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Short report

# Homozygous variants in *SYCP2L* cause premature ovarian insufficiency

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## ABSTRACT

**Background** The genetic causes of the majority of cases of female infertility caused by premature ovarian insufficiency (POI) are unknown.

**Objective** To identify the genetic causes of POI in 110 patients.

**Methods** Whole-exome sequencing was performed on 110 patients with POI, and putative disease-causative variants were validated by Sanger sequencing. Bioinformatic and in vitro functional analyses were performed for functional characterisation of the identified candidate disease-causative variants.

**Results** We identified two homozygous variants (NM\_001040274: c.150\_151del (p.Ser52Profs\*7), c.999A>G (p.Ile333Met)) in *SYCP2L* in two patients, which had co-segregated with POI in these families. Bioinformatic analysis predicted that the two variants are deleterious, and in vitro functional analysis showed that mutant *SYCP2L* proteins exhibited mislocalisation and loss of function.

**Conclusions** *SYCP2L* is a novel gene found to be responsible for human POI. Our findings provide a potential molecular marker for POI and improve the understanding of the genetic basis of female infertility.

## INTRODUCTION

Premature ovarian insufficiency (POI) is characterised by the depletion or loss of normal ovarian function in women under 40 years of age; it is a frequent cause of female infertility.<sup>1</sup> POI typically manifests with at least a 4-month history of oligomenorrhoea/amenorrhoea and elevated plasma levels of follicle-stimulating hormone (FSH; >25 IU/L).<sup>2</sup> The aetiologies of POI can be classified as genetic, autoimmune or other factors (eg, environmental factors and iatrogenic factors);<sup>3</sup> however, most POI cases are idiopathic, with only 20%–25% being associated with a definitive genetic cause.<sup>4</sup>

During meiosis, diploid germ cells undergo two reducing divisions to generate haploid oocytes, which then progress through the initial stages of the first meiotic prophase and arrest at the diplotene stage prior to ovulation.<sup>5</sup> Several studies have demonstrated that variants in meiosis-associated genes such as *DMC1*, *PSMC3IP*, *TEX11* and *MEIOB* can result in oocyte loss and cause infertility in humans.<sup>6–9</sup> *SYCP2L* (*SYCP2*-like) is an *SYCP2* homologue that is involved in synaptonemal complex (SC) assembly during the prophase of meiosis. Although *Sycp2l* knockout (KO)

mice exhibit accelerated reproductive ageing, it is unknown whether variants in *SYCP2L* affect female fertility.<sup>10</sup>

Herein, we performed whole-exome sequencing (WES) using samples from 110 patients with POI and identified two *SYCP2L* variants in two individuals from two unrelated consanguineous families. Bioinformatics and in vitro functional analyses were performed to evaluate the effