activity normally induced by ligand binding to the LHCGR. Of interest, we demonstrated maturation of 3 GV oocytes to the MII stage with subsequent fertilization and embryo cleavage. Unfortunately, none of these in vitro matured oocytes developed to the blastocyst stage.

Ten years ago, only a handful of LHCGR mutations had been reported. Deeper understanding, higher suspicion and improved testing for these mutations have enabled the identification of many more cases. Much is left to discover about the LHCGR and its effects on fertility. Further research is warranted not only to clarify all of these aspects but also to provide a means for assisting these women to conceive. Crystallization structures of the rhodopsin and segments of other GPC receptors have provided valuable insight. Ultimately crystallization studies of active and inactive LHCGR, conformational dynamics of the receptor in solution, transfection vectors and computational analysis may provide the most information.

References

- McGee EA, Hsueh AJ. Initial and cyclic recruitment of ovarian follicles. *Endocr Rev*. 2000;21:200–14.
- Hsieh M, Lee D, Panigone S, et al. Luteinizing hormone-dependent activation of the epidermal growth factor network is essential for ovulation. *Mol Cell Biol*. 2007;27:1914–24.
- Otsuka F, McTavish KJ, Shimasaki S. Integral role of GDF-9 and BMP-15 in ovarian function. *Mol Reprod Dev*. 2011;78:9–21.
- Ascoli M, Fanelli F, Segaloff DL. The lutropin/choriogonadotropin receptor, a 2002 perspective. *Endocr Rev*. 2002;23:141–74.
- Amhold IJ, Lofrano-Porto A, Latronico AC. Inactivating mutations of luteinizing hormone beta-subunit or luteinizing hormone receptor cause oligo-amenorrhea and infertility in women. *Horm Res*. 2009;71:75–82.
- Qiao J, Han B, Liu BL, et al. A splice site mutation combined with a novel missense mutation of LHCGR cause male pseudohermaphroditism. *Hum Mutat*. 2009;30:E855–65.
- Latronico AC, Chai Y, Amhold IJ, Liu X, Mendonca BB, Segaloff DL. 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*. 1998;12:442–50.
- Troppmann B, Kleinau G, Krause G, Gromoll J. Structural and functional plasticity of the luteinizing hormone/choriogonadotrophin receptor. *Hum Reprod Update*. 2013;19:583–602.
- Gether U. Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. *Endocr Rev*. 2000;21:90–113.
- Puett D, Li Y, Angelova K, et al. Structure-function relationships of the luteinizing hormone receptor. *Ann N Y Acad Sci*. 2005;1061:41–54.
- Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocr Rev*. 1996;17:121–55.
- Ben-Ami I, Komsky A, Bern O, Kasterstein E, Komarovskiy D, Ron-El R. In vitro maturation of human germinal vesicle-stage oocytes: role of epidermal growth factor-like growth factors in the culture medium. *Hum Reprod*. 2011;26:76–81.
- Peluffo MC, Ting AY, Zamah AM, et al. Amphiregulin promotes the maturation of oocytes isolated from the small antral follicles of the rhesus macaque. *Hum Reprod*. 2012;27:2430–7.
- Park JY, Su YQ, Ariga M, Law E, Jin SL, Conti M. EGF-like growth factors as mediators of LH action in the ovulatory follicle. *Science*. 2004;303:682–4.
- Rutkowski DT, Ott CM, Polansky JR, Lingappa VR. Signal sequences initiate the pathway of maturation in the endoplasmic reticulum lumen. *J Biol Chem*. 2003;278:30365–72.
- Wu SM, Hallermeier KM, Laue L, et al. Inactivation of the luteinizing hormone/chorionic gonadotropin receptor by an insertional mutation in Leydig cell hypoplasia. *Mol Endocrinol*. 1998;12:1651–60.
- Hong S, Phang T, Ji I, Ji TH. The amino-terminal region of the luteinizing hormone/choriogonadotropin receptor contacts both subunits of human choriogonadotropin: I. MUTATIONAL ANALYSIS. *J Biol Chem*. 1998;273:13835–40.
- Song Y, Yi C, Lee C, et al. Interaction and activation of luteinizing hormone receptor. *Indian J Exp Biol*. 2002;40:424–33.
