

# Paternal Origin and Subsequent Maternal Inheritance of a Novel FOXL2 Frameshift Variant Underlie Accelerated Ovarian Aging: A Three-Generation BPES-I Family Study

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## Research Article

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# Abstract

**Background:** Type I Blepharophimosis-Ptoxis-Epicanthus Inversus Syndrome (BPES-I) is characterized by eyelid malformations and Premature Ovarian Insufficiency (POI). While *FOXL2* variants are the primary cause, the impact of paternal origin followed by maternal inheritance on ovarian aging trajectories remains poorly defined. This study characterizes a three-generation Chinese family with a novel *FOXL2* variant to elucidate its structural consequences and impact on the ovarian reserve trajectory.

**Methods:** Comprehensive clinical and hormonal assessments were performed. Whole Exome Sequencing (WES) and Sanger sequencing identified the causal variant, which was classified using ACMG 2015 guidelines and structurally mapped via AlphaFold3. Wild-type and mutant plasmids were transfected into HeLa cells to assess mRNA/protein expression via RT-qPCR and Western blotting. Downstream targets were validated by RT-qPCR.

**Results:** A novel heterozygous *FOXL2* frameshift variant, c.962\_967delinsCT (p.Gln321Profs\*34), was identified and classified as Pathogenic (De Baere Class D). Segregation analysis revealed the variant originated from the maternal grandfather (paternal origin) and was subsequently transmitted maternally to the mother, aunt, and proband. Functionally, the mutant exhibited significant mRNA and protein decay, confirming haploinsufficiency. Clinically, the family exhibited a striking acceleration of the ovarian aging trajectory: the mother and the aunt experienced fertility and early menopause at 40-41 years, whereas the proband presented with infertility and severe POI at 26 years. This anticipation-like pattern highlights the dosage-sensitive nature of *FOXL2* in maintaining the primordial follicle pool. The mutant protein failed to sustain the expression of key folliculogenesis regulators, including *CYP19A1* and *FST*.

**Conclusion:** We firstly report a paternally derived *FOXL2* variant can drive severe BPES-I via maternal inheritance. The convergence of clinical phenotyping, wet-lab validation, and public bioinformatics data establishes a robust model of *FOXL2*-driven accelerated ovarian aging, advocating for universal AMH surveillance in female carriers regardless of variant origin.

## Highlights

- First report of a paternally derived *FOXL2* Class D frameshift variant (c.962\_967delinsCT) causing overt BPES-I via two generations of maternal inheritance, directly challenging the canonical paternal bias in *FOXL2*-related premature ovarian insufficiency (POI).
- Documented an anticipation-like acceleration of ovarian aging, with a 14-year advancement in ovarian failure onset (early menopause at 40 in the mother vs. severe POI at 26 in the proband), clarifying the nonlinear trajectory of *FOXL2*-driven follicular depletion.
- Mechanistically validated that the variant triggers haploinsufficiency via nonsense-mediated mRNA decay, disrupting core folliculogenesis pathways and accelerating the transition from slow follicular attrition to rapid reserve loss.
- Clinically demonstrate that parental origin is an unreliable predictor of ovarian outcomes in *FOXL2* carriers, advocating for universal longitudinal AMH surveillance and early fertility counseling for all at-risk females regardless of variant origin or maternal menopausal age.

## 1. Introduction

Premature Ovarian Insufficiency (POI) affects approximately 1% of women under 40 and represents a major cause of female infertility<sup>[1]</sup>. Among the monogenic causes of POI, variants in the Forkhead box L2 (*FOXL2*) gene are

prominent, particularly in individuals presenting with Blepharophimosis-Ptoxis-Epicanthus Inversus Syndrome (BPES) [2]. BPES is clinically subdivided into Type I (BPES-I), which includes both eyelid malformations and POI, and Type II (BPES-II), which involves only ocular features [3].

*FOXL2* encodes a transcription factor essential for granulosa cell differentiation and follicular maintenance [4]. Genotype-phenotype correlations have been extensively studied; notably, BPES-I has historically exhibited a paternal inheritance bias. It has been hypothesized that *FOXL2* may be subject to ovarian-specific genomic imprinting, where the maternal allele is preferentially expressed in the ovary. Under this model, a variant inherited from the father (paternal origin) would leave only the (potentially imprinted/silent) maternal allele active, leading to haploinsufficiency and severe POI. Conversely, a variant inherited from the mother (maternal origin) would spare the active paternal allele, potentially resulting in milder ovarian phenotypes (BPES-II) or later-onset POI [5]. However, clinical observations of overt POI in maternally inherited cases challenge this simplistic view [6].

Furthermore, the trajectory of ovarian decline in *FOXL2* variant carriers remains unclear. While "genetic anticipation" is classically linked to trinucleotide repeat disorders, an anticipation-like pattern (earlier menopause in younger generations) has been anecdotally reported in BPES families [7]. Whether this reflects true biological acceleration or ascertainment bias is unknown, particularly in families where the variant originates from a paternal source but is passed through the maternal line across generations.

Ovarian aging is defined as the progressive, synchronized decline in follicular quantity (depleted ovarian reserve) and quality (impaired oocyte competence), culminating in the exhaustion of primordial follicles [8]. This process follows a non-linear, biphasic trajectory: a slow attrition phase followed by rapid depletion [8, 9]. Pathological acceleration manifests as early menopause (40–45 years) or POI (< 40 years) [10]. Understanding how the parental origin of *FOXL2* variants modulates this trajectory is vital for risk stratification.

In this study, we investigated a three-generation Chinese family with BPES-I. We identified a novel *FOXL2* frameshift variant (c.962\_967delinsCT, p.Gln321Profs\*34) belonging to De Baere Class D. Crucially, segregation analysis revealed the variant originated from the maternal grandfather (paternal origin) and was transmitted maternally to affected female relatives. We demonstrate that this paternal-to-maternal transmission cascade leads to accelerated ovarian aging in the proband compared to her mother. Through in vitro functional assays and re-analysis of public transcriptomic datasets, we elucidate the molecular basis of this phenotype, providing insights into the complex interplay between variant origin, inheritance pattern, and ovarian reserve depletion.

## 2. Materials and Methods

### 2.1 Ethical Compliance and Clinical Evaluation

The study was approved by the Ethics Committee of the Second Xiangya Hospital of Central South University (Approval No. 2022-KT110). Written informed consent was obtained from all participants. A three-generation family was recruited. Clinical evaluation included ophthalmic examination, menstrual history, and hormonal profiling (FSH, LH, E2, AMH). Ovarian reserve was assessed via transvaginal ultrasound (AFC).

### 2.2 Genetic Analysis

Genomic DNA was extracted from peripheral blood leukocytes. Whole Exome Sequencing (WES) was performed on the Salus Pro platform (ShenZhen Salus Biomed Ltd). Candidate variants were filtered against dbSNP, 1000 Genomes,

and gnomAD. The identified *FOXL2* variant was validated by Sanger sequencing. Segregation analysis was performed to trace the variant's origin through the paternal grandfather (II-3) to the mother (III-2) and proband (IV-2).

## 2.3 In Silico Analysis

The variant was classified according to ACMG 2015 guidelines<sup>[11]</sup>. Protein structural domains (Forkhead Domain, Polyalanine tract) were mapped using UniProt annotations. Evolutionary conservation was assessed using ClustalW<sup>[12]</sup>. The three-dimensional impact of the truncation was predicted using AlphaFold3<sup>[13]</sup>.

## 2.4 Bioinformatic analysis of public transcriptomic datasets

To contextualize our experimental findings, we re-analyzed the publicly available transcriptomic dataset GSE154185<sup>[14]</sup>, which profiles gene expression in murine granulosa cells following *Foxl2* knockdown (KD) via siRNA. Raw counts were processed using DESeq2 to identify Differentially Expressed Genes (DEGs;  $|\log_2 \text{Fold Change}| > 1$ , adjusted  $P < 0.05$ ). Gene Ontology (GO) enrichment analysis for biological processes was performed using the clusterProfiler package in R. Key DEGs involved in steroidogenesis and folliculogenesis were visualized using heatmaps and volcano plots.

## 2.5 Plasmid Construction and Cell Transfection

The wild-type (WT) human *FOXL2* cDNA was cloned into the pEGFP-N1 vector. The p.Gln321Profs\*34 variant (MUT) was generated via site-directed mutagenesis. HeLa cells were cultured in DMEM with 10% FBS. Transfections were performed using PEI at 70–80% confluence.

## 2.6 RNA Extraction and RT-qPCR

Total RNA was extracted 24 hours post-transfection using Trizol. cDNA was synthesized using HiScript II Reverse Transcriptase. Quantitative PCR was performed using ChamQ SYBR Master Mix on a CFX96 Touch system. Primers targeted *FOXL2*, *CYP19A1*, *FST*, *TP63*, *CCND1*, *WNT2B*, and *GAPDH* (internal control).

## 2.7 Protein Extraction and Western Blotting

Protein lysates were harvested 48 hours post-transfection. Equal amounts of protein were separated by SDS-PAGE and transferred to PVDF membranes. Membranes were incubated with primary antibodies: anti-GFP (1:1000) and anti-GAPDH (1:5000). HRP-conjugated secondary antibodies were used, and signals were detected via chemiluminescence. Band intensities were quantified using ImageJ.

## 2.8 Statistical Analysis

Data are presented as Mean  $\pm$  SD. Differences between WT and MUT groups were analyzed using Student's t-test.  $P < 0.05$  was considered statistically significant. All analyses were performed using GraphPad Prism 10.0.

## 3. Results

To unravel the molecular basis of the observed anticipation-like progression in this unique pedigree, we integrated deep clinical phenotyping with a multi-tiered investigative pipeline. We hypothesized that a high-impact *FOXL2* variant, originating from the paternal lineage but exerting its effect through maternal transmission, drives this accelerated ovarian aging trajectory. Here, we delineate the segregation of this variant, characterize its structural consequences via cutting-edge AI-powered proteostasis modeling (AlphaFold3), and validate its pathogenicity through convergent in vitro functional assays and public transcriptomic meta-analysis.

### 3.1 Clinical Phenotype and Transmission Route

The proband (IV-2), a 26-year-old woman, presented with primary infertility, classic BPES ocular features, and severe POI (AMH: 0.08 ng/mL; FSH: 35.59 mIU/mL). Her mother (III-2), also exhibiting BPES ocular features, experienced natural menopause at age 40 (classified as Early Menopause per STRAW + 10 criteria) <sup>[15]</sup>. The maternal aunt (III-3) exhibited ocular features and had children, with no reported POI. The maternal grandfather (II-3, deceased) had documented BPES ocular features but no ovarian phenotype (male). (Fig. 1A )

The proband's ovarian failure (POI at 26 years) occurred 14 years earlier than the early menopause of her mother (40 years) and her aunt (41 years). This represents a significant acceleration of the ovarian aging trajectory across one generation. (Table 1).

Table 1  
Clinical and Genetic Characteristics of the Family

| Individual | Role                 | Age | Ocular BPES | Menopause Age | AMH (ng/mL) | FSH (mIU/mL) | FOXL2Status  | Origin of Variant    |
|------------|----------------------|-----|-------------|---------------|-------------|--------------|--------------|----------------------|
| II-3       | Maternal Grandfather | 78  | Yes         | N/A           | N/A         | N/A          | Heterozygous | Paternal Origin      |
| III-2      | Mother               | 52  | Yes         | 40            | N/A         | N/A          | Heterozygous | Paternal Inheritance |
| III-3      | Maternal Aunt        | 50  | Yes         | 41            | N/A         | N/A          | Heterozygous | Paternal Inheritance |
| IV-2       | Proband              | 28  | Yes         | 26            | 0.08        | 35.59        | Heterozygous | Maternal Inheritance |

### 3.2 Genetic Identification of a Novel Frameshift Variant

Pedigree analysis unequivocally demonstrated the variant originated from the maternal grandfather (II-3, paternal origin) and was transmitted maternally to his daughters and granddaughter (Fig. 1A).

To pinpoint the underlying molecular defect driving this phenotype, we performed whole exome sequencing, which revealed a novel heterozygous deletion/insertion in FOXL2(NM\_023067.4): c.962\_967delinsCT, predicting a frameshift at codon 321 followed by a premature stop 34 amino acids downstream (p.Gln321Profs\*34). This variant was absent in the gnomAD, 1000 Genomes, and in-house control databases, and segregated perfectly with the BPES-I phenotype within the family. According to ACMG 2015 guidelines, it was classified as Pathogenic (PVS1 + PM2+PM4 + PP3+PP4).

Sanger sequencing confirmed the heterozygous FOXL2 variant c.962\_967delinsCT in the proband (IV-2) and her mother (III-2), and her aunt (III-3). The variant was absent in the unaffected father (III-1). Pedigree analysis unequivocally demonstrated the variant originated from the maternal grandfather (II-3, paternal origin) and was transmitted maternally to his daughters (III-2, III-3) and granddaughter (IV-2) (Fig. 1B and 1D).

Structurally, the variant localizes downstream of the intact forkhead domain (FHD; residues 54–148) and polyalanine tract (residues 221–234), assigning it to De Baere Class D. AlphaFold3 modeling revealed that the mutant protein (354 aa) lacks 22 residues at the C-terminus compared to the wild-type (376 aa), disrupting the irregular coiled-coil region essential for transcriptional activation while preserving the core DNA-binding architecture (Fig. 2).

### 3.3 Functional Haploinsufficiency Validation

RT-qPCR showed significantly reduced *FOXL2* mRNA in MUT-transfected cells vs. WT ( $P < 0.05$ ), indicating NMD (Fig. 3A). Western blotting confirmed reduced protein expression of the truncated variant (~ 66 kDa) vs. WT (~ 68 kDa) ( $P < 0.05$ ) (Fig. 3).

### 3.4 Downstream Dysregulation

To corroborate our in vitro findings, we analyzed GSE154185, modeling *Foxl2* loss-of-function. We identified 467 DEGs (250 upregulated, 217 downregulated; Supplementary Figure S1B). GO enrichment revealed significant perturbations in "gonad development," "steroid hormone biosynthesis," and "Wnt signaling pathway" (Supplementary Figure S1C). Crucially, key genes downregulated in the *Foxl2*-KD dataset (*Cyp19a1*, *Fst*, *Tp63*, *Ccnd1*, *Wnt2b*) overlapped precisely with genes suppressed by our mutant variant in vitro (Supplementary Figure S1A). This convergence strongly supports the pathogenicity of the variant and its role in disrupting ovarian homeostasis.

As downstream targets, MUT transfection significantly downregulated *CYP19A1*, *FST*, *TP63*, *CCND1*, and *WNT2B* ( $P < 0.05$ ) (Fig. 4A), confirming disruption of folliculogenesis pathways (Fig. 4B).

