Whole-exome sequencing in patients with premature ovarian insufficiency: early detection and early intervention

Hongli Liu 1†, Xiaoli Wei 2†, Yanwei Sha 3†, Wensheng Liu 4, Haijie Gao 3, Jin Lin 3, Youzhu Li 5, Yaling Tang 6, Yifeng Wang 4*, Yanlong Wang 1* and Zhiying Su 3*

Abstract

Background: The loss of ovarian function in women, referred to as premature ovarian insufficiency (POI), is associated with a series of concomitant diseases. POI is genetically heterogeneous, and in most cases, the etiology is unknown.

Methods: Whole-exome sequencing (WES) was performed on DNA samples obtained from patients with POI, and Sanger sequencing was used to validate the detected potentially pathogenic variants. An in silico analysis was carried out to predict the pathogenicity of the variants.

Results: We recruited 24 patients with POI and identified variants in POI-related genes in 14 patients, including bi-allelic mutations in DNAH6, HFM1, EIF2B2, BNC, and LRPPRC and heterozygous variants in BNC1, EIF2B4, FOXL2, MCM9, FANCA, ATM, EIF2B3, and GHR. No variants in the above genes were detected in the WES data obtained from 29 women in a control group without POI. Determining a clear genetic etiology could significantly increase patient compliance with appropriate intervention strategies.

Conclusions: Our study confirmed that POI is a genetically heterogeneous condition and that whole-exome sequencing is a powerful tool for determining its genetic etiology. The results of this study will aid researchers and clinicians in genetic counseling and suggests the potential of WES for the detection of POI and thus early interventions for patients with POI.

Keywords: Premature ovarian insufficiency, Pathogenic variants, Whole-exome sequencing, Early intervention, Compliance
**Background**
Premature ovarian insufficiency (POI) is defined as amenorrhea before the age of 40 and is characterized by FSH levels greater than 25 IU/L in two measurements for at least 4 weeks [1]. POI is a severe disorder affecting approximately 1% of women of childbearing age worldwide [2]. Genetic defects are a common cause of POI, as has been proven in a large number of studies. Currently, with the development of technology, more than 100 genes have been found to be associated with POI [3–6].

Whole-exome sequencing (WES) is widely used to identify the genetic etiologies of various diseases [7]. However, gynecologists diagnose and treat POI only based on a patient’s symptoms, and genetic methods have not been widely used to identify the genetic causative factors of idiopathic POI. With the significant decrease in the price of WES, it has emerged as a powerful tool with potential for clinical applications in the early detection of POI and timely intervention for patients with POI. Moreover, a clear genetic etiology could significantly improve treatment compliance.

In this study, we performed WES of DNA samples obtained from 24 patients with POI and found variants in POI-related genes in 14 patients. Therefore, it is possible to conduct etiological testing for patients with POI through WES, which could help in the timely intervention of POI.

**Results**

**Clinical features of patients with POI**
All 24 patients were diagnosed with sporadic POI according to standard criteria (detailed in the Methods section). Based on the physical examination results, none of the patients showed an obvious abnormality in physical development. The ovaries of all patients could be detected by transvaginal color Doppler ultrasound examination. However, all patients had abnormal hormone levels. All patients with POI had a normal 46, XX karyotype. The clinical characteristics of the POI patients are shown in Table 1.

**Whole-exome sequencing analysis of patients with premature ovarian insufficiency**
To characterize the genetic pathogenesis of POI, we performed WES of the DNA samples of the 24 POI patients. We retained the variants with a minor allele frequency of less than 1% in the dbSNP, 1000G, ESP6500, and gnomAD databases. The retained variants were filtered according to the selected candidate genes involved in POI. We identified 19 variants in 12 genes, including compound heterozygous variants in DNAH6, HFM1, EIF2B2, BNC1, and LRPPRC and nine heterozygous variants in BNC1, EIF2B4, FOXL2, MCM9, FANCA, ATM, EIF2B3, and GHR, from 14 patients (Table 2). None of the above genes were detected in the WES data from 29 women in the control group.

