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CASE REPORT

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

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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 *LHCGR* gene may result in mild or complete loss of receptor function. Insertions in exon-1 of *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 (LHCGR) gene sequencing was performed. Gene sequence analysis revealed a 54bp homozygous insertion (GCTGCTGAAGCTGCTGCTGCTGCTGCAGCTGCTGAAGCTGCTGCTGCTGCTGCA) in the exon-1 of *LHCGR* gene. This mutation might have caused a decrease in receptor function in the present infertile patient, thus resulting in poor ovarian response.

Keywords

LHCGR , insertions , exon-1 , poor ovarian response

Open Peer Review

Approval Status ? ?

	1	2
version 1	?	?
18 Mar 2015	view	view
1. Han Zhao , Shandong University, Shandong, China 2. Zi-Jiang Chen , Shandong University, Jinan, China		
Any reports and responses or comments on the article can be found at the end of the article.		

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Introduction

Luteinizing hormone (LH) is a key regulator of the female menstrual cycle¹ 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^{2,3}. Therefore, LH along with its receptor complex has a cardinal role during reproductive processes^{4,5}. 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, granulosa cells of the ovaries²⁰. The receptor activation and inactivation are brought about by the conformation of amino acids in their localized regions⁶.

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)⁷. 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 function⁸.

LHCGR gene mutations were first studied in men with Leydig cell hypoplasia, later they were extended to female reproductive disorders¹¹. 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 population⁹⁻¹¹. 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-müllerian 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 reserves¹¹.

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 antimüllerian 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^{11,12}. 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:48ml, 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 susten 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 formation again.

Figure 1. Laparoscopic image showing ovary with thick capsule.

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.

Grant information

The author(s) declared that no grants were involved in supporting this work.

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We would like to thank Dr. Kavitha Lakshmi and Dr. Kavitha for their help in coordinating the patient/s.

Supplementary material

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

Available: [here](#)

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Zi-jiang Chen

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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 (CTGCTGAAGCTGCTGCTGCTGCTGCAG)₂, but not (GCTGCTGAAGCTGCTGCTGCTGCTGCA)₂, which did not change the amino acid sequence but consist with the previous reported insertions of 6bp (CTGCAG), 33bp and 27bp (CTGCTGAAGCTGCTGCTGCTGCTGCAG). 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 (CTGCTGAAGCTGCTGCTGCTGCTGCAG, 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 confirm that I have read this submission and 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

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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 confirm that I have read this submission and 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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