Published in final edited form as:

Fertil Steril. 2011 August ; 96(2): e125–e130. doi:10.1016/j.fertnstert.2011.05.057.

Inherited mutation of the luteinizing hormone/choriogonadotropin receptor (*LHCGR*) in empty follicle syndrome

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Abstract

Objective—To test by genomic analysis whether empty follicle syndrome (EFS) in a family with two affected sisters has a genetic basis.

Design—Whole-exome sequencing in the context of clinical genetics.

Setting—University hospital.

Patient(s)—Two women (36 and 32 years old at the time of the study) with EFS.

Intervention(s)—Genetic counseling based on autosomal recessive inheritance.

Main Outcome Measure(s)—Discovery of a mutation in the LH/choriogonadotropin receptor (*LHCGR*) as the cause of EFS.

Result(s)—A novel missense mutation in *LHCGR*, p.N400S, was homozygous in sisters with EFS and/or infertility, but not in their unaffected siblings or parents. The mutation was not present in 500 ancestry-matched control subjects. Asparagine at residue 400 is highly conserved and its substitution by serine predicted to alter critical interactions that stabilize *LHCGR*.

Conclusion(s)—We describe a genetic basis for EFS and provide strong evidence for the existence of genuine EFS in some patients. A mutation impairing the function of *LHCGR* explains the lack of response of these patients to repeated administration of β -hCG.

Keywords

Empty follicle syndrome; luteinizing hormone/choriogonadotropin receptor; infertility; next-generation sequencing

Since its description by Coulam et al. in 1986 (1), the nature of empty follicle syndrome (EFS) has been controversial. The condition was defined as the failure to retrieve oocytes from mature ovarian follicles after ovulation induction for in vitro fertilization (IVF), even after meticulous aspiration and repeated flushing, despite apparently normal follicular development and E_2 levels (2). The frequency of EFS was estimated to range from <1% to 7% among patients presenting for IVF treatment (3, 4). Suggested causes of EFS included oocyte atresia despite normal hormonal response (6), genetic or chromosomal factors (7, 8), and defects in hCG activity (9, 10); but iatrogenic causes and technical issues were also posited (5), particularly errors in hCG administration (11, 12), including inappropriate dosage or timing (13) or missed injection (14).

In 2008, Stevenson and Lashen (15) classified EFS as either “false” or “genuine” according to the demonstration of the optimum levels of β -hCG on the day of oocyte retrieval. They defined “genuine EFS” as failure to retrieve oocytes from mature ovarian follicles after ovulation induction for IVF after apparently normal follicular development and steroidogenesis in the presence of optimum β -hCG levels on the day of oocyte retrieval, and “false EFS” as failure to retrieve oocytes in the presence of low β -hCG due to an error in the administration of hCG or to its bioavailability on the day of oocyte retrieval (15). They estimated that more than two-thirds of EFS cases were false, leading other investigators to suggest that EFS does not exist as a natural entity (16). On the other hand, the existence of genuine EFS was supported by the observation of empty follicles, resembling preantral follicles and devoid of oocytes, in a patient with primary infertility (5), by the observation of familial EFS (7), and by the observation of EFS in association with a chromosomal translocation (8).

The purpose of the present project was to carry out genomic analysis of EFS in a family originally reported by Onalan et al. in 2003 (7), which was defined in Stevenson and Lashen’s review as representing genuine EFS (15). We show that the affected sisters in this family are homozygotes for a mutation in the LH/choriogonadotropin receptor, *LHCGR*. This result provides insights into the pathogenesis of infertility and supports the existence of EFS as a natural entity in some patients.

MATERIALS AND METHODS

Subjects

As described by Onalan et al. (7), two sisters with primary infertility from a Turkish family underwent controlled ovarian hyperstimulation and IVF cycles at the same center and by the same team. Although parents denied consanguinity they originated from a small town, making distant kinship very likely. At the time of EFS, one sister (individual II-8 in Fig. 1A) was 36 years old with a 17-year history of primary infertility, and the other sister (individual II-10 in Fig. 1A) was 32 years old with a 14-year history of primary infertility. The first patient underwent one IVF cycle, but despite optimal β -hCG bioavailability, no oocytes or cumulus-corona complexes were retrieved by follicular aspiration. A rescue dose of β -hCG and repeated aspiration 35 hours later did not succeed in recovering oocytes. In the second patient, two cycles of intracytoplasmic sperm injection were attempted within a 3-month interval, but both resulted in EFS despite administration of rescue doses of β -hCG. During all IVF cycles, both sisters had normal follicular development, E_2 levels, and bioavailable β -

hCG plasma levels (7). Karyotypes of both sisters were 46,XX. Gonadotropin and E₂ levels were normal, with no evidence of hypogonadotrophic hypogonadism, nor any history of anosmia, primary amenorrhoea, polycystic ovaries, hyperandrogenism, systemic disorder, or neurologic defect. Despite normal pubertal development oligomenorrhea was present in both sisters. Family history revealed that two other sisters (II-4 and II-6 of Fig. 1A) had problems with conception, but these individuals did not pursue IVF. In the original publication, the two sisters with EFS were reported as having moderate hearing loss (7). However, we did not detect hearing loss by pure tone audiogram or by auditory brainstem response in either sister (Supplemental Fig. 1). Genetic analysis of this family was approved by the Ethics Committee of Ankara University and by the human subjects committees of the University of Miami and the University of Washington. Informed consent was obtained from each of the participants.

Molecular Genetics

Genomic DNA from affected individual II-8 was extracted from blood, sonicated to ~200-bp fragments, and used to make a library for paired-end sequencing (Illumina). After quality control, the library was hybridized to biotinylated cRNA oligonucleotide baits from the Sure-Select Human All Exon kit (Agilent Technologies), purified by streptavidin-bound magnetic beads, amplified, and sequenced as previously described (17). The exome design covers 38 Mb of human genome corresponding to the exons and flanking intronic regions of 23,739 genes in the National Center for Biotechnology Information Consensus CDS database (September 2009 release) as well as 700 miRNAs from the Sanger v13 database and 300 noncoding RNAs (18). We generated a total of 5.6 Gb of sequence as paired-end 76-bp reads from one lane of an Illumina Genome Analyzer IIX with Illumina Pipeline v. 1.6. We used MAQ v.0.7.1 to discard reads of inadequate sequence quality or depth and those with identical start and end sites. Candidate variants were confirmed with Sanger sequencing using a Beckman Coulter CEQ8000 automated sequencer.

RESULTS

Exome sequencing of genomic DNA of affected individual II-8 (Fig. 1A) revealed 1,759 rare variants (Table 1), 358 of which were missense, nonsense, splice, or frameshift mutations. Of these 358 mutations, only six mutations were homozygous in II-8; all other mutations were heterozygous. The six homozygous mutations were evaluated by Sanger sequencing in all enrolled family members (I-1, I-2, II-6, II-10, II-12, and II-13) and tested for cosegregation with EFS (Fig. 1A). Only one mutation was homozygous in all three affected sisters, but not homozygous in their unaffected sister, an unaffected brother, or either of their parents.

The mutation that perfectly cosegregated with EFS in the family was *LHCGR* c.1199A>G (p.N400S) at chr2:48,915,737T>C (hg19) in *LHCGR* (Fig. 1B). This mutation was not present in 500 healthy control subjects from the same geographic region of Turkey and has not been reported in any other population. The mutation is at a site that is highly conserved both among *LHCGR* sequences of mammals, birds, and fish (Fig. 1C) and among multiple members of the glycoprotein hormone receptor (GHR) family (Fig. 1C). *LHCGR* p.N400S is predicted to be a damaging mutation by multiple in silico bioinformatics tools: score 0.994 as a probably damaging mutation on PolyPhen2 (19), predicted to affect protein function with a score of $P=.03$ on SIFT (20), and $P=.975$ for being a disease-associated allele on MutationTaster (21).

LHCGR p.N400S lies in the second transmembrane helix of the receptor (Fig. 2A) (22–24). Three-dimensional modeling of *LHCGR* shows that the side chain of residue 400 is directed outwards from the helix (Fig. 2B). Asparagine at residue 400 interacts with leucine at

position 452, methionine at position 487, and tryptophan at position 491. These residues are also highly conserved in LHCGR as hubs to maintain the structural stability of the protein (25). If asparagine is replaced by a smaller serine at residue 400, distances between this residue and its neighbors will be altered, potentially leading to abnormal conformation of the protein. The consequences of mutations in the most conserved residues of LHCGR were previously evaluated experimentally, in the context of structural analysis. For LHCGR with a mutation (tryptophan) introduced at N400, receptor expression level was 32% that of wild-type protein and hCG-saturation binding level was 60% that of wild-type protein, suggesting that hormone-stimulated activity of the mutant was impaired (25).

DISCUSSION

LHCGR is a glycoprotein hormone receptor belonging to the G-protein coupled receptor family. Loss-of-function mutations of LHCGR lead to Leydig cell hypoplasia and pseudohermaphroditism in males (Fig. 2A; Supplemental Table 1). In a few studies, sisters of these affected males were also genotyped, and those with the same biallelic *LHCGR* mutations were found to be infertile (26). One brother (II-1) in the present family is fertile and not known to have the identified *LHCGR* mutation (Fig. 1A). Analogous to the human EFS phenotype, female mice lacking *LHCGR* had no preovulatory follicles or corpora lutea, likely secondary to defects in follicular maturation and ovulation (27).

Together, these observations provide strong evidence that LHCGR p.N400S causes EFS in the family we evaluated and that the condition is inherited as a recessive genetic trait. The mutant allele cosegregates with EFS in the family, it is absent in ethnicity-matched controls, the critical amino acid residue is highly conserved, and earlier in vitro studies showed that a mutation at this residue results in reduced β -hCG binding to the receptor. A conclusion to be drawn from these results is that genuine EFS due to mutation of *LHCGR* cannot be resolved with repeated administration of β -hCG, because the receptor is impaired.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported in part by grants R01DC009645 and R01DC005641 from the National Institute of Deafness and other Communication Disorders of the National Institutes of Health with a supplement from the American Recovery and Reinvestment Act.

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FIGURE 1.

Familial empty follicle syndrome (EFS). (A) Sisters II-8 and II-10 are affected with EFS. Sisters II-4 and II-6 are infertile but did not undertake IVF. *LH/choriogonadotropin receptor* (*LHCGR*) genotypes are indicated for enrolled relatives. The affected sisters are homozygous for the mutant allele, and their unaffected sister and both parents are heterozygous carriers. (B) Sequences of the wild-type, heterozygous, and mutant alleles of *LHCGR*. (C) Conservation of *LHCGR*: Asparagine at residue 400 is conserved among mammals, birds, and fish. This residue is also highly conserved in the second transmembrane helix of other glycoprotein hormone receptors.

FIGURE 2.

Structure of LHCGR with loss-of-function mutations. **(A)** A total of 17 loss-of-function missense mutations of LH/choriogonadotropin receptor (LHCGR; blue and purple circles) have been reported to cause Leydig cell hypoplasia and pseudohermaphroditism in males (complete list is available in Supplemental Table 1). LHCGR p.N400S (*red*) is the novel mutation associated with empty follicle syndrome in this study. Mutations identified in infertile sisters of affected males in earlier studies are indicated with purple circles. EC = extracellular domain; TM = transmembrane domain (number of TM region is shown under the structure); IC = intracellular domain. The two-dimensional structure of LHCGR was created at the Sequence-Structure-Function Analysis database (22–24). **(B)** The interaction of Asn400 (*red*) with Met487 (*purple*), Trp491 (*purple*), and Leu452 (*green*) is critical to maintaining the structural stability of LHCGR (25). A prediction from the structure is that if a smaller serine residue replaces asparagine at residue 400, distances between the side chains of these amino acids would increase, disrupting their interaction.

TABLE 1

Variants revealed by whole exome sequencing in a patient with empty follicle syndrome (EFS).

	SBP	Indel	Total
Total rare variants	1,201	557	1,759
Nonsense, missense, frameshift, or splice variants	348	10	358
Variants homozygous in patient	5	1	6
Variants cosegregating with EFS in family	1	0	1

Note: Indel = small insertions or deletions; SBP = single basepair changes.

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