Sanger sequencing was performed on samples from a subset of patients (POI-1, 3, and 6–9) and their family members to validate the variants and investigate inheritance. POI-1 harbored two compound heterozygous variants in DNAH6, c.2407C > A and c.8680G > A. Her unaffected mother carried the heterozygous variant c.2407C > A, and her father carried the heterozygous variant c.2407C > A, and her father carried the heterozygous variant

| Case ID | Age (Years) | Menopause age (Years) | BMI (kg/m2) | Primary or secondary amenorrhea | FSH (IU/L) | LH (IU/L) | E2 (pg/mL) | Karyotype | Ovary size (right/ left, mm) | Follicle |
|---------|-------------|-----------------------|-------------|---------------------------------|------------|-----------|------------|-----------|-----------------------------|---------|
| POI-1   | 35          | 33                    | 25.20       | secondary                       | 35.92      | 16.61     | 24         | 46,XX     | 21*16/22*18                 | Rare    |
| POI-3   | 29          | 27                    | 28.25       | secondary                       | 161.18     | 56.92     | 21         | 46,XX     | 18*11/16*11                 | Absent  |
| POI-6   | 30          | 28                    | 24.14       | secondary                       | 73.77      | 27.56     | 79         | 46,XX     | 19*11/18*12                 | Absent  |
| POI-7   | 23          | 13                    | 23.80       | primary                         | 127.54     | 39.5      | 34         | 46,XX     | 15*10/13*9                  | Absent  |
| POI-8   | 25          | 15                    | 25.71       | primary                         | 92.93      | 43.38     | 13         | 46,XX     | 10*6/11*5                   | Absent  |
| POI-9   | 24          | 18                    | 25.46       | secondary                       | 102.8      | 38.39     | 20         | 46,XX     | 18*9/21*8                   | Absent  |
| POI-11  | 32          | 31                    | 22.31       | secondary                       | 48.65      | 40.24     | 18         | 46,XX     | 19*12/17*15                 | Rare    |
| POI-12  | 28          | 27                    | 25.65       | secondary                       | 86.57      | 35.77     | 22         | 46,XX     | 16*11/15*9                  | Rare    |
| POI-14  | 30          | 28                    | 22.83       | secondary                       | 110.38     | 51.60     | 16         | 46,XX     | 17*14/16*10                 | Absent  |
| POI-17  | 25          | 24                    | 22.86       | secondary                       | 55.24      | 36.83     | 15         | 46,XX     | 20*14/21*17                 | Absent  |
| POI-18  | 27          | 26                    | 27.55       | secondary                       | 60.45      | 50.46     | 18         | 46,XX     | 18*10/17*8                  | Rare    |
| POI-21  | 22          | 14                    | 23.07       | primary                         | 107.28     | 38.55     | 24         | 46,XX     | 11*6/12*5                   | Absent  |
| POI-23  | 26          | 25                    | 25.81       | secondary                       | 38.50      | 24.69     | 15         | 46,XX     | 17*15/16*13                 | Rare    |
| POI-24  | 16          | 13                    | 24.31       | primary                         | 49.66      | 35.52     | 33         | 46,XX     | 10*5/11*4                   | Absent  |

Abbreviation: FSH Follicle-stimulating hormone, LH Luteinizing hormone, E2 Estradiol
variant c.8680G > A, indicating that the two variants in POI-1 were inherited, one each, from her parents (Fig. 1a). Compound heterozygous variants c.3100G > A and c.1006 + 1G > T of HFM1 were confirmed in POI-6. The heterozygous c.3100G > A variant was identified in her mother, and the heterozygous c.1006 + 1G > T variant was identified in her father (Fig. 1c). Heterozygous variants c.1724A > T in BNC1 (Fig. 1b), c.1397G > A in EIF2B4 (Fig. 1d), c.676G > A in FOXL2 (Fig. 1e), and c.2488G > A in MCM9 (Fig. 1f) were identified in POI patients and their fathers. Moreover, as expected, the mothers of these four patients harbored wild-type alleles.