- Dufau ML, Liao M, Zhang Y. Participation of signaling pathways in the derepression of luteinizing hormone receptor transcription. *Mol Cell Endocrinol*. 2010;314:221–7.
- Lebon G, Warne T, Tate CG. Agonist-bound structures of G protein-coupled receptors. *Curr Opin Struct Biol*. 2012;22:482–90.
- Kosugi S, Mori T, Shenker A. The role of Asp578 in maintaining the inactive conformation of the human lutropin/choriogonadotropin receptor. *J Biol Chem*. 1996;271:31813–7.
- Richter-Unruh A, Martens JW, Verhoef-Post M, et al. Leydig cell hypoplasia: cases with new mutations, new polymorphisms and cases without mutations in the luteinizing hormone receptor gene. *Clin Endocrinol (Oxf)*. 2002;56:103–12.
- Richter-Unruh A, Korsch E, Hiort O, Holterhus PM, Themmen AP, Wudy SA. Novel insertion frameshift mutation of the LH receptor gene: problematic clinical distinction of Leydig cell hypoplasia from enzyme defects primarily affecting testosterone biosynthesis. *Eur J Endocrinol*. 2005;152:255–9.
- Segaloff DL. Diseases associated with mutations of the human lutropin receptor. *Prog Mol Biol Transl Sci*. 2009;89:97–114.
- Martens JW, Verhoef-Post M, Abelin N, et al. A homozygous mutation in the luteinizing hormone receptor causes partial Leydig cell hypoplasia: correlation between receptor activity and phenotype. *Mol Endocrinol*. 1998;12:775–84.
- Laue LL, Wu SM, Kudo M, et al. Compound heterozygous mutations of the luteinizing hormone receptor gene in Leydig cell hypoplasia. *Mol Endocrinol*. 1996;10:987–97.
- Ryu K, Lee H, Kim S, et al. Modulation of high affinity hormone binding. Human choriogonadotropin binding to the exodomain of the receptor is influenced by exoloop 2 of the receptor. *J Biol Chem*. 1998;273:6285–91.
- Ji I, Lee C, Song Y, Conn PM, Ji TH. Cis- and trans-activation of hormone receptors: the LH receptor. *Mol Endocrinol*. 2002;16:1299–308.
- Kossack N, Simoni M, Richter-Unruh A, Themmen AP, Gromoll J. Mutations in a novel, cryptic exon of the luteinizing hormone/chorionic gonadotropin receptor gene cause male pseudohermaphroditism. *PLoS Med*. 2008;5:e88.
- Menon KM, Menon B. Structure function and regulation of gonadotropin receptors - a perspective. *Mol Cell Endocrinol*. 2012;356:88–97.
- Piersma D, Verhoef-Post M, Look MP, et al. Polymorphic variations in exon 10 of the luteinizing hormone receptor: functional consequences and associations with breast cancer. *Mol Cell Endocrinol*. 2007;276:63–70.
- Atger M, Misrahi M, Sar S, Le Flem L, Dessen P, Milgrom E. Structure of the human luteinizing hormone-choriogonadotropin receptor gene: unusual promoter and 5' non-coding regions. *Mol Cell Endocrinol*. 1995;111:113–23.
- Reshef E, Lei ZM, Rao CV, Pridham DD, Chegini N, Luborsky JL. The presence of gonadotropin receptors in nonpregnant human uterus, human placenta, fetal membranes, and decidua. *J Clin Endocrinol Metab*. 1990;70:421–30.
- Shemesh M. Actions of gonadotrophins on the uterus. *Reproduction*. 2001;121:835–42.

35. Zhang M, Shi H, Segaloff D, Van Voorhis BJ. Expression and localization of luteinizing hormone receptor in the female mouse. *Biol Reprod*. 2001;64:179–87.
36. Griselet V, Tsampalas M, Berndt S, et al. Evidence for cross-talk between the LH receptor and LH during implantation in mice. *Reprod Fertil Dev*. 2013;25:511–22.
37. Mishra S, Lei ZM, Rao CV. A novel role of luteinizing hormone in the embryo development in cocultures. *Biol Reprod*. 2003;68:1455–62.
38. Lin DX, Lei ZM, Li X, Rao CV. Targeted disruption of LH receptor gene revealed the importance of uterine LH signaling. *Mol Cell Endocrinol*. 2005;234:105–16.
39. Rao CV, Lei ZM. Consequences of targeted inactivation of LH receptors. *Mol Cell Endocrinol*. 2002;187:57–67.
40. Pakarainen T, Zhang FP, Poutanen M, Huhtaniemi I. Fertility in luteinizing hormone receptor-knockout mice after wild-type ovary transplantation demonstrates redundancy of extragonadal luteinizing hormone action. *J Clin Invest*. 2005;115:1862–8.