A NOVEL COMPOUND HETEROZYGOUS *FSHR* VARIANT ASSOCIATED WITH PRIMARY AMENORRHEA: THE FIRST CASE REPORT IN VIETNAM

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Primary amenorrhea is a rare condition with heterogeneous causes, including anatomic and sexual development problems, ovarian insufficiency, hypothalamus or pituitary illnesses, and other endocrine gland disorders. Genetic etiology may involve chromosomal abnormalities or gene mutations. Variants in the follicle-stimulating hormone receptor (FSHR) gene are extremely rare, with only a few cases reported worldwide. We describe a 32-year-old Vietnamese female with primary amenorrhea and repeated in vitro fertilization failure due to absent ovarian response. She exhibited a small uterus on ultrasound, and hormonal testing results of ovarian insufficiency status and a normal 46,XX karyotype. Clinical exome sequencing revealed an in trans compound heterozygous FSHR variant: c.656_659del and c.263C>T, which were classified as likely pathogenic and variant of uncertain significance with high possibility of pathogenicity, accordingly. Despite gonadotropin stimulation at a high dose, the woman failed to produce mature oocytes. This study reports the first Vietnamese case with primary amenorrhea that is related to novel FSHR variants. The findings expand the FSHR mutation spectrum and underscore the importance of genetic testing and counseling in unexplained ovarian insufficiency.

Keywords: Primary amenorrhea, ovarian insufficiency, *FSHR* gene, clinical exome sequencing, genetic counseling.

I. INTRODUCTION

Primary amenorrhea (PA), defined as the absence of menarche by age 15 in females with normal secondary sexual characteristics, affects approximately 0.3% of women of reproductive age.[1] Its etiologies are diverse, including anatomical anomalies and endocrine disorders that may be caused by genetic factors. Chromosomal abnormalities such as Turner syndrome account for nearly 30% of cases, while monogenic disorders contribute to a smaller portion.[1] Genes may be involved in premature ovarian insufficiency (POI)/PA, including *AIRE, BMP15, CLPP, CYP17A1, CYP19A1, EIF2B2, EIF2B4, EIF2B5, FIGLA, FOXL2, FSHR, GALT, HFM1, LMNA, MCM8, MCM9, NOBOX, NR5A1, PSMC3IP, SOHLH1, STAG3, …*

The *FSHR* gene encodes the follicle-stimulating hormone receptor, which is critical for ovarian folliculogenesis and steroidogenesis. Pathogenic variants in this gene caused ovarian dysgenesis (OMIM: 233300) and ovarian hyperstimulation syndrome (OMIM: 608115). Since Aittomäki et al. first described a *FSHR* mutation in Finnish families in 1995,[2] a limited number of pathogenic *FSHR* variants have been reported worldwide, primarily among

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European and East Asian populations.[3-8] Recent studies in Chinese and Korean cohorts have identified novel *FSHR* variants associated with POI, underscoring the need for adequate genetic diagnosis.[5-8]

There have been no case reported in Vietnam to date. In this report, we present the first Vietnamese female with PA harboring two novel heterozygous *FSHR* variants. The primary objectives of this study are to describe the clinical, hormonal, and genetic characteristics of this case, expand current knowledge of rare genetic etiologies of POI/ PA beyond common causes such as Turner syndrome, and underscore the role of comprehensive genetic testing and counseling in informing personalized fertility prognosis.

II. CASE PRESENTATION

1. Clinical Findings

A 32-year-old woman (Figure 1, II.1) came to our clinic for preconception genetic counseling due to primary amenorrhea and recurrent failed in vitro fertilization. She had no history of chronic illness, surgery, or systemic disease. She mentioned that she had undergone two failed in vitro fertilization attempts at a private hospital previously. Both IVF cycles were unsuccessful due to a lack of ovarian response to gonadotropins, even with higher doses. Previous laboratory findings before our examination included an anti-Müllerian hormone (AMH) level of 1.49 ng/mL. Hormone test (performed using Cobas E601) on day 2 of the artificial menstrual cycle revealed follicle-stimulating hormone (FSH) was appropriately measured at 5.31 mIU/mL (follicular phase: 3.5-12.5), while luteinizing hormone (LH) was notably reduced at 0.45 IU/L (2.4-12.6). Estradiol was markedly low at 8.82 pg/mL (12-165), whereas progesterone (0.829 nmol/L; 0.6-4.7) and testosterone (0.55 nmol/L; 0.22-2.99) were within reference ranges (8/2024). Karyotype analysis demonstrated a normal 46,XX result. Genetic analysis for four single-nucleotide polymorphisms (SNPs) in the *FSHR* gene associated with ovarian response (rs6165 GA, rs6166 GG, rs1394205 AA, rs10835638 GT) to gonadotrophin stimulation in a private laboratory showed no variant.

At our center, the physical examination showed an average body habitus, Tanner stage III breast and pubic hair development, and no dysmorphic feature. According to the Practice Committee of the American Society for Reproductive Medicine on the current evaluation of amenorrhea,[9] we performed a subclinical assessment. Results showed AMH 1.5 ng/mL, FSH 40.6 mIU/mL (elevated), LH 28.1 mIU/mL (elevated), estradiol <18.4 pmol/L (low), with normal progesterone, prolactin, and testosterone (10/02/2025, using testing method Pro-1-A2-e801-2-2). Pelvic ultrasound revealed a small uterus with antral follicle count of 4+3. Preliminary diagnosis: Primary amenorrhea, suspected primary ovarian insufficiency. Family historyhad no abnormality; her younger sister has regular menstrual cycle (Figure 1).

The woman was counseled to undergo clinical exome sequencing (CES-4503 genes), which includes genes associated with primary ovarian depletion or poor ovarian response.

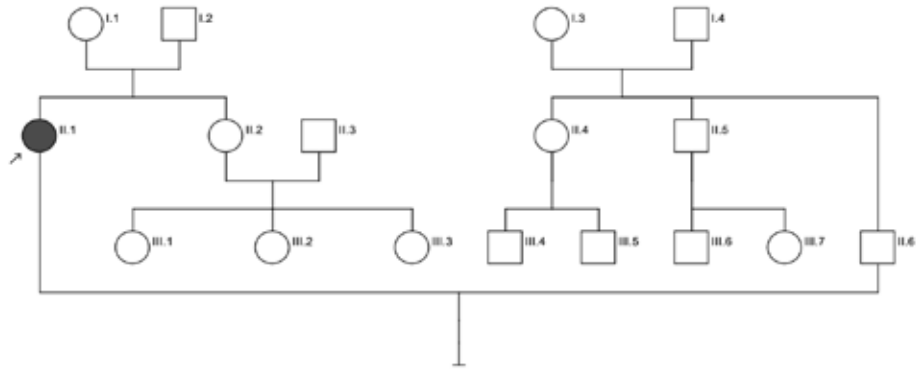

Figure 1. Pedigree of the patient

2. Testing Method and Genetic Results

Testing method: Next-generation sequencing of 4503 genes in the coding genome. Enzymatic fragmentation of genomic DNA and enrichment with a DNA capture probe were performed. New England Biolabs (USA) kit-prepared CES libraries were enhanced using IDTDNA targeted xGen probes. A minimum mean coverage of 100x and at least 95% of target genes covered more than 10X were sequenced in the Illumina Nextseq System (USA). Variants were detected by aligning sequencing data to GRCh38. Variants with frequency below 1% would be examined in the genomic database, including the 1000 Exome Sequencing Project (ESP). All gnomAD database variations with a minor allele frequency (MAF) < 1% and deleterious variants from HGMD®, ClinVar, or internal Biodatabank were evaluated. Genotyping showed significant variation between the coding exons and the +/-10 nucleotides of the gene's junction region (OMIM® data). According to the American College of Medical Genetics and Genomics (ACMG) guideline/ClinGen SVI recommendations, variants were classified as pathogenic, likely pathogenic, variant of uncertain significance [VUS], likely benign, or benign. Direct Sanger sequencing verified low-quality and/or unclear genetic variants. Gene rearrangements (fusions, inversions, etc.) and alterations of short tandem repeats, pseudogenes, and mosaic variants may not be investigated. The test does not examine mitochondrial genes.

Genetic analysis identified novel biallelic variants in *FSHR*: NM_000145.4:c.656_659del and NM_000145.4:c.263C>T (Table 1). Sanger sequencing of first-degree relatives confirmed the in-*trans* phase: the father was heterozygous for c.656_659del (Figure 2A), the mother was heterozygous for c.263C>T (Figure 2B), and an unaffected younger sister tested negative for both variants (Figures 2C and 2D). Neither variant has been previously reported in the literature, on ClinVar, or in our internal database. These findings support an autosomal recessive inheritance pattern, consistent with the patient's clinical phenotype of PA/POI.[4] Copy-number variant sequencing was analyzed concurrently and revealed no abnormality with seq (X,1-22)×2.

