CASE REPORT

Case Report: A 54 base pair inactivating mutation of \textit{LHCGR} in a 28-year old woman with poor ovarian response [version 1; peer review: 2 approved with reservations]

Ravi Krishna Cheemakurthi\textsuperscript{1}, Gottumukkala Achyuta Rama Raju\textsuperscript{2}, Thota Sivanaryana\textsuperscript{1}, Kalagara Madan\textsuperscript{3}, Kota Murali Krishna\textsuperscript{3}, Godi Sudhakar\textsuperscript{4}

\textsuperscript{1}Department of Genetics, Krishna IVF Clinic, Visakhapatnam, Andhra Pradesh, 530002, India
\textsuperscript{2}Gynecology Division: Director, Krishna IVF Clinic, Visakhapatnam, Andhra Pradesh, 530002, India
\textsuperscript{3}Department of Embryology, Krishna IVF Clinic, Visakhapatnam, Andhra Pradesh, 530002, India
\textsuperscript{4}Department of Human Genetics, Andhra University, Visakhapatnam, Andhra Pradesh, 530002, India

\textbf{Abstract}

The luteinizing hormone/choriogonadotropin (LH/CG) receptor plays an important role in male and female infertility. Many studies have demonstrated that mutations at specific sites in \textit{LHCGR} gene may result in mild or complete loss of receptor function. Insertions in exon-1 of \textit{LHCGR} gene were first studied in male Leydig cell hypoplasia and later extended to female reproductive disorders. Previous studies have shown that these insertions play an important role in intrauterine insemination (IUI) and in vitro fertilization (IVF) outcome. Here we report a 54bp insertion in a 28-year old woman with infertility, recurrent cyst formation and failed stimulated IUI cycles. As the patient showed a blunted response to the ovarian stimulation and human chorionic gonadotropin (hCG) stimulation test, follicle stimulating hormone receptor (FSHR) and luteinizing hormone/choriogonadotropin (LHGR) gene sequencing was performed. Gene sequence analysis revealed a 54bp homozygous insertion (GCTGCTGAAGCTGCTGCTGCTGCTGCAGCTGCTGAAGCTGCTGCTGCTGCTGCA) in the exon-1 of \textit{LHCGR} gene. This mutation might have caused a decrease in receptor function in the present infertile patient, thus resulting in poor ovarian response.

\textbf{Keywords}

LHCGR, insertions, exon-1, poor ovarian response
Corresponding author: Gottumukkala Achyuta Rama Raju (Krishnaivf@gmail.com)

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Introduction

Luteinizing hormone (LH) is a key regulator of the female menstrual cycle and is produced by the anterior pituitary gland of gonadotroph cells. During ovulation, the LH surge is not only essential for the release of an egg from the follicle, but also regulates the formation of the corpus luteum. The corpus luteum in turn produces progesterone, which helps in the maintenance of pregnancy. Therefore, LH along with its receptor complex has a cardinal role during reproductive processes. The heterodimeric glycoproteins LH and chorionic gonadotrophin (CG) are mediated by luteinizing hormone/choriogonadotrophin receptor (LHCGR), which is a member of the G-Coupled receptor protein family. This receptor is expressed by the LHCGR gene located on chromosome 2p21 that consists of 11 or 12 exons and codes for around 600 amino acids. The LH receptor is located on Leydig cells in males, and theca, granulose cells of the ovaries. The receptor activation and inactivation are brought about by the conformation of amino acids in their localized regions.

Women, whose LH receptor is down regulated with GnRH agonists or antagonists, may have decreased concentrations of luteinizing hormone and follicle stimulating hormone. This results in poor outcome due to low endogenous LH levels. Results from our earlier study demonstrated more number of oocytes and clinical pregnancies after use of recombinant LH (rLH) combined with recombinant follicle stimulating hormone (rFSH) in assisted reproductive therapy (ART). Although supplementation of LH is beneficial in a selected category of patients, a number of studies have also demonstrated that LHCGR mutations at specific amino acid regions result in mild or complete loss of receptor function.

