A novel homozygous mutation in the *FSHR* gene is causative for primary ovarian insufficiency

Hongli Liu, M.D., Ph.D.,[a,b,c,d] Xiaofei Xu, M.D., Ph.D.,[a,b,c,d] Ting Han, M.D., Ph.D.,[a,b,c,d] Lei Yan, M.D., Ph.D.,[a,b,c,d] Lei Cheng, M.S.,[a,b,c,d] Yingying Qin, M.D., Ph.D.,[a,b,c,d] Wen Liu, M.D., Ph.D.,[a,b,c,d] Shidou Zhao, M.D., Ph.D.,[a,b,c,d] and Zi-Jiang Chen, M.D., Ph.D.[a,b,c,d,e,f]

[a] Center for Reproductive Medicine, Shandong University; [b] Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University; [c] National Research Center for Assisted Reproductive Technology and Reproductive Genetics; [d] Key Laboratory of Reproductive Endocrinology, Shandong University, Ministry of Education, Jinan; [e] Center for Reproductive Medicine, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University; and [f] Shanghai Key Laboratory for Assisted Reproduction and Reproductive Genetics, Shanghai, People's Republic of China

Objective: To identify the potential *FSHR* mutation in a Chinese woman with primary ovarian insufficiency (POI).
Design: Genetic and functional studies.
Setting: University-based reproductive medicine center.
Patient(s): A POI patient, her family members, and another 192 control women with regular menstruation.
Intervention(s): Ovarian biopsy was performed in the patient. Sanger sequencing was carried out for the patient, her sister, and parents. The novel variant identified was further confirmed with the use of control subjects.
Main Outcome Measure(s): Sanger sequencing and genotype analysis to identify the potential variant of the *FSHR* gene; hematoxylin and eosin staining of the ovarian section to observe the follicular development; Western blotting and immunofluorescence to detect FSH receptor (FSHR) expression; and cyclic adenosine monophosphate (cAMP) assay to monitor FSH-induced signaling.
Result(s): Histologic examination of the ovaries in the patient revealed follicular development up to the early antral stage. Mutational screening and genotype analysis of the *FSHR* gene identified a novel homozygous mutation c.175C>T (p.R59X) in exon 2, which was inherited in the autosomal recessive mode from her heterozygous parents but was absent in her sister and the 192 control women. Functional studies demonstrated that in vitro the nonsense mutation caused the loss of full-length FSHR expression and that p.R59X mutant showed no response to FSH stimulation in the cAMP level.
Conclusion(s): The mutation p.R59X in FSHR is causative for POI by means of arresting folliculogenesis. (Fertil Steril® 2017;108: 1050–5. ©2017 by American Society for Reproductive Medicine.)
Key Words: Primary ovarian insufficiency, *FSHR* gene, mutation, ovarian follicle

Primary ovarian insufficiency (POI), also known as premature ovarian failure or premature menopause, is defined as a primary ovarian defect characterized by amenorrhea before 40 years of age with serum FSH level >40 IU/L (1, 2). It is thought that 1% of women under 40 years of age and 0.1% under 30 years of age are affected by POI (3). It is a clinically and etiologically heterogeneous condition. Chromosomal abnormalities have accounted for 12% of cases (4), and the incidence of familial cases has been estimated to be 12.7% (5), both of which indicate a strong genetic component. Furthermore, variants causative for POI have been identified in dozens of genes, including *BMP15*, *PGRMC1*, *GDF9*, *NOBOX*, *FIGLA*, and *NR5A1* by means of candidate gene sequencing and *STAG3*, *HFM1*, *MCM8*, *MCM9*, *CSB-PGBD3*, and *MSH5* by means of whole exome sequencing (6–8). Although genetic etiology has been found in 25% of cases, more than 50% of cases are idiopathic (9).

The pituitary glycoprotein hormone FSH plays a pivotal role in mammalian reproduction through binding and

Received March 21, 2017; revised August 21, 2017; accepted September 7, 2017; published online November 20, 2017.
H.L. has nothing to disclose. X.X. has nothing to disclose. T.H. has nothing to disclose. L.Y. has nothing to disclose. L.C. has nothing to disclose. Y.Q. has nothing to disclose. W.L. has nothing to disclose. S.Z. has nothing to disclose. Z.-J.C. has nothing to disclose.
Funding provide by grants from the National Key Research and Developmental Program of China (2017YFC1001100), National Natural Science Foundation of China (81522018, 81471509, 81270662, and 81571505), Young Scholars Program of Shandong University (2016WLJH26), and Fundamental Research Funds of Shandong University.
Reprint requests: Shidou Zhao, M.D., Ph.D., 44 Wenhua Xi Road, Jinan, Shandong, 250012, People's Republic of China (E-mail: shidouzhao@sdu.edu.cn).

Fertility and Sterility® Vol. 108, No. 6, December 2017 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2017.09.010

activating its specific receptor located on target cells. The normal function of the FSH receptor (FSHR) is considered to be essential for follicular development and estradiol production in females (10). FSHR is a G-protein–coupled receptor, which facilitates a number of intracellular signaling pathways after being activated by FSH. In the classic signaling pathway, FSH-FSHR first activates the heterotrimeric Gs protein and then stimulates the effector adenylyl cyclase with subsequent increase in production of the second messenger cyclic adenosine monophosphate (cAMP), activation of protein kinase A, phosphorylation of ERK protein, and activation of transcription of target genes (11, 12).

Fshr-knockout female mice displayed thin uteri and small ovaries and were sterile owing to follicular arrest before antral follicle formation (13). The importance of the *FSHR* gene in human reproduction is evident and it has been considered that *FSHR* was the first single gene to cause nonsyndromic POI (14). Diverse inactivating mutations and polymorphisms have been described in the *FSHR* gene, most of which lead to a POI phenotype (14–26). Actually, inactivating mutations at different domains of FSHR resulted in distinct loss of function (27). Mutations located in the extracellular domain (ECD), such as p.A189V, p.I160T, p.D224V, p.P348R, and p.V221G, affected ligand binding and subsequent signaling (14, 16, 17, 20, 28), whereas mutations outside the ECD, such as p.R573C, p.L601V and p.A419T, did not greatly affect the ligand-binding ability of the receptor but decreased cAMP production stimulated by FSH (16–18). *FSHR* mutations have been largely reported in Finnish patients with POI (29), but are extremely rare in patients of Chinese descent. In the present study, we report a novel *FSHR* mutation (p.R59X) in a Chinese woman with POI. The nonsense mutation caused the loss of full-length FSHR expression and function.

MATERIALS AND METHODS

Ethics Approval

The study procedures were approved by the Institutional Review Board of Reproductive Medicine of Shandong University. All of the DNA and tissue samples were handled in accordance with the National Regulation of Clinical Sampling in China. Written informed consent was obtained from each subject.

Clinical and Endocrinologic Characteristics

The patient was a 30-year-old woman of Chinese origin presenting with POI. She had no spontaneous menstruation, and her menstrual cycle was induced with the use of cyclic estrogen-progestin therapy from the age of 18 years. No family history of primary amenorrhea or infertility was reported. The patient's mother suffered from menopause at the age of 47 years after radiochemotherapy for cervical carcinoma. The patient had a 28-year-old sister with regular menstruation. After exogenous estrogen and progesterone therapy, her breasts developed to Tanner II from Tanner I. The patient was 167 cm in height, 58 kg in weight, and 20.7 kg/m^2 in body mass index (BMI). At the first visit, her endocrinologic evaluation revealed high levels of FSH (71.22 IU/L) and LH (29.74 IU/L), but low levels of E_2 (<5 pg/mL), T (0.09 ng/dL) and antimüllerian hormone (0.485 ng/mL). Pelvic ultrasonography showed a hypoplastic uterus (29 × 18 mm) and two apparently shrunken ovaries measuring 15 × 9 mm on the right and 13 × 9 mm on the left. Morphology of ovary was poorly displayed and two/three follicles measuring 2∼3 mm were detected in the right and left ovary, respectively. The patient had a normal 46,XX karyotype.

Ovarian Biopsy Under Laparoscopy

Laparoscopy was performed for ovarian tissue biopsy. Visual examination found a small uterus and two small gray ovaries without any growing follicle or corpus luteum, which was consistent with the result of ultrasound imaging. A small piece of tissue ∼125 mm^3 in size was extracted from each ovary.

Histological Analysis of Ovarian Tissue

The two fragments of the ovaries were fixed in Bouin solution for 24 hours and then embedded in paraffin. Then the samples were cut into 5-μm sections and hematoxylin and eosin staining was carried out with the use of standard procedures. The morphology was observed under a microscope (Olympus), and photomicrographs were captured with a digital camera (Olympus).

Sanger Sequencing of the *FSHR* Gene

Genomic DNA was extracted from peripheral blood samples with the use of QIAamp DNA mini kit (Qiagen) according to the manufacturer's protocol. The entire coding sequence and exon-intron boundaries of the human *FSHR* gene (NM_000145) were polymerase chain reaction (PCR) amplified with the use of specific primer pairs. All *FSHR* primers are presented in Supplemental Table 1 (available online at www.fertstert.org). Detailed information regarding PCR conditions is available upon request. The PCR-amplified products were purified with the use of an Avanti J-20 XP centrifuge (Beckman Coulter), and subsequent sequencing was completed with the use of a Bigdye terminator kit with the ABI 3730-Avant Genetic Analyzer (Applied Biosystems). The results were compared with the *FSHR* gene sequence with the use of Sequencer software version 4.9. The novel variant was confirmed with three independent PCR runs by sequencing both in forward and reverse direction.