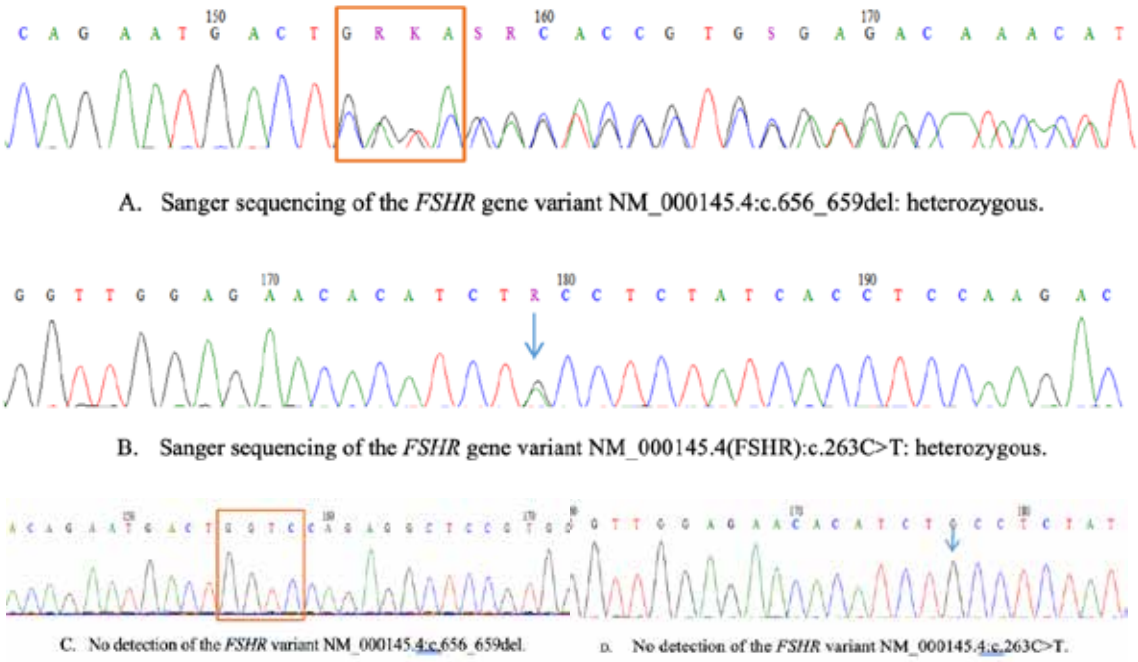

Figure 2. Sanger sequencing results for father (A), mother (B), and younger sister (C, D)

Variants Interpretation according to the American College of Medical Genetics and Genomics (ACMG) guideline/ClinGen SVI recommendations:[4,10]

Table 1. Variant information and classification

The variant in the *FSHR* gene (NM_000145.4)	c.656_659del	c.263C>T
Exon	8/10	3/10
Mutation type	A frameshift deletion	A Substitution
Molecular sequence	Nonsense (Null variant/ premature termination codon)	Missense
Frequency (gnomAD)	Not Found	0.000000684
Classification	Likely Pathogenic (PVS1, PM2_supporting)	VUS 5 points - high possibility of pathogenicity (PM2_ supporting, PM3, PP2, PP4)

Note: PVS1: Null variant in a gene where LOF is a known mechanism of disease. PM2_supporting: this variant is very rare/absent from large population databases (gnomAD) (PM2_supporting). Missense variant in a gene with a low rate of benign missense mutations and for which a missense mutation is a common mechanism of a disease (PP2). The clinical phenotype and subclinical findings (PA, hypergonadotropic hypogonadism, failed ovarian stimulation) and family history are consistent with FSHR-associated ovarian dysfunction (PP4). This variant was detected in trans with a likely pathogenic variant in a patient with POI (this case) (PM3).

III. DISCUSSION

Primary ovarian insufficiency is diagnosed in women under 40 years of age when they experience menstrual disturbances (absent or irregular periods) for at least four months combined with elevated follicle-stimulating hormone (FSH) levels greater than 25 IU/L measured on two separate occasions at least four weeks apart.[11] The diagnostic workup includes measuring additional hormones such as estradiol (typically low, <50 pg/mL) and AMH, performing a pregnancy test to exclude pregnancy, and conducting a pelvic ultrasound to assess ovarian morphology and antral follicle count.[12] Once POI is confirmed, physicians perform etiological investigations, including karyotype analysis to detect chromosomal abnormalities like Turner syndrome, *FMR1* gene testing for Fragile X premutation, and autoimmune screening for thyroid and adrenal disorders, though 50-90% of cases remain idiopathic with no identifiable cause.[12] Genetic variants associated with POI are increasingly identified with the advancement of next-generation sequencing technology, including the *FSHR* gene.