LHCGR gene mutations were first studied in men with Leydig cell hypoplasia, later they were extended to female reproductive disorders. Studies have shown that inactivating base pair insertions in exon-1 of LHCGR gene are associated with low oocyte yield and also negatively associated with intrauterine insemination (IUI) and in vitro fertilization (IVF) outcome in infertile population. A recent investigation by Bentov et al., 2012 reported a 6bp insertion in exon-1 of LHCGR gene which was associated with recurrent IUI and IVF failures. Although many factors like age, low anti-mullerian hormone (AMH) etc. are indications for decreased oocyte yield, poor ovarian response and low ovarian reserves, recent publications have focused on FSHR and LHCGR mutations/polymorphisms. A study was initiated to delineate the possible role of LHCGR dysfunction in patients with poor ovarian response, low and moderate ovarian reserves.

Materials and methods

Patient selection

A total of 114 patients was screened for LH receptor gene polymorphisms/mutations with the following inclusion criteria: number of antral follicle count <8, normal testosterone levels, low antimullerian hormone (AMH) and poor response to previous infertility treatment. Out of 114 patients, 3 patients showed a 6bp homozygous insertion and 23 patients showed heterozygous 6bp insertion as described previously. However, a 54bp insertion in exon-1 of LHCGR gene was detected in a patient with a clinical history of poor response to human menopausal gonadotrophin (hMG) stimulation and recurrent cyst formation.

The study was approved by the institutional ethics committee and informed consent was obtained from the participants.

Case history and treatment

A 28-year old Indian woman with a marital life of 5 years suffering with infertility referred to the Krishna IVF clinic for evaluation and treatment. Physical examination revealed normal breast development, normal external and internal female genitalia with irregular menstrual cycles. The patient has a sibling with two children.

At day 2, basal ultrasound examination showed an anteverted uterus volume: 48 ml, a right side ovary volume of 14.43 ml with a growing follicle and a left side ovary volume of 6.32 ml. Antral follicle count was 6 combining both ovaries. The patient underwent diagnostic laparoscopy and hysteroscopy and the ovaries had a normal volume with thick capsules as shown (Figure 1). On patient’s request, gonadotrophin/IUI was planned three weeks after laparoscopy. Ultrasound examination detected a large functional cyst which was managed using oral contraceptive pill. In the first cycle, the patient was super ovulated with a dose of hMG of 150 units/day. No response was observed after 4 days and 6 days of stimulation. As the AMH (0.2 ng/dl) and the ovarian reserves were low and there was no other option available, in the next cycle patient was super ovulated with a hMG dose of 225 units/day for 10 days. On the 9th day of stimulation, one follicle with 18 to 20 mm diameter, 2 follicles with 1 to 9 mm and endometrial thickness of 10 mm were observed. A trigger dose of hCG 5000 IU was administered. Luteal support was provided with sosten 200 mg vaginally. The patient had started bleeding p/v from 6 days after trigger dose of hCG. A day 2 scan in the subsequent cycle showed an ovarian cyst again.

Figure 1. Laparoscopic image showing ovary with thick capsule.
Because of the discrepancy between AFC, AMH and the ovarian volume, and the poor response to IUI stimulated cycles and the recurrent cyst formation, hCG stimulation test and LHGR gene sequencing were performed.

**hCG stimulation test**
The hCG stimulation test was performed in a natural cycle. Baseline data of the patient hormonal profile were recorded. These showed serum FSH 10 mIU/ml, serum LH 8 mIU/ml, serum dehydroepiandrosterone (DHEA-S) 3.71 mcg/ml, serum testosterone 20 ng/dl, serum prolactin 14.1 ng/ml, indicating normal values, and serum AMH showed a value of <0.2 ng/ml. hCG increases the serum concentrations of androstendione and testosterone by acting through LHGR. A dose of 10,000 units of hCG was administered subcutaneously. A blunted response to hCG was observed after 4 hours of administration as there was no significant change in the serum testosterone concentration (22 ng/dl) compared to baseline values. Based on the patient’s poor response to ovarian stimulation and hCG stimulation test, the patient was further referred to the molecular genetics department to investigate the possible LHGR mutations/polymorphisms.