To confirm the novel mutation identified in the *FSHR* gene, we further screened 192 fertile women with normal levels of FSH and E_2. At the same time, the suspected variant was examined by means of Sanger sequencing in the patient's sister and parents. Amino acid sequences from other species were obtained from the Uniprot database (www.uniprot.org), and the conservation analysis was conducted with the use of ClustalW2 website (www.ebi.ac.uk/Tools/msa/clustalw2/).

Genotype Analysis

Genomic DNA including the novel variant and its neighboring region was PCR amplified, and the primer sequences were as

follows: forward: 5′-CGGTAGTGGGAACAAGCAAG-3′; reverse: 5′-TGCAGAAAGTTTGGCTGACC-3′. The 493-bp PCR products were purified with the use of DNA Purification Kit (Axygen) and then digested with the use of restriction enzyme Taq I, which produced DNA fragments of 318 bp and 175 bp. The novel variant at site 175 in the *FSHR* gene led to the loss of cleavage site of Taq I. The PCR products before and after Taq I digestion were analyzed by means of electrophoresis in 2% agarose gels, stained with ethidium bromide, and visualized under an ultraviolet transilluminator (Bio-Rad).

Plasmid Construction

The wild-type vector was constructed by means of inserting human *FSHR* cDNA directly into a pSG5 expression vector as described previously (14). The mutant expression vector for p.R59X was generated with the use of the Quikchange Lightning Site-Directed Mutagenesis Kit (Stratagene), and DNA sequencing was conducted to confirm the desired mutation.

Cell Culture and Transfection

The human embryonic kidney 293T (HEK293T) cells were cultured in Dulbecco Modified Eagle Medium (Hyclone) supplemented with 10% fetal bovine serum (Hyclone) and 1% penicillin-streptomycin in an atmosphere of 5% CO_2 at 37°C. For functional experiments, the desired constructs were transfected into cells with the use of lipofectamine 3000 reagent (Invitrogen), and the assays were carried out at indicated time points.

Western Blotting

HEK293T cells were seeded in a 10-cm dish for 24 hours to grow to 75%–80% confluence and then transfected with 10 μg desired plasmids (mock vector, wild-type FSHR, or p.R59X mutant plasmids). Forty-eight hours after transfection, cells were lysed with the use of Minute Plasma Membrane Protein Isolation Kit (Invent Biotechnologies) according to the manufacturer's protocol, and plasma membrane protein was harvested. Equal amounts of protein were resolved through sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Millipore). The membranes were blocked and then incubated with primary antibodies against human FSHR (1:1,000; Proteintech) or Na-K-ATPase (1:100,000, Abcam). The blots were finally captured with the use of the Chemidoc MP System (Bio-Rad).

Immunofluorescence

HEK293T cells were seeded on cover slips in 24-well plates and then transfected with desired plasmids. Forty-eight hours after transfection, cells were fixed with 4% paraformaldehyde for 20 minutes at room temperature and permeabilized with the use of 0.3% Triton X-100 for 40 minutes before incubating with 10% bovine serum albumin and FSHR antibody (1:200; Abcam). Fluorescein isothiocyanate–conjugated IgG was used as the secondary antibody (1:100; ZSGB-BIO). The cells were then counterstained with DAPI and visualized under a fluorescence microscope (BX53; Olympus).

cAMP Assay

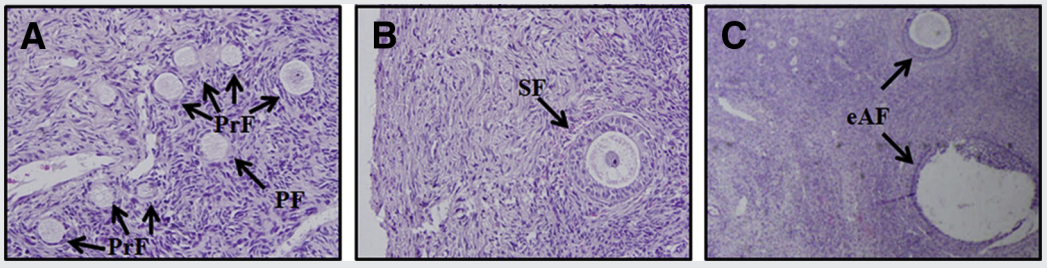
FSH-induced cAMP production was measured with the use of the Glosensor cAMP Assay kit (Promega) according to the manufacturer's instructions. HEK293T cells were seeded in a 6-well plate for 24 hours to grow to 75%–80% confluence and then cotransfected with 0.7 μg Glosensor 22-F plasmid and 2 μg desired plasmids (mock vector, wild-type FSHR, or p.R59X mutant plasmids). Twenty-four hours after transfection, cells were seeded in 96-well plates at a density of 2.0×10^4 cells/well and incubated for another 24 hours. Then the cAMP levels were measured in cells stimulated with human FSH (100 IU/L) for 45 minutes or in cells after stimulation with different doses of FSH (0–5,000 IU/L) for 20 minutes with the use of a luminescence counter (PE). Three independent experiments were conducted.

RESULTS

Histologic Observation

Many small follicles in primordial, primary, and secondary stages were observed in the ovary section of the POI patient

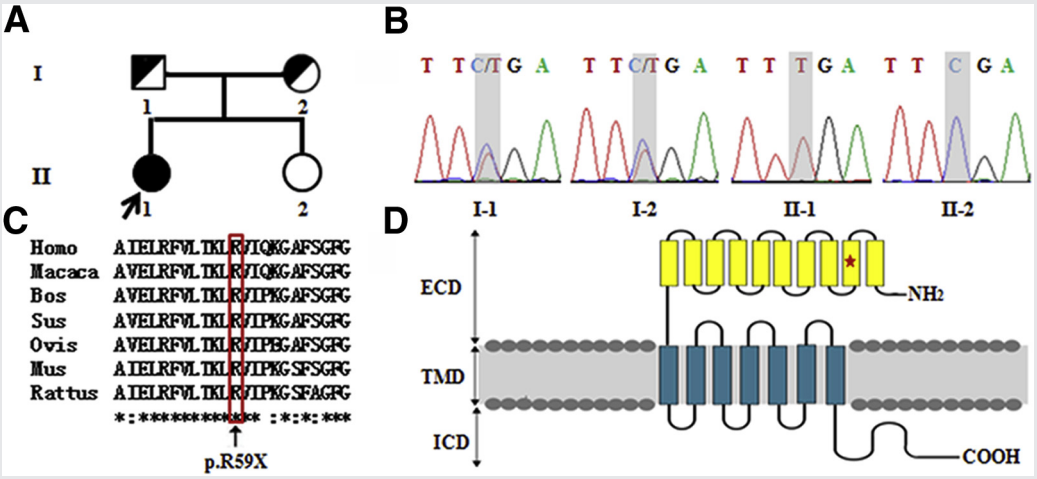
FIGURE 1



Hematoxylin and eosin staining of the ovaries of the patient. (**A**) Section of the ovarian cortex, containing several primordial (PrF) and primary (PF) follicles (×400). (**B**) Secondary follicle (SF): central oocyte surrounded by 2–3 layers of granulosa cells (×100); (**C**) Early antral follicle (eAF): two small antral follicles measuring 0.625 mm and 0.25 mm in diameter (×40).

Liu. A novel FSHR *mutation identified in POI. Fertil Steril 2017.*

FIGURE 2



Identification of a novel mutation c.175C>T (p.R59X) in the *FSHR* gene. (**A**) Pedigree of the primary ovarian insufficiency patient. The proband is indicated by an arrow. (**B**) Sanger sequencing confirmed the homozygous variant of the subject as well as the heterozygous status of her parents. (**C**) FSHR N-terminus alignment among different species. (**D**) Schematic presentation of the FSHR protein and structure. The novel mutation is indicated by a red star. ECD = extracellular domain; TMD = transmembrane domain; ICD = intracellular domain.

Liu. A novel FSHR *mutation identified in POI. Fertil Steril 2017.*

(Figs. 1A and 1B). Particularly, two early antral follicles were found (Fig. 1C). However, corpora lutea and mature graafian follicles were absent. These results suggested that primary amenorrhea and infertility of the patient may have been due to a block in follicular maturation.

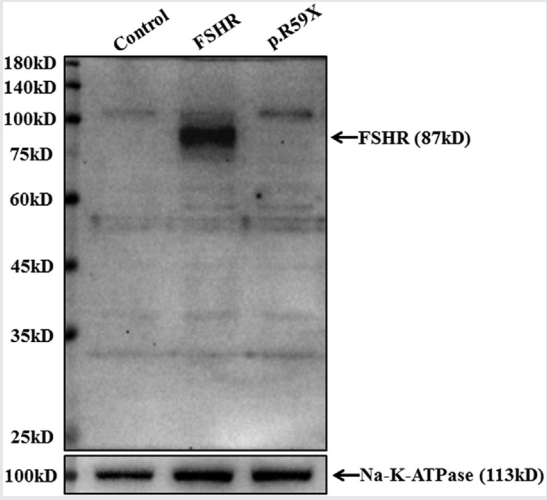
Novel Mutation Identified in the *FSHR* Gene

Based on the clinical characteristics and histologic results, mutation screening of the *FSHR* gene was carried out for the patient and other family members. Sequencing results revealed a homozygous C to T at site 175 of the *FSHR* gene in the patient, whereas both of her parents were heterozygous carriers and her unaffected sister showed a normal genotype (Figs. 2A and 2B), which indicated an autosomal recessive inheritance pattern. None of the 192 control women carried the same variant. The genotype of the patient and her family members was confirmed by means of restriction enzyme cleavage and electrophoresis (Supplemental Fig. 1, available online at www.fertstert.org). The amino acid involved was highly conserved among species (Fig. 2C). Theoretically, this novel nonsense mutation c.175C>T in exon 2 resulted in a premature stop codon (p.R59X) at the N-terminal extracellular domain of the receptor, leading to the absence of the extracellular hormone-binding region, seven transmembrane helixes, and the intracellular domain (Fig. 2D).