The FSH receptor, a G protein-coupled receptor, is encoded by the *FSHR* gene, which comprises 10 exons and nine introns. Exons 1-9 encode the extracellular domain, whereas exon 10 encodes the C-terminal portion of the extracellular domain, the transmembrane region, and the intracellular domain. The extracellular domains confer selectivity and high affinity in ligand binding, while the transmembrane domains facilitate G protein receptor activation and signal transduction.[13] Pathogenic variants in *FSHR* are an exceptionally rare cause of primary amenorrhea, accounting for less than 1% of cases.[1,3,6] LOF mutations disrupt receptor expression, ligand binding, or downstream signaling, leading to ovarian resistance to FSH and hypergonadotropic hypogonadism.[4] The c.656_659del variant introduces a premature termination codon, strongly supporting loss-of-function, whereas the c.263C>T missense change remains a variant of uncertain significance. Both variants are located on the transmembrane receptor domain. In this case, the affected proband is compound heterozygous for a variant present in each of her parents, and her unaffected sibling did not inherit the variants. The patient's genotype is consistent with autosomal recessive inheritance.

In literature, Aittomäki et al. first described recessive *FSHR* mutations in Finnish women with hereditary ovarian failure,[2] Meduri et al. later provided functional evidence linking receptor defects to ovarian dysgenesis,[4] Desai et al. reviewed multiple variants with variable effects on receptor function,[3] and Yoo et al. described two Korean sisters with PA carrying novel *FSHR* mutations (c.1364T>G and c.374T>G), both confirmed by Sanger sequencing.[5] A Chinese family with two

siblings, both carrying compound heterozygous pathogenic variants of *FSHR*: c.182T>A (p.Ile61Asn) and c.2062C>A (p.Pro688Thr). Both siblings had amenorrhea, infertility, and resistance to gonadotropin (Gn) stimulation but showed high anti-Müllerian hormone levels and early antral follicles. These findings collectively emphasize the underrepresentation of Southeast Asian data and the value of genetic testing in this region.[6-8] In women with PA, elevated FSH/LH, and low estradiol, *FSHR* sequencing (in a panel of genes associated with PA/POI) should be included in the investigation after normal cytogenetic testing. This highlights the importance of applying next-generation sequencing to identify the causes of unknown POI. Molecular diagnosis could provide a more obvious prognosis of pregnancy ability and alternative reproductive strategies. Women with *FSHR* mutations often have a poor response to exogenous gonadotropins, explaining recurrent IVF failure; in vitro maturation or oocyte donation may represent more viable reproductive options. In genetic counseling, identifying causative variants enables family screening and guides reproductive planning, which may include carrier screening for the partner.

In women with primary amenorrhea, elevated FSH/LH and low estradiol, *FSHR* sequencing within a targeted gene panel should be performed after normal cytogenetic testing. Identification of causative variants guides reproductive counseling, enables family screening, and informs clinical decision-making, including consideration of alternative reproductive strategies such as in vitro maturation or oocyte donation.

In summary, this case highlights the importance of integrating clinical, endocrine, and genetic data in diagnosing unexplained ovarian insufficiency and underscores the need for functional validation and population studies to better understand *FSHR*-related primary amenorrhea in Southeast Asia. Finally, from a research perspective, the identification of these novel variants underscores the urgent need for functional validation studies and for establishing genetic population research in Vietnam to better define prevalence and mutational spectra.

IV. CONCLUSION

We report the first Vietnamese case of primary amenorrhea associated with a novel compound heterozygous variant in *FSHR*, comprising a frameshift deletion (c.656_659del, p.Gly219GlufsTer4) and a missense variant (c.263C>T, p.Ala88Val). The frameshift mutation is likely pathogenic, while the missense variant is classified as a variant of uncertain significance with high pathogenic potential. This report broadened the mutational spectrum of *FSHR* and highlighted the importance of evaluating the *FSHR* gene as a potential cause of primary amenorrhea or inadequate response to ovarian stimulation. Functional validation studies and population-level genomic screening in Vietnam are needed to characterize *FSHR*-associated ovarian dysfunction better.

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DISCLOSURE

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