**Genome analysis**
Genomic DNA from peripheral blood of patient’s was isolated using a modified salting out method as described previously1. All the coding exons of the gene were amplified by polymerase chain reaction using primers designed using primer express software from Applied Biosystems, the amplified product was observed on 2% agarose gel electrophoresis and purified with Exo-Sap enzyme from Thermo Scientific1. The purified product was sequenced using big dye cycle sequencing kit on 3500 genetic analyzer and analyzed using Seq Scape (Life Technologies, USA).

The sequencing results revealed a 54bp insertion (GCTGCTGAAGCTGCTGCTGCTGCTGCAG) in the exon-1 of LHGR, which facilitated the activation of different cell groups in the ovaries. LH triggers follicular maturation, ovulation and formation of corpus luteum and increases progesterone secretion in luteal phase1,2,3,14. Different chronological, hormonal, functional biomarkers are used to assess the ovarian response during controlled ovarian stimulation (COS)1,2. Along with these biomarkers, the possible roles of LHGR mutations associated with different female reproductive disorders like oligo amenorrhea, recurrent pregnancy loss, ovarian resistance, infertility and IVF outcome have been studied previously10,11,16,17. A 6bp (CTGCAG, amino acids: LQ) exon-1 insertion was first described in LHGR after nucleotide position 54 in Leydig cell hypoplasia10,18. Later, a homozygous 33bp and a 27bp insertions (CTGCTGAAAGCTGCTGCTGCTGCTGCTGAAAGCTGCTGCTGCTGCTGCA) in the exon-1 of LHGR of an infertile woman. Inactivating mutations of the LH/HR are known to affect signaling pathways, decrease LH and its receptor binding. Improper folding of this receptor protein structure in the endoplasmic reticulum may result in decreased LHR expression which leads to reduced cell response12. One of the above stated mechanisms might have played a role in the poor ovarian response in the present case. The serum hCG stimulation test also showed a blunted response indicating reduced receptor function. The data from the present case are highly significant as the involvement of a possible genetic cause was delineated at an early stage of the infertility treatment. LHGR mutations may lead to a reproductive catastrophe. Supplementation of LH in patients with LHGR polymorphisms might offer a benefit for those who opt for IVF treatment. However the concept of LH supplementation may not yield a good response in such a type of homozygous LHGR mutations. A blunted response might be observed at a very high doses with mature and immature oocytes. In

**Results and discussion**
LH regulates a number of reproductive functions in males as well as in females. LH and CG hormones act through the LH/CG receptor which facilitates the activation of different cell groups in the ovaries. LH triggers follicular maturation, ovulation and formation of corpus luteum and increases progesterone secretion in luteal phase1,2,3,14.

Here we present a homozygous mutation with a 54bp insertion (GCTGCTGAAAGCTGCTGCTGCTGCTGCTGAAAGCTGCTGCTGCTGCTGCA) in the exon-1 of LHGR of an infertile woman. Inactivating mutations of the LH/HR are known to affect signaling pathways, decrease LH and its receptor binding. Improper folding of this receptor protein structure in the endoplasmic reticulum may result in decreased LHR expression which leads to reduced cell response. The data from the present case are highly significant as the involvement of a possible genetic cause was delineated at an early stage of the infertility treatment. LHGR mutations may lead to a reproductive catastrophe. Supplementation of LH in patients with LHGR polymorphisms might offer a benefit for those who opt for IVF treatment. However the concept of LH supplementation may not yield a good response in such a type of homozygous LHGR mutations. A blunted response might be observed at a very high doses with mature and immature oocytes. In
such situations, in vitro maturation of oocytes, where in the dependence of hCG is less to cause the follicle maturation, can be an option for such patients. This study concludes that examination of LHCGR mutations/polymorphisms would be a useful investigation for classifying response, prognosis and management in infertile patients with low ovarian reserves.