Loss of FSHR Expression Caused by p.R59X Mutation

The expression of FSHR in HEK293T cells transfected with mock vector, wild-type FSHR, or p.R59X mutant was determined by means of Western blotting. Mature form of FSHR at 87 kD was detected in cells expressing wild-type FSHR, but not in cells transfected with mock vector or p.R59X mutant, indicating that the p.R59X mutation resulted in the loss of full-length FSHR expression (Fig. 3). In contrast to wild-type FSHR, the expression of the mutant FSHR was not observed with the use of immunofluorescence (Supplemental Fig. 2, available online at www.fertstert.org).

FIGURE 3



Expression of wild-type FSHR and p.R59X mutant in HEK293T cells, determined by Western blotting. The cells transiently transfected with mock vector, wild-type FSHR, and p.R59X mutant were harvested and the FSHR expression detected by Western blotting. The membrane protein Na-K-ATPase served as a loading control. The molecular weight markers are shown on the left.

Liu. A novel FSHR *mutation identified in POI. Fertil Steril 2017.*

FSH-Induced cAMP Production

The FSH-induced signaling was measured in the cAMP level after FSH stimulation in HEK293T cells transfected with mock vector, wild-type FSHR, or p.R59X mutant. Whereas cells expressing wild-type FSHR responded to FSH (100 IU/L) in a dynamic way and the cAMP level reached the peak at ~20 minutes after FSH stimulation, cells transfected with mock vector or p.R59X mutant showed no response to FSH stimulation in cAMP level (Fig. 4A). In addition, FSH elicited cAMP accumulation in cells transfected with wild-type FSHR in a dose-dependent manner, but no increase in cAMP production was detected in cells transfected with mock vector or p.R59X mutant even at high concentrations of FSH (500, 1,000, and 5,000 IU/L; Fig. 4B). These results indicated that the mutation led to the loss of FSHR function.

DISCUSSION

In this study, we identified a novel homozygous mutation in the *FSHR* gene which occurred in a patient with POI who presented primary amenorrhea. However, it should be noted that there existed many follicles in the ovaries of the patient, including two or three follicles at early antral stage (2–3 mm at ultrasonography; data not shown). The homozygous variant c.175C>T in the *FSHR* gene might result in a truncated protein with 59 amino acids, leading to absence of the extracellular hormone-binding region, seven transmembrane helixes, and the intracellular domain. Furthermore, no expression of FSHR was detected by means of Western blotting and immunofluorescence in cells transfected with p.R59X mutant, and the mutant did not respond to FSH stimulation in the cAMP assay. These results suggest that p.R59X is a loss-of-function mutation.

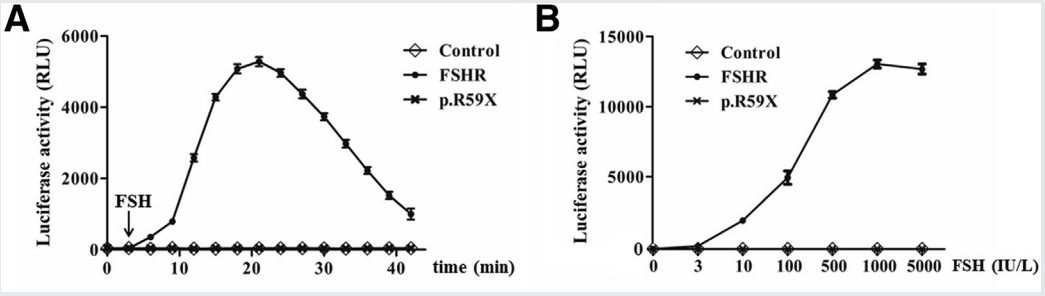
POI is characterized by hypergonadotropic hypoestrogenic amenorrhea and typically represents the end result of premature depletion of the follicular pool. Generally speaking, follicles are absent in the ovaries of POI patients. However, histologic examination of the ovaries in the present patient showed follicular development up to the early antral stage. Therefore, the etiology of this POI patient may result from folliculogenesis arrest instead of a lack of primordial follicles.

FSHR is detected in granulosa cells from primary follicles and is sustained throughout folliculogenesis (30). After binding with its ligand FSH, FSHR facilitates multiple signaling pathways to trigger follicle maturation and estrogen production (31). Although the activation of FSHR is considered to be essential for the development of antral follicles in mammalian ovary (32), it has been reported that FSH can also promote preantral follicle growth in rodents (33, 34). However, follicles could develop to preantral stages in $Fshr^{-/-}$ female mice (13), indicating that FSH-FSHR signaling pathways are not necessary for preantral follicle formation in mice.