### Analysis of the novel variants identified in patients with POI

We evaluated the 19 variants by in-silico analysis. First, we assessed the frequency of the variants. Data from the gnomAD database, a rich and informative database containing exome data from the ExAc and 1000G databases, in addition to data from many other databases, suggest that all of these variants were rare, and six of them were not present in the database. These results indicate that the frequency of these variants in the population is extremely low, which is compatible with the incidence of POI. Moreover, 16 of the 19 variants were predicted to be deleterious by five prediction tools, Polyphen-2, SIFT, MutationTaster, CADD, and DANN (Table 2).

### Discussion

In this study, we performed WES of the DNA samples of 24 POI patients and identified pathogenic variants associated with POI in 14 of the patients. Our study further supports the notion that genetic variants of several genes are important in the pathogenesis of POI and may be the main reason for sporadic cases of unknown etiology.

Previous studies have shown that dynein axonemal heavy chain 6 (DNAH6) is involved in generating the force required for ciliary beating, and mutations in this gene may cause primary ciliary dyskinesia, non-obstructive azoospermia, or sperm morphological defects [8–11]. By using a high-resolution array comparative genomic hybridization platform, a 171 kb deletion in DNAH6 was identified as the main etiology of POI in a patient [12]. POI-1 harbored two compound heterozygous DNAH6 variants, c.2407C > A and c.8680G > A.

| Case ID | Zygosity | Gene | Ref mRNA No. | Mutation type | Variants | Amino acid change | gnomAD   | PolyPhen/SIFT/MutationTaster/CADD/DANN |
|---------|----------|------|--------------|---------------|----------|-----------------|----------|--------------------------------------|
| POI-1   | Het.     | DNAH6 | NM_001370.2  | missense      | c.2407C > A | p.Q803K         | 0.000004062 | B/T/P/T/T                             |
|         | Het.     |       |              |               | c.8680G > A | p.V2894M        | 0        | D/D/D/D/D/D                            |
| POI-3   | Het.     | BNC1  | NM_001717.4  | missense      | c.1724A > T | p.D575V         | 0.0003   | D/T/D/D/D                             |
| POI-6   | Het.     | HFM1  | NM_001017975.6 | missense      | c.3100G > A | p.G1034S        | 0        | D/D/D/D/D                             |
|         | Het.     |       |              | splice-site   | c.1006 + 1G > T | – | 0.00000523 | –/–/D/D/D |
| POI-7   | Het.     | EIF2B4| NM_015636.3  | missense      | c.1397G > A | p.R466Q         | 0.00007716 | D/T/D/D/D                             |
| POI-8   | Het.     | FOXL2 | NM_023067.4  | missense      | c.676G > A | p.A226T         | 0        | D/D/P/D/D                             |
| POI-9   | Het.     | MCM9  | NM_017696.3  | missense      | c.2488G > A | p.A830T         | 0.00002173 | B/T/P/T/T                             |
| POI-10  | Het.     | FANCA | NM_000135.4  | missense      | c.2340T > G | p.H780Q         | 0        | D/P/D/T/T                             |
| POI-11  | Het.     | ATM   | NM_000051.4  | missense      | c.2367C > G | p.N789K         | 0        | B/T/P/T/T                             |
| POI-12  | Het.     | EIF2B8| NM_014239.4  | missense      | c.76G > A   | p.G26S          | 0.00008134 | D/T/D/T/D                             |
| POI-13  | Het.     |       |              | c.922G > A   | p.V308M     | 0.00008122 | D/D/D/D/D/D                     |
| POI-14  | Het.     |       |              | c.922G > A   | p.V308M     | 0.00008122 | D/D/D/D/D/D                     |
| POI-15  | Het.     |       |              | c.922G > A   | p.V308M     | 0.00008122 | D/D/D/D/D/D                     |
| POI-16  | Het.     |       |              | c.922G > A   | p.V308M     | 0.00008122 | D/D/D/D/D/D                     |
| POI-17  | Het.     |       |              | c.922G > A   | p.V308M     | 0.00008122 | D/D/D/D/D/D                     |
| POI-18  | Het.     |       |              | c.922G > A   | p.V308M     | 0.00008122 | D/D/D/D/D/D                     |
| POI-22  | Het.     |       |              | c.922G > A   | p.V308M     | 0.00008122 | D/D/D/D/D/D                     |
| POI-23  | Het.     |       |              | c.922G > A   | p.V308M     | 0.00008122 | D/D/D/D/D/D                     |
| POI-24  | Het.     |       |              | c.922G > A   | p.V308M     | 0.00008122 | D/D/D/D/D/D                     |