## 4. Discussion

This study reports a novel *FOXL2* Class D frameshift variant (c.962\_967delinsCT) in a Chinese family with BPES-I. A key strength is the precise delineation of the transmission route: the variant originated from the maternal grandfather (paternal origin) and was passed maternally through two generations. Our findings offer critical insights into the interplay between variant origin, inheritance pattern, and ovarian phenotype.

First, we demonstrate that a paternally originated *FOXL2* variant can cause severe BPES-I when transmitted maternally, challenging the simplistic interpretation of the "paternal bias." While the paternal bias suggests paternal variants drive POI, our family shows the same variant (originating paternally) can cause a spectrum of ovarian phenotypes—from parity in the aunt (III-3) to early menopause in the mother (III-2) and severe POI in the proband (IV-2)—when inherited maternally. This suggests that parental origin alone is an incomplete predictor of ovarian outcome. Instead, the dosage of functional *FOXL2* protein, modulated by genetic modifiers or stochastic factors during follicular development, likely determines the phenotype. The preserved FHD and polyalanine tract in our Class D variant allow partial functionality, but the C-terminal truncation proves insufficient for long-term reserve maintenance.

Second, we characterize an anticipation-like acceleration of ovarian aging in this paternal-to-maternal transmission cascade. The progression from early menopause in the mother (40 y) to POI in the proband (26 y) compresses the ovarian lifespan significantly. While classic anticipation involves repeat expansions, *FOXL2* polyalanine tracts are stable. The observed worsening likely reflects the dosage-sensitive nature of the variant combined with modifier effects. Haploinsufficiency (confirmed by NMD) appears to cross a critical threshold in the proband. This highlights that even variants originating paternally can lead to severe, accelerating ovarian aging when transmitted through the maternal line.

Third, our functional assays delineate the molecular mechanism. Downregulation of *CYP19A1* and *FST* confirms impaired steroidogenesis and disrupted TGF- $\beta$  signaling<sup>[16, 17]</sup>. Suppression of *WNT2B-CCND1* and *TP63* suggests inhibited granulosa proliferation and compromised oocyte integrity<sup>[18, 19]</sup>. This convergence explains how Class D variants disrupt ovarian homeostasis.

**Molecular mechanisms driving accelerated ovarian aging in a paternal-to-maternal transmission cascade**

A key strength of this study is the convergence of evidence from clinical observation, cellular assays, and public transcriptomic re-analysis. Our finding that the p.Gln321Profs\*34 variant disrupts *CYP19A1*, *FST*, and *WNT2B* expression was robustly validated by re-analysis of the GSE154185 dataset. This public data, derived from *Foxl2* knockdown in primary granulosa cells, provides an in vivo-relevant context that complements our HeLa cell transfection model. The overlap confirms that the C-terminal truncation in Class D variants impairs the transcriptional program essential for maintaining the follicular pool. This integrated approach elevates our findings beyond a single family report, positioning the variant within the broader molecular pathology of *FOXL2*-related ovarian aging.

### **Clinical implications: redefining surveillance based on transmission route**

Viewing BPES-I through the lens of accelerated ovarian aging reframes management. Ovarian aging is continuous. The mother and the aunt's early menopause is a sentinel event indicating shortened ovarian lifespan and long-term health risks (cardiovascular, skeletal) <sup>[15, 20]</sup>. Our family demonstrates that the origin of the variant (paternal grandfather) and maternal transmission do not preclude severe POI in the next generation. Therefore, we propose that all female *FOXL2* variant carriers, regardless of the grandparental origin (paternal or maternal) or the mother's menopausal age, require longitudinal ovarian aging surveillance. Serial AMH measurement from late adolescence tracks depletion rates. Identifying the inflection point between slow attrition and rapid decline provides a critical window for fertility preservation counseling and timely hormone replacement therapy to mitigate consequences of accelerated aging.

### **Limitations**

Focus on a single family and use of HeLa cells (non-granulosa) are limitations. Future studies with larger cohorts and iPSC-derived ovarian models are warranted.

## **5. Conclusion**

In conclusion, we identified a novel *FOXL2* Class D frameshift variant transmitted via a paternal-to-maternal cascade causing BPES-I. The variant induces haploinsufficiency, disrupting key ovarian pathways and accelerating ovarian aging across generations. These results challenge the notion that paternal origin guarantees severe POI, highlighting the critical need for early, aggressive ovarian reserve assessment in all female carriers, irrespective of variant origin or maternal phenotype.

## **Declarations**

**Ethics approval and consent to participate:** Approved by the Ethics Committee of the Second Xiangya Hospital of Central South University (2022-KT110). Written informed consent was obtained from all participants.

**Consent for publication:** Written informed consent for publication of clinical details and images was obtained from the participants.

**Availability of data and materials:** The datasets used during the current study are available from the corresponding author on reasonable request. The *FOXL2* variant has been submitted to ClinVar (Accession number: [SCV007651229]).