The first homozygous mutation (p.A189V), which led to a dramatic reduction of FSHR function, was reported in the Finnish population (14). Subsequent studies showed that small follicles before the antral stage were present in all of the ovarian biopsies of nine patients with this p.A189V mutation, but mature follicles also were observed in one of these patients, which might be attributed to residual receptor activity (35, 36). This mutation is prevalent in Finland but is uncommon in other populations (29). Furthermore, other FSHR mutations also are extremely rare in POI patients of other ethnicities. Owing to the partial loss of FSHR function, patients with compound heterozygous mutations in the *FSHR* gene also had antral follicles (16, 17). In contrast, a block of follicular growth after the primary stage was observed in a patient with a complete loss of FSHR function (19). These results suggest that in humans, FSH is required for follicular growth after the primary stage. In the present study, the follicular development in the patient proceeded up to the early antral stage, indicating partial function of the FSHR in vivo. It is thus possible that forced translation of the transcript by reading through the premature stop codon occurs in vivo.

In conclusion, we identified a novel mutation c.175C>T (p.R59X) in the *FSHR* gene, and the loss of FSHR expression and function was causative for POI by means of arresting folliculogenesis.

FIGURE 4



Cyclic adenosine monophosphate (cAMP) production stimulated by FSH in HEK293T cells. (**A**) The cells transfected with mock vector, wild-type FSHR, and p.R59X mutant were stimulated with the use of human FSH (100 IU/L) for 45 minutes, and cAMP was measured at each time point. The arrow indicates the start point of FSH stimulation. (**B**) The cells transfected with mock vector, wild-type FSHR, and p.R59X mutant were stimulated with different doses of FSH (0–5,000 IU/L) and cAMP was measured after 20 minutes.

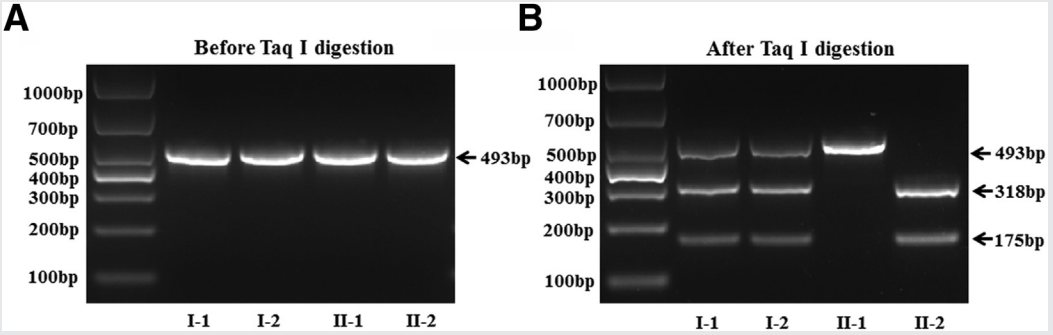
Liu. A novel FSHR mutation identified in POI. Fertil Steril 2017.

Acknowledgments: The authors thank the patient, her family members, and the 192 control women for their participation in this study.