### Abbreviation:

PolyPhen http://genetics.bwh.harvard.edu/pph2/. D: Probably damaging (> = 0.957), P: possibly damaging (0.453 <=pp2_hdiv<=0.956) B: benign (pp2_hdiv<=0.452), SIFT http://sift.bii.a-star.edu.sg/. D: Deleterious (sift<=0.05); T: tolerated (sift>0.05), MutationTaster http://www.mutationtaster.org/. A" ("disease_causing_automatic"); "D" ("disease_causing"); "N" ("polymorphism"); "P" ("polymorphism_automatic", CADD https://cadd.gs.washington.edu/download. D: Damaging; T: Tolerable, DANN https://cblics.uci.edu/public_data/DANN/. D: Damaging; T: Tolerable.
Therefore, DNAH6 is an attractive candidate pathogenic gene for POI.

Basonuclin 1 (BNC1), a zinc finger protein, is abundant in the germ cells of the testis and ovary. BNC1 plays a regulatory role in rRNA transcription during mouse oogenesis, and deletion of the gene (BNC1) that expresses BNC1 protein in mice leads to female subfertility, suggesting that BNC1 is essential for oogenesis [13–15]. Haploinsufficiency of BNC1 has been reported as an etiology of human autosomal dominant POI [16]. Another study found a 1597.8 kb deletion in BNC1 in a patient with POI [17]. POI-3 carried the heterozygous BNC1 variant c.1724A > T, and POI-21 carried the biallelic BNC1 variants c.1703A > T and c.1574T > C. All three variants were predicted to be disease-causing substitutions. In general, both heterozygous and complex heterozygous mutations in BNC1 are pathogenic factors for POI, so it is important to pay particular attention to haploinsufficiency caused by heterozygous mutations.

Helicase for meiosis 1 (HFM1) is an ATP-dependent DNA helicase that is mainly expressed in germ-line cells. Defects in the gene HFM1 cause premature ovarian failure [18–21]. POI-6 harbored the compound heterozygous variants c.3100G > A and c.1006 +1G > T. Both variants were rare and were predicted to be disease causing. Thus, we suspected that these variants are the main pathogenic determinants in POI-6.
Eukaryotic translation initiation factor 2B (eIF2B), a multi-subunit protein comprising two sets of α, β, γ, δ, and ε subunits, is a guanine nucleotide exchange factor (GEF) specific for eIF2 and a key regulator of mRNA translation. EIF2B2, EIF2B3, and EIF2B4 are the β, γ, and δ subunits of EIF2B, respectively. All three participate in protein synthesis and exchange GDP and GTP for activation and deactivation [22]. Compound heterozygous variants in EIF2B2 have been identified as a cause of POI in one of four patients by next-generation sequencing [23]. The c.1117C > T (p.Arg373Cys) variant in EIF2B4 was shown to be associated with premature ovarian failure in two patients at the ages of 13 and 18 years, respectively [24, 25]. POI-11, who carried a heterozygous c.2340 T > G variant in FANCA, was married at 30 years of age and had secondary amenorrhea and POI when trying to conceive.

