Supplementary methods

Variant filtering

Identification of candidate pathogenic variants was performed as described previously [1,2]. Briefly, rare candidate variants with below 1% prevalence in three public databases (1000 Genomes variant database, NHLBI-GO exome sequencing project, and Exome Aggregation Consortium), were predicted to be deleterious by at least one *in silico* tool, such as MutationTaster, CADD (Combined Annotation Dependent Depletion), and PolyPhen-2 (Polymorphism Phenotyping v2), were homozygous or compound heterozygous in patients and heterozygous or wild-type in parents and mothered sisters, and/or were previously associated with female infertility.

Bioinformatics analysis

Evolutionary conservation analysis was performed by aligning the amino acid sequences of the SYCP2L protein among different species from the GenBank database (https://www.ncbi.nlm.nih.gov/homologene/). Structural analysis of SYCP2L (NP_001035364.2) was performed using SWISS-MODEL (https://swissmodel.expasy.org).

Plasmid construction

Full-length *SYCP2L* was obtained from human ovarian cDNA by PCR and inserted into the pcDNA3.1/V5-His expression vector as described previously, leading to the production of fusion proteins with V5 at the N-terminus of SYCP2L (SYCP2L-WT).[2] The identified mutations (c.150_151del and c.999A>G) were introduced into the

SYCP2L-WT plasmid vector by oligonucleotide-mediated mutagenesis using a MutExpress II Fast Mutagenesis Kit (Vazyme, Guangzhou, China). All expression constructs were sequenced to confirm the presence of the desired mutation and exclude PCR-induced mutations.

Western blotting

Cells were harvested 48 h after transfection and homogenised using RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with Protease Inhibitor Cocktail (Thermo Fisher Scientific, USA). Proteins extracted from the transfected cells were blotted onto a polyvinylidene difluoride membrane and incubated overnight at 4°C with anti-V5 antibody (1:1000 dilution, Cell Signaling Technology, Danvers, MA, USA). The next morning, the membrane was incubated with secondary antibodies (goat anti-rabbit IgG; 1:5,000 dilution; Abways, Shanghai, China). Finally, the blots were developed with an ECL Western blotting kit (Pierce Biotechnology, Rockford, IL, USA).

Reference

1. He WB, Tu CF, Liu Q, Meng LL, Yuan SM, Luo AX, He FS, Shen J, Li W, Du J, Zhong CG, Lu GX, Lin G, Fan LQ, Tan YQ. DMC1 mutation that causes human non-obstructive azoospermia and premature ovarian insufficiency identified by whole-exome sequencing. *J Med Genet* 2018;55(3):198-204.
2. He WB, Du J, Yang XW, Li W, Tang WL, Dai C, Chen YZ, Zhang YX, Lu GX, Lin G, Gong F, Tan YQ. Novel inactivating mutations in the FSH receptor cause premature ovarian insufficiency with resistant ovary syndrome. *Reprod Biomed Online* 2019;38(3):397-406.