REFERENCES

1. Rebar RW. Premature ovarian failure. Obstet Gynecol 2009;113:1355–63.
2. Goswami D, Conway GS. Premature ovarian failure. Hum Reprod Update 2005;11:391–410.
3. Coulam CB, Adamson SC, Annegers JF. Incidence of premature ovarian failure. Obstet Gynecol 1986;67:604–6.
4. Jiao X, Qin C, Li J, Qin Y, Gao X, Zhang B, et al. Cytogenetic analysis of 531 Chinese women with premature ovarian failure. Hum Reprod 2012;27:2201–7.
5. van Kasteren YM, Hundscheid RD, Smits AP, Cremers FP, van Zonneveld P, Braat DD. Familial idiopathic premature ovarian failure: an overrated and underestimated genetic disease. Hum Reprod 1999;14:2455–9.
6. Qin Y, Jiao X, Simpson JL, Chen ZJ. Genetics of primary ovarian insufficiency: new developments and opportunities. Hum Reprod Update 2015;21:787–808.
7. Qin Y, Guo T, Li G, Tang TS, Zhao S, Jiao X, et al. CSB-PGBD3 mutations cause premature ovarian failure. PLoS Genet 2015;11:e1005419.
8. Guo T, Zhao S, Zhao S, Chen M, Li G, Jiao X, et al. Mutations in MSH5 in primary ovarian insufficiency. Hum Mol Genet 2017;26:1452–7.
9. Persani L, Rossetti R, Cacciatore C. Genes involved in human premature ovarian failure. J Mol Endocrinol 2010;45:257–79.
10. Channing CP, Schaerf FW, Anderson LD, Tsafriri A. Ovarian follicular and luteal physiology. Int Rev Physiol 1980;22:117–201.
11. Jiang X, Dias JA, He X. Structural biology of glycoprotein hormones and their receptors: insights to signaling. Mol Cell Endocrinol 2014;382:424–51.
12. Ulloa-Aguirre A, Zariñán T, Pasapera AM, Casas-González P, Dias JA. Multiple facets of follicle-stimulating hormone receptor function. Endocrine 2007;32:251–63.
13. Dierich A, Sairam MR, Monaco L, Fimia GM, Gansmuller A, Lemeur M, et al. Impairing follicle-stimulating hormone (FSH) signaling in vivo: targeted disruption of the FSH receptor leads to aberrant gametogenesis and hormonal imbalance. Proc Natl Acad Sci U S A 1998;95:13612–7.
14. Aittomäki K, Lucena JL, Pakarinen P, Sistonen P, Tapanainen J, Gromoll J, et al. Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. Cell 1995;82:959–68.
15. Gromoll J, Simoni M, Nordhoff V, Behre HM, De Geyter C, Nieschlag E. Functional and clinical consequences of mutations in the FSH receptor. Mol Cell Endocrinol 1996;125:177–82.
16. Beau I, Touraine P, Meduri G, Gougeon A, Desroches A, Matuchansky C, et al. A novel phenotype related to partial loss of function mutations of the follicle stimulating hormone receptor. J Clin Invest 1998;102:1352–9.
17. Touraine P, Beau I, Gougeon A, Meduri G, Desroches A, Pichard C, et al. New natural inactivating mutations of the follicle-stimulating hormone receptor: correlations between receptor function and phenotype. Mol Endocrinol 1999;13:1844–54.
18. Doherty E, Pakarinen P, Tiitinen A, Kiilavuori A, Huhtaniemi I, Forrest S, et al. A novel mutation in the FSH receptor inhibiting signal transduction and causing primary ovarian failure. J Clin Endocrinol Metab 2002;87:1151–5.
19. Meduri G, Touraine P, Beau I, Lahuna O, Desroches A, Vacher-Lavenu MC, et al. Delayed puberty and primary amenorrhea associated with a novel mutation of the human follicle-stimulating hormone receptor: clinical, histological, and molecular studies. J Clin Endocrinol Metab 2003;88:3491–8.
20. Allen LA, Chermann JC, Pakarinen P, Kotlar TJ, Huhtaniemi IT, Jameson JL, et al. A novel loss of function mutation in exon 10 of the FSH receptor gene causing hypergonadotrophic hypogonadism: clinical and molecular characteristics. Hum Reprod 2003;18:251–6.
21. Kuechler A, Hauffa BP, Köninger A, Kleinau G, Albrecht B, Horsthemke B, et al. An unbalanced translocation unmasks a recessive mutation in the follicle-stimulating hormone receptor (FSHR) gene and causes FSH resistance. Eur J Hum Genet 2010;18:656–61.
22. Nakamura Y, Maekawa R, Yamagata Y, Tamura I, Sugino N. A novel mutation in exon8 of the follicle-stimulating hormone receptor in a woman with primary amenorrhea. Gynecol Endocrinol 2008;24:708–12.
23. Achrekar SK, Modi DN, Meherji PK, Patel ZM, Mahale SD. Follicle stimulating hormone receptor gene variants in women with primary and secondary amenorrhea. J Assist Reprod Genet 2010;27:317–26.
24. Woad KJ, Prendergast D, Winship IM, Shelling AN. FSH receptor gene variants are rarely associated with premature ovarian failure. Reprod Biomed Online 2013;26:396–9.
25. Katari S, Wood-Trageser MA, Jiang H, Kalynchuk E, Muzumdar R, Yatsenko SA, et al. Novel inactivating mutation of the FSH receptor in two siblings of Indian origin with premature ovarian failure. J Clin Endocrinol Metab 2015;100:2154–7.
26. Bramble MS, Goldstein EH, Lipson A, Ngun T, Eskin A, Gosschalk JE, et al. A novel follicle-stimulating hormone receptor mutation causing primary ovarian failure: a fertility application of whole exome sequencing. Hum Reprod 2016;31:905–14.
27. Zariñán T, Perez-Solís MA, Maya-Núñez G, Casas-González P, Conn PM, Dias JA, et al. Dominant negative effects of human follicle-stimulating hormone receptor expression-deficient mutants on wild-type receptor cell surface expression. Rescue of oligomerization-dependent defective receptor expression by using cognate decoys. Mol Cell Endocrinol 2010;321:112–22.
28. Banerjee AA, Achrekar SK, Joseph S, Pathak BR, Mahale SD. Functional characterization of two naturally occurring mutations V(221)G and T(449)N in the follicle stimulating hormone receptor. Mol Cell Endocrinol 2017;440:69–79.
29. Jiang M, Aittomäki K, Nilsson C, Pakarinen P, Iitiä A, Torresani T, et al. The frequency of an inactivating point mutation (566C>T) of the human follicle-stimulating hormone receptor gene in four populations using allele-specific hybridization and time-resolved fluorometry. J Clin Endocrinol Metab 1998;83:4338–43.
30. Oktay K, Briggs D, Gosden RG. Ontogeny of follicle-stimulating hormone receptor gene expression in isolated human ovarian follicles. J Clin Endocrinol Metab 1997;82:3748–51.
31. Simoni M, Gromoll J, Nieschlag E. The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology. Endocr Rev 1997;18:739–73.
32. McGee EA, Hsueh AJ. Initial and cyclic recruitment of ovarian follicles. Endocr Rev 2000;21:200–14.
33. McGee EA, Perlas E, LaPolt PS, Tsafriri A, Hsueh AJ. Follicle-stimulating hormone enhances the development of preantral follicles in juvenile rats. Biol Reprod 1997;57:990–8.
34. Hardy K, Fenwick M, Mora J, Laird M, Thomson K, Franks S. Onset and heterogeneity of responsiveness to FSH in mouse preantral follicles in culture. Endocrinology 2017;158:134–47.
35. Aittomäki K, Herva R, Stenman UH, Juntunen K, Ylöstalo P, Hovatta O, et al. Clinical features of primary ovarian failure caused by a point mutation in the follicle-stimulating hormone receptor gene. J Clin Endocrinol Metab 1996;81:3722–6.
36. Rannikko A, Pakarinen P, Manna PR, Beau I, Misrahi M, Aittomäki K, et al. Functional characterization of the human FSH receptor with an inactivating Ala189Val mutation. Mol Hum Reprod 2002;8:311–7.

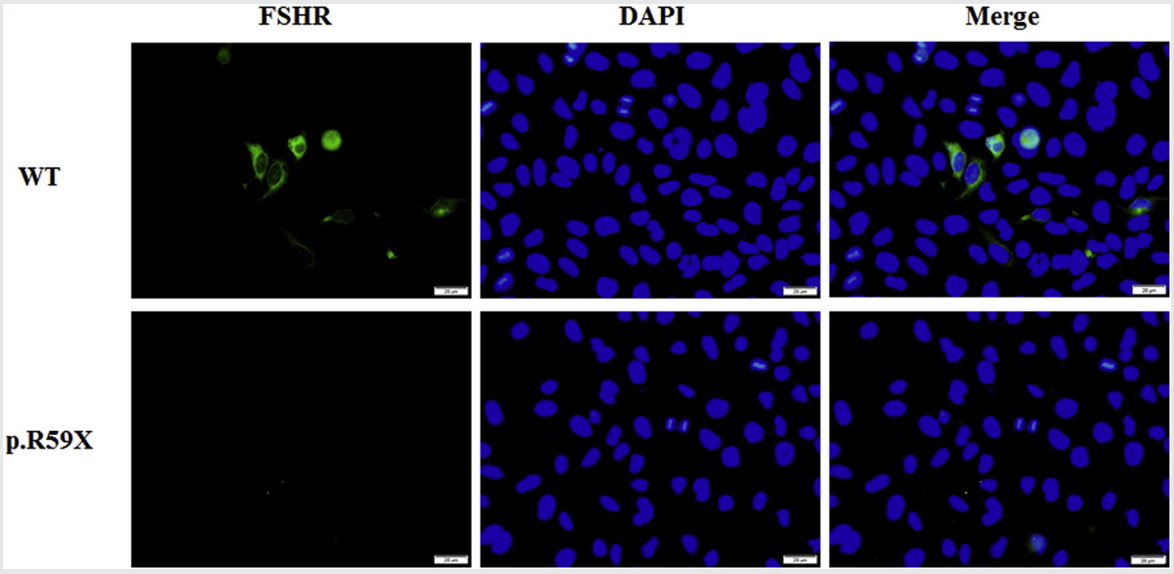
SUPPLEMENTAL FIGURE 1



Genotype analysis of the family members. (**A**) A 493-bp fragment of the FSHR gene was detected before Taq I enzyme digestion. (**B**) The polymerase chain reaction products after digestion by Taq I were displayed. The upper arrow indicates the 493-bp full-length fragment. The middle and lower arrows indicate the 318-bp and 175-bp fragments produced by Taq I enzyme digestion.

Liu. A novel FSHR *mutation identified in POI. Fertil Steril 2017.*

SUPPLEMENTAL FIGURE 2



Expression of wild-type (WT) FSHR and p.R59X mutant in HEK293T cells detected by means of immunofluorescence. FSHR protein (*green*) was detected in cells transfected with wild-type *FSHR*, but not in cells transfected with mutant *FSHR*. Cell nuclei were counterstained with DAPI (*blue*). Scale bars = 20 μm.

Liu. A novel FSHR *mutation identified in POI. Fertil Steril 2017.*