**Competing interests:** The authors declare that they have no competing interests.

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**Authors' contributions:** Suping Li, Mengjiao Hu, Ru Xu, and Hualin Huang conceived and designed the study. Jiaqi Sun, Qi Qi and Wenxin Chen contributed to the clinical data collection and interpretation. Hualin Huang and Jianlin Chen assisted with bioinformatic analysis, validation and manuscript editing. All authors contributed to the article and approved the submitted version.

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## References

1. Webber L, Davies M, Anderson R, et al. ESHRE Guideline: management of women with premature ovarian insufficiency[J]. Hum Reprod. 2016;31(5):926–37.
2. Crisponi L, Deiana M, Loi A, et al. The putative forkhead transcription factor FOXL2 is mutated in blepharophimosis/ptosis/epicanthus inversus syndrome[J]. Nat Genet. 2001;27(2):159–66.
3. De Baere E, Dixon MJ, Small KW, et al. Spectrum of FOXL2 gene mutations in blepharophimosis-ptosis-epicanthus inversus (BPES) families demonstrates a genotype–phenotype correlation[J]. Hum Mol Genet. 2001;10(15):1591–600.
4. Uda M, Ottolenghi C, Crisponi L, et al. Foxl2 disruption causes mouse ovarian failure by pervasive blockage of follicle development[J]. Hum Mol Genet. 2004;13(11):1171–81.
5. Bunyan DJ, Thomas NS. Screening of a large cohort of blepharophimosis, ptosis, and epicanthus inversus syndrome patients reveals a very strong paternal inheritance bias and a wide spectrum of novel FOXL2 mutations[J]. Eur J Med Genet. 2019;62(7):103668.
6. Harris SE, Chand AL, Winship IM, et al. Identification of novel mutations in FOXL2 associated with premature ovarian failure[J]. Mol Hum Reprod. 2002;8(8):729–33.
7. Beysen D, De Jaegere S, Amor D, et al. Identification of 34 novel and 56 known FOXL2 mutations in patients with Blepharophimosis syndrome[J]. Hum Mutat. 2008;29(11):E205–19.
8. Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: mechanisms and clinical consequences[J]. Endocr Rev. 2009;30(5):465–93.
9. Wallace WHB, Kelsey TW. Human ovarian reserve from conception to the menopause[J]. PLoS ONE. 2010;5(1):e8772.
10. Qin Y, Jiao X, Simpson JL, et al. Genetics of primary ovarian insufficiency: new developments and opportunities[J]. Hum Reprod Update. 2015;21(6):787–808.
11. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology[J]. Genet Med. 2015;17(5):405–24.

12. Thompson JD, Gibson TJ, Higgins DG. Multiple sequence alignment using ClustalW and ClustalX[J]. *Curr Protoc Bioinformatics*, 2002,Chap. 2:2–3.
13. Abramson J, Adler J, Dunger J, et al. Accurate structure prediction of biomolecular interactions with AlphaFold 3[J]. *Nature*. 2024;630(8016):493–500.
14. Gene Expression Omnibus. GSE154185. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE154185> [EB/OL]
15. Harlow SD, Gass M, Hall JE, et al. Executive summary of the Stages of Reproductive Aging Workshop + 10: addressing the unfinished agenda of staging reproductive aging[J]. *J Clin Endocrinol Metab*. 2012;97(4):1159–68.
16. Pannetier M, Fabre S, Batista F, et al. FOXL2 activates P450 aromatase gene transcription: towards a better characterization of the early steps of mammalian ovarian development[J]. *J Mol Endocrinol*. 2006;36(3):399–413.
17. Georges A, L'Hôte D, Todeschini AL et al. The transcription factor FOXL2 mobilizes estrogen signaling to maintain the identity of ovarian granulosa cells[J]. *Elife*, 2014,3.
18. Gustin SE, Hogg K, Stringer JM, et al. WNT/ $\beta$ -catenin and p27/FOXL2 differentially regulate supporting cell proliferation in the developing ovary[J]. *Dev Biol*. 2016;412(2):250–60.
19. Huang C, Zhao S, Yang Y et al. TP63 gain-of-function mutations cause premature ovarian insufficiency by inducing oocyte apoptosis[J]. *J Clin Invest*, 2023,133(5).
20. Shuster LT, Rhodes DJ, Gostout BS, et al. Premature menopause or early menopause: long-term health consequences[J]. *Maturitas*. 2010;65(2):161–6.

## Figures

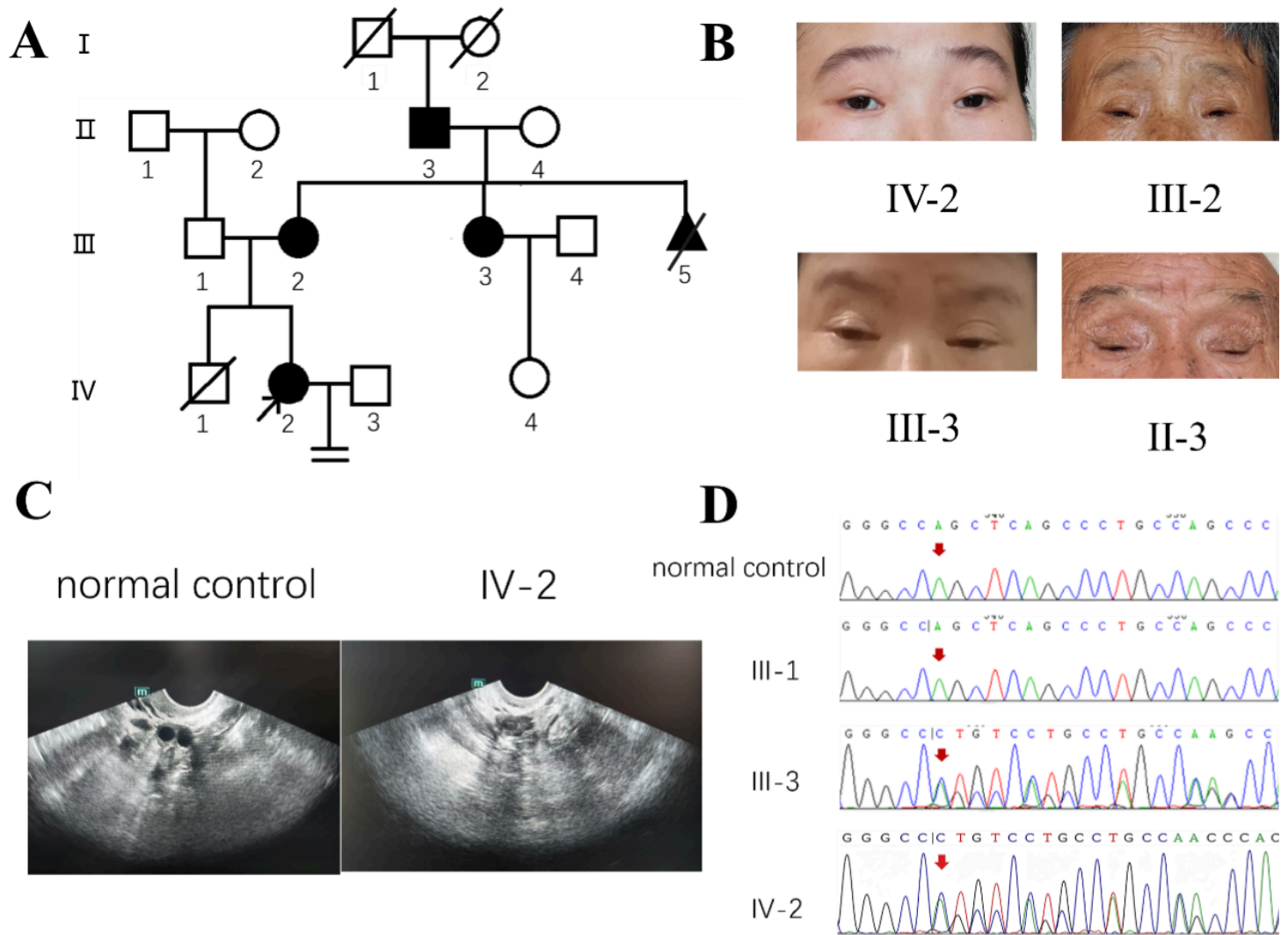


**Figure 1**

**Pedigree, clinical phenotype, and Sanger sequencing chromatograms.**

(A) Four-generation pedigree. The arrow indicates the proband (IV-2). Filled symbols denote individuals with BPES ocular features. The variant originated from the maternal grandfather (II-3, paternal origin) and was transmitted maternally to his daughters (III-2, III-3) and granddaughter (IV-2). (B) Ocular features of the proband (ptosis, blepharophimosis, epicanthus inversus). (C) Transvaginal ultrasound images: the mother (III-2, early menopause at 40) had occasional antral follicles, while the proband (IV-2, POI at 26) had no visible antral follicles (AFC=0). (D) Sanger sequencing chromatograms confirming the heterozygous 6-bp delins in the proband and mother, absent in the unaffected father.

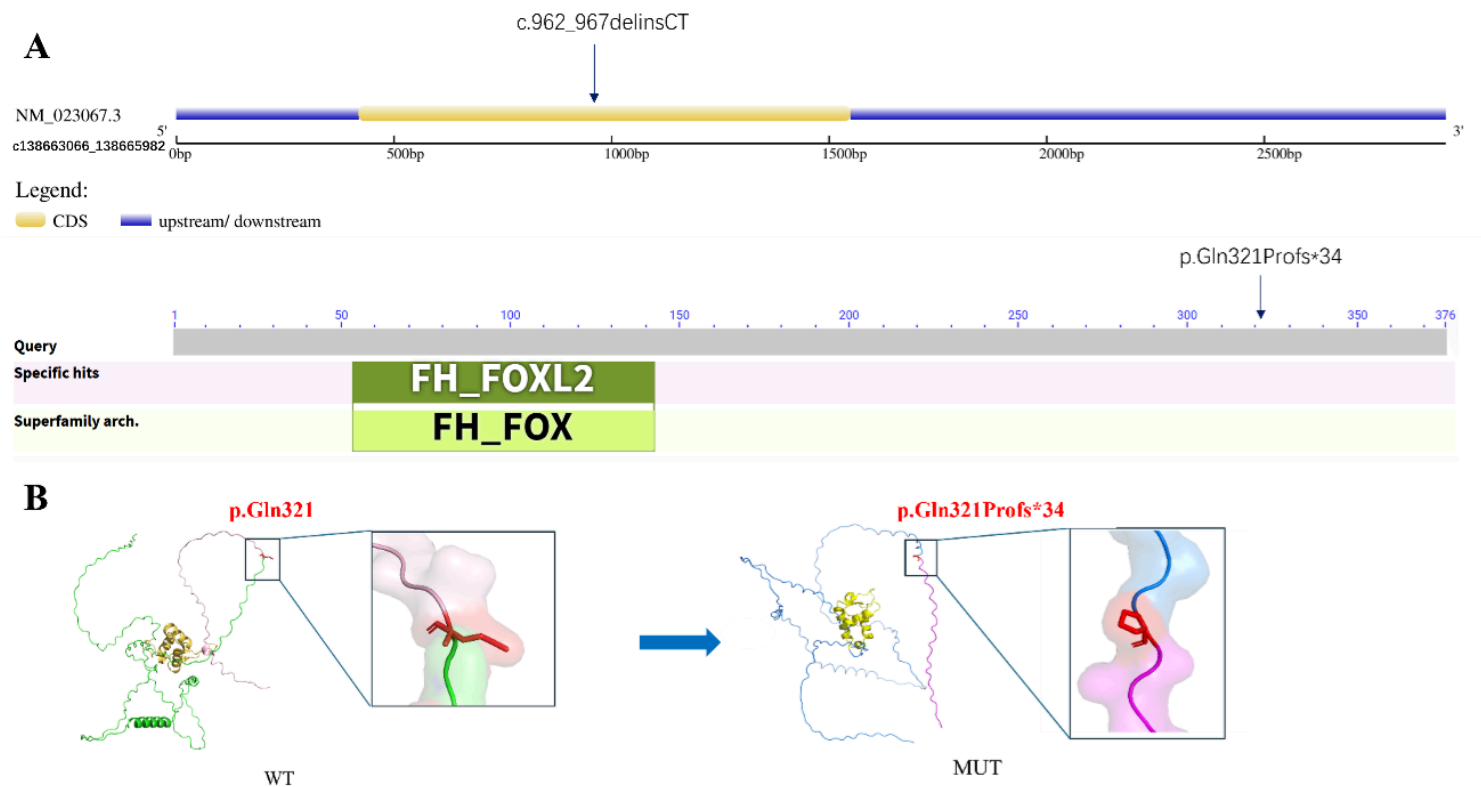


**Figure 2**

**Structural characterization of the *FOXL2* c.962\_967delinsCT variant.**

(A) Schematic of *FOXL2* domains: the variant localizes downstream of the forkhead domain (FHD) and polyalanine tract, defining it as Class D. (B) AlphaFold3 structural models: the mutant (MUT, 354 aa) lacks 22 C-terminal residues (red asterisk) compared to wild-type (WT, 376 aa), disrupting the coiled-coil activation domain. Abbreviations: EM, early menopause; POI, premature ovarian insufficiency; FHD, forkhead domain.

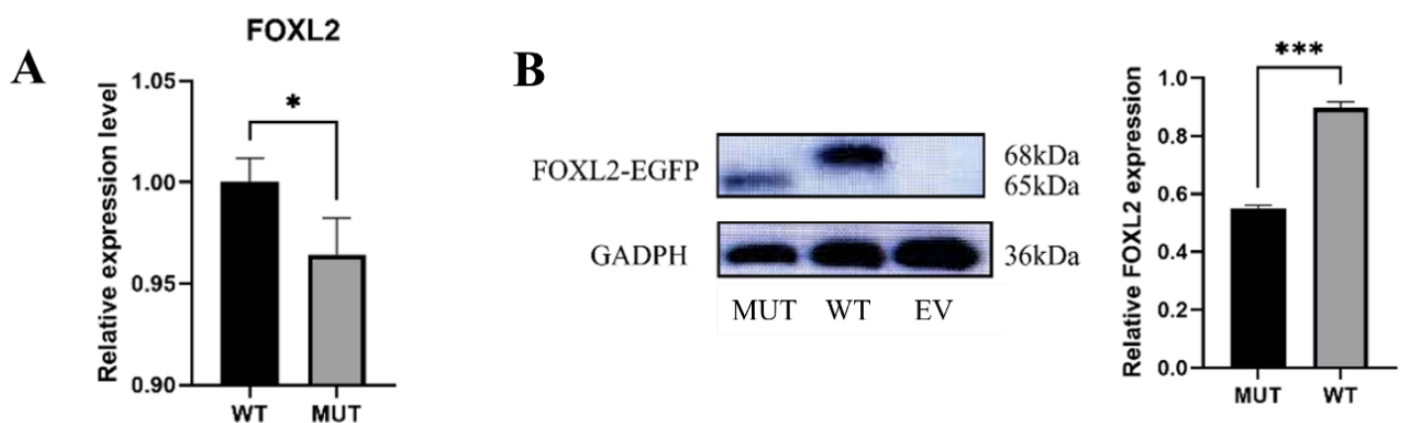
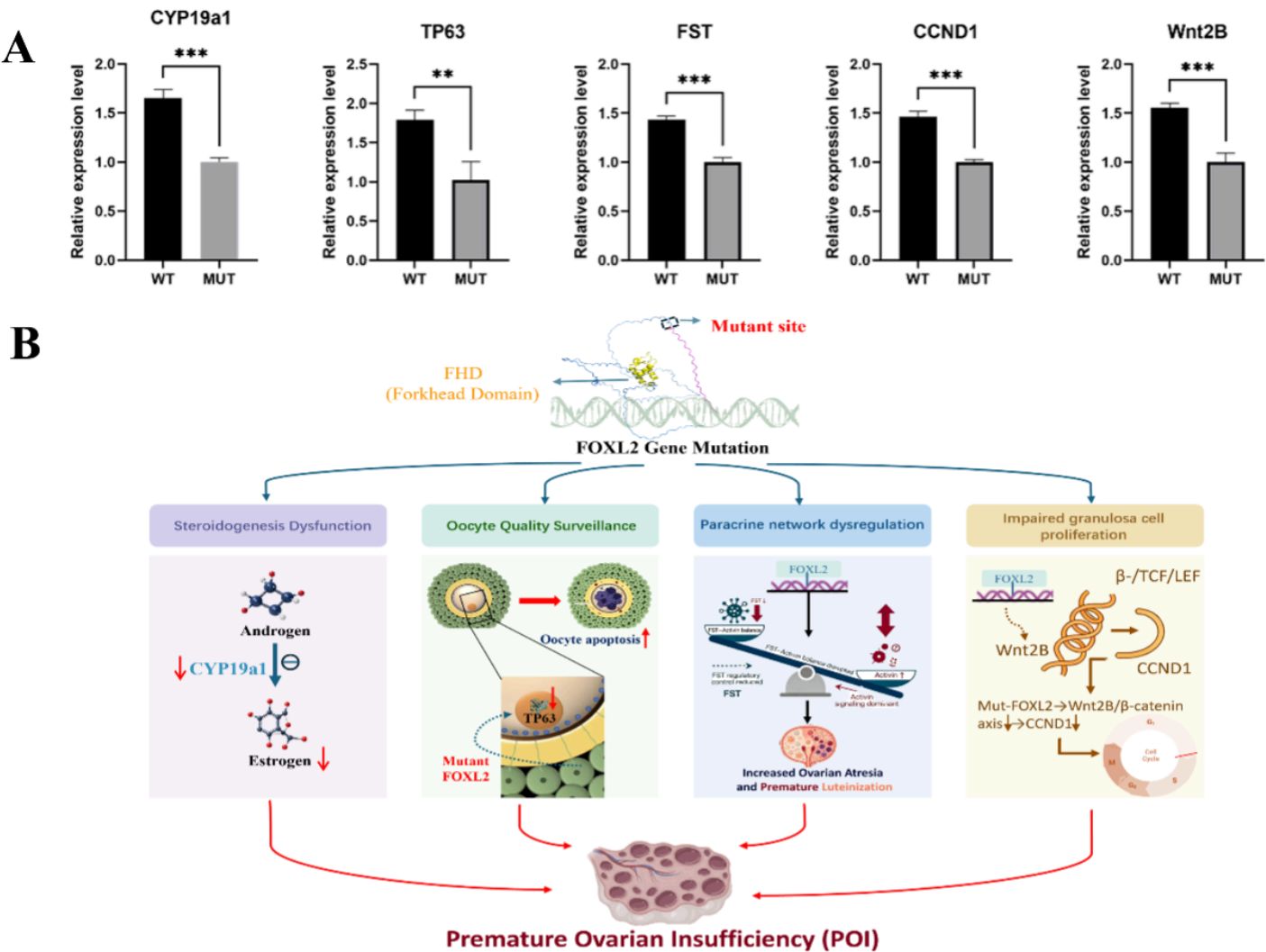


**Figure 3**

**The p.Gln321Profs\*34 variant induces haploinsufficiency.**

(A) RT-qPCR showing significant *FOXL2* mRNA decay in MUT-transfected cells vs. WT ( $P < 0.05$ ,  $n = 3$ ). (B) Western blot revealing reduced protein levels of the truncated MUT (~65 kDa) vs. WT (~68 kDa); GAPDH as loading control.

Densitometric quantification confirming reduced MUT protein expression ( $P<0.05$ ).



## Supplementary Files

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