Consent
Informed written consent to publish clinical data was obtained from the patient.

Author contributions
Ravi Krishna: carried out the research.
Thota Sivanarayana: played a role in standardization and provided necessary technical support.
Madan: reviewed the literature and played an important role in designing the whole study.

Murali Krishna: wrote the manuscript.
Sudhakar: helped us in developing the technique and writing the manuscript.
Rama Raju: designed the project and provided the whole clinical support.

All authors agreed to the final content of the manuscript.

Competing interests
No competing interests were disclosed.

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Supplementary material
Gene sequence analysis revealing a 54bp homozygous insertion (GCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGAGCTGCTGAAGCTGCTGCTGCTGCTGCTG) in the exon-1 of LHCGR gene.

Available: here

References


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Zi-Jiang Chen
Center for Reproductive Medicine, Shandong Provincial Hospital, Shandong University, Jinan, China

Correction from F1000Research – 22nd July 2015:

This report was mistakenly published with the summary status of ‘Approved’ and has now been corrected to the referee’s intended status of ‘Approved with Reservations’. No changes have been made to the text of the report. We apologise for this error.

This is an interesting report in which a 54bp homozygous insertion in exon-1 of LHCGR have been found that are associated with poor ovarian response. That should be helpful for understanding the effect of short sequence repeat in LHCGR on ovarian response. But there still are a number of concerns that need to be addressed.

1. The sequence of 54bp insertion should be (CTGCTGAAAGCTGCTGCTGCTGCTGCA), but not (GCTGCTGAAGCTGCTGCTGCTGCTGCA), which did not change the amino acid sequence but consist with the previous reported insertions of 6bp (CTGCAG), 33bp and 27bp (CTGCTGAAAGCTGCTGCTGCTGCTGCA). Moreover, it would be interesting to discuss why the 6bp to 54bp duplication in the same region adversely affect LHCGR response to hCG/LH.

2. How about the LHCGR sequence of the patient’s parents and sibling? Whether the insertion is an autosomal recessive disorder or a kind of dynamic mutation?

3. “Insertions in exon-1 of LHCGR gene were first studied in male Leydig cell hypoplasia.” In present study, the patient with 54bp insertion had ovaries with thick capsule. Is there some relationship?

4. There are some typos and awkward phrasing in the manuscript. E.g. “Later, a homozygous 33bp and a 27bp insertions (CTGCTGAAAGCTGCTGCTGCTGCTGCA, amino acids:) …”. There should be the sequence of amino acids, right? So please review carefully for those.

Competing Interests: No competing interests were disclosed.
I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 01 May 2015

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Han Zhao
Shandong Provincial Key Laboratory of Reproductive Medicine, National Research Center for Assisted Reproductive Technology and Reproductive Genetics, Shandong University, Shandong, Jinan, China

The article has identified a 54bp homozygous insertion in exon-1 of LHCGR gene which was related to poor ovarian response and concluded that screening of LHCGR mutations/polymorphisms would be useful for prognosis and management of patients with low ovarian reserves. However, there are several concerns that must be adequately addressed and require a detailed response or additional data.

1. The authors performed sequencing of LHCGR gene in patients with history of poor ovarian response, but detailed criterion of poor ovarian response was not characterized.

2. The article mentioned that the patient with the 54bp homozygous insertion in exon-1 of LHCGR gene has a sibling with two children. Whether the sibling has been sequenced of LHCGR gene or not? The result of the sibling could help understand the origin and the function of the homozygous insertion.

3. As described in the article, the patient with LHCGR gene insertion had a history of poor ovarian response and recurrent cyst formation. Why not try transvaginal oocyte retrieval which might improve the outcome?

4. A minor detail is that although the language is excellent for a paper from a non-English speaking country, there are still a lot of grammatical errors that would benefit from editing by a native speaker.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
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