ATM is a serine/threonine kinase belonging to the PI3/P14-kinase family. It is a cell cycle checkpoint kinase that plays a crucial role in cell cycle checkpoint signaling pathways, which are required for the cellular response to DNA damage and genome stability [41]. ATM is involved in ovarian function, and ATM deficiency can induce premature ovarian failure [42]. The gonads of patients with ATM defects are hypoplastic with germ cell deficiencies [43]. Deletion of the atm locus in mice accelerated primordial follicle degradation at prophase of meiosis I during oogenesis, leading to primordial and maturing follicles and oocyte deficiency [44]. POI-12 carried the heterozygous ATM variant c.2367C > G and had secondary amenorrhea and POI.

Growth hormone receptor (GHR) is a transmembrane receptor for growth hormone. It binds to GH and undergoes conformational changes, which eventually result in activation of the JAK2/STAT-5/IGF-I signaling pathway [45]. The litter size of GHR-knockout mice was significantly reduced due to ovarian defects [46]. POI-23 carried a heterozygous GHR variant, c.282G > A, and had secondary amenorrhea. We predicted that the ovarian function of this patient was affected by haploinsufficiency.

WES is an unbiased genetic approach that has advantages for identifying the genetic etiologies of POI in patients without obvious somatic anomalies [47–50]. In addition to identifying the genetic pathogenesis of patients with unexplained POI, WES also shows broad potential for applications in the screening and early diagnosis of patients with POI. POI patients suffer both mentally and physically due to poor treatment outcomes and unclear etiologies. Clear genetic etiologies will allow us to develop more effective treatment strategies and significantly improve patient compliance.

POI has major impacts on the reproductive ability and physical and mental health of affected patients. Hormone replacement therapy can partially relieve the symptoms induced by POI. However, there are limited effective treatments for the associated impaired reproductive capacity. Therefore, it is important to recognize POI in patients as early as possible to establish a fertility reserve (e.g., oocyte cryopreservation). With the significant decrease in the price and comprehensive spectrum of pathogenic genes, WES
has good potential for application in the early detection and intervention of POI.

Conclusions
In summary, we recruited 24 patients with POI and identified pathogenic variants in 14 of these patients. In approximately 60% (14/24) of the sporadic cases in our study, we were able to identify potentially pathogenic mutations, which shows the utility of WES for determining the genetic pathogenesis of POI. Our research showed that WES is an effective method for identifying the genetic etiology in patients with idiopathic POI, which may offer a theoretical basis for the early detection and intervention in patients with potential idiopathic POI in the future.

Methods
Patients and control subjects
We recruited 24 patients with POI for this study. POI was diagnosed if a patient had amenorrhea for at least 4 months under the age of 40 years and two consecutive follicle stimulating hormone (FSH) measurements > 25 IU/L taken 4 weeks apart [1]. Patients who had significant POI-related risk factors were excluded, including karyotype abnormalities, autoimmune disorders, a history of radiotherapy, chemotherapy, or pelvic surgery, and so on. After the patients provided written informed consent, we performed WES on DNA isolated from the peripheral blood samples of the POI patients to identify any disease-associated variants. All procedures involving human participants were performed in accordance with the standards of the Ethics Committee of the Women and Children’s Hospital of Xiamen University, Zhijiang Hospital of Southern Medical University, and the First Affiliated Hospital of Xiamen University, and in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Written informed consent was obtained from each study participant.

Hormone measurements
Blood hormone (FSH, LH, and E2) levels were measured using a UniCel Dxi 800 immunochemistry analyzer (Beckman Coulter Inc., USA) according to the manufacturer’s instructions and requirements.

WES analysis and sanger sequencing validation
WES was performed as described elsewhere. Variants fulfilling the following criteria were retained: (a) missense, nonsense, frameshift, or splice site variants; (b) allele frequencies < 1% in the dbSNP (http://www.ncbi.nlm.nih.gov/snp/), 100 Genomes (http://browser.1000genomes.org/index.html), ESP6500 (http://evs.gs.washington.edu/EVS/), and Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/) databases. Sanger sequencing was used to validate the variants found in the WES analysis of the patients and their families.

Abbreviations
POI: Premature ovarian insufficiency; WES: Whole-exome sequencing; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; gnomAD: Genome aggregation database; PolyPhen-2: Polymorphism Phenotyping v2; SIFT: Sorting Intolerant From Tolerant; CADD: Combined Annotation Dependent Depletion; DANN: Domain-Adversarial Training of Neural Networks

Acknowledgments
The authors thank all the patients and their family members for their interest and cooperation in the study.

Authors’ contributions
HL, YS, YL, and YT collected the clinical samples and performed the clinical diagnosis. XW and WL analysed the WES data and wrote the manuscript. HG and JL performed the data analysis. YW, WW, and ZS conceived the study. All authors read and approved the final manuscript.

Funding
This work was supported by the Fujian Medical Innovation Fund Project (No. 2019-CXB-35) and the Natural Science Foundation of Fujian Province of China (grant no. 2017 J01361).

Availability of data and materials
The data underlying the findings of this article will be shared on reasonable request to the corresponding author.

Ethics approval and consent to participate
The study protocol was performed in accordance with the tenets of the Declaration of Helsinki and was approved by the ethics committees of the Women and Children’s Hospital of Xiamen University, Zhijiang Hospital of Southern Medical University, and the First Affiliated Hospital of Xiamen University. Written informed consent was obtained from each participant.

Consent for publication
Not applicable.

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

Author details
1Department of Gynecology, Key Clinical Discipline of Fujian province, Women and Children’s Hospital, School of Medicine, Xiamen University, Xiamen 361005, Fujian, China. 2School of Pharmaceutical Sciences, Xiamen University, Xiamen 361005, Fujian, China. 3Department of Reproductive Medicine, Women and Children’s Hospital, School of Medicine, Xiamen University, Xiamen 361005, Fujian, China. 4Department of Gynecology and Obstetrics, Zhijiang Hospital, Southern Medical University, Guangzhou 510000, Guangdong, China. 5Department of Reproductive Medicine Center, the First Affiliated Hospital of Xiamen University, Xiamen 361003, Fujian, China. 6Department of Obstetrics and Gynecology, the First Affiliated Hospital of Xiamen University, Xiamen 361003, China.

Received: 23 June 2020 Accepted: 11 September 2020
Published online: 22 September 2020

References
1. European Society for Human R, Embryology guideline group on POI, Webber L, Davies M, Anderson R, Bartlett I, Braat D, Cartwright B, Cifkova R, de Munck Keizer-Schrama S, et al. ESHRE guideline: management of women with premature ovarian insufficiency. Hum Reprod. 2016;31(5):926–37.
2. Chae-Kim JI, Gavriloa-Jordan L. Premature ovarian insufficiency: procreative management and preventive strategies. Biomedicines. 2018;7(1):2.
3. Qin Y, Jiao X, Simpson JL, Chen ZJ. Genetics of primary ovarian insufficiency: new developments and opportunities. Hum Reprod Update. 2015;21(6):787–808.

4. França MM, Mendonca BB. Genetics of primary ovarian insufficiency in the next-generation sequencing era. J Endocr Soc. 2019;4(2):1.

5. Yatsenko SA, Rakovic A. Genetics of human female infertility. Biol Reprod. 2019;101(3):549–66.

6. Jiao X, Ke H, Qin Y, Chen ZJ. Molecular genetics of premature ovarian insufficiency. Trends Endocrinol Metab. 2018;29(11):795–807.

7. Goh G, Choi M. Application of whole exome sequencing to identify disease-causing variants in inherited human diseases. Genomics Inform. 2012;10(4):214–9.

8. Otean A, Schaffer AJ, Bajly PV, Brody SL. Quantifying Ciliary dynamics during assembly reveals stepwise waveform maturation in airway cells. Am J Respir Cell Mol Biol. 2018;59(4):511–22.

9. Tu C, Nie H, Meng L, Yuan S, He W, Luo A, Li H, Li W, Du J, Lu G, et al. Identification of DNAH6 mutations in infertility men with multiple morphological abnormalities of the sperm flagella. Sci Rep. 2019;9(1):15864.

10. Gershoni M, Hauser R, Yogev L, Lebahi O, Azem F, Yaveh Z, Pietrokovski S, Kleiman SE. A familial study of azospermic men identifies three novel causative mutations in three new human azospermia genes. Genet Med. 2017;19(9):998–1006.

11. Li L, Sha YW, Xu M, Mei LB, Qu P, Ji ZY, Lin SB, Su ZY, Wang C, Yin C, et al. Identification of DNAH6 mutations in infertility men with multiple morphological abnormalities of the sperm flagella. Sci Rep. 2019;9(1):15864.

12. Norling A, Hirschberg AL, Rodriguez-Walberg KA, Iwasson E, Wedell A, Barbaro M. Identification of a duplication within the GDF9 gene and novel candidate genes for primary ovarian insufficiency (POI) by a customized high-resolution array comparative genomic hybridization platform. Hum Reprod. 2014;29(8):1818–27.

13. Tseng H, Green H. Basonuclin: a keratinocyte protein with multiple paired zinc fingers. Proc Natl Acad Sci U S A. 1992;89(21):10311–5.

14. Tian Q, Kopf GS, Brown RS, Tseng H. Function of basonuclin in increasing oocyte maturation in a cohort of premature ovarian failure cases. Hum Reprod. 2018;26(9):1319–27.

15. Tsuiko O, Noukas M, Zilina O, Hensen K, Tapanainen JS, Magi R, Kals M, Ketterer DM, Matic J, Chipkin J, Jiang HY, et al. MCM9 mutations are associated with ovarian failure, short stature, and chromosomal instability. Am J Hum Genet. 2014;95(6):754–62.

16. Fauchereau F, Shalev D, Windlich P, Ploski R, Wielgolaj M. Premature ovarian insufficiency as a variable feature of blepharophimosis, ptosis, and epicanthus inversus syndrome with premature ovarian insufficiency. Mol Genet Genomic Med. 2019;6(1):80–9.

17. Harris SE, Chand AL, Winship IM, Gorski K, Attorniaki K, Shearing AN. Identification of novel mutations in FOXL2 associated with premature ovarian failure. Mol Hum Reprod. 2002;8(7):239–33.

18. Tsuiko O, Noukas M, Zilina O, Hensen K, Tapanainen JS, Magi R, Kals M, Ketterer DM, Matic J, Chipkin J, Jiang HY, et al. MCM9 mutations are associated with ovarian failure, short stature, and chromosomal instability. Am J Hum Genet. 2014;95(6):754–62.

19. De Baeere E, Lemercier B, Christin-Maitre S, Duval D, Messiaen L, Fellous M, Veitia R. FOXL2 mutation screening in a large panel of POF patients and XX males. J Med Genet. 2002;39(8):483.

20. Veitia R. FOXL2 mutation screening in a large panel of POF patients and XX males. J Med Genet. 2002;39(8):483.

21. Zhai Y, Xu BC, Maheshwari HG, He L, Reed M, Lozykowski M, Okada S, Wu BL, et al. Consanguineous familial study revealed biallelic FIGLA mutation associated with premature ovarian insufficiency. J Ovarian Res. 2018;11(1):48.
48. Heddar A, Dessen P, Flatters D, Misrahi M. Novel STAG3 mutations in a Caucasian family with primary ovarian insufficiency. Mol Gen Genomics. 2019;294(6):1527–34.

49. Zhao M, Feng F, Chu C, Yue W, Li L. A novel EIF4ENIF1 mutation associated with a diminished ovarian reserve and premature ovarian insufficiency identified by whole-exome sequencing. J Ovarian Res. 2019;12(1):119.

50. Renault L, Patino LC, Magnin F, Delenier B, Young J, Laisse P, Binart N, Beau I. BMPR1A and BMPR1B missense mutations cause primary ovarian insufficiency. J Clin Endocrinol Metab. 2020;105(4):e1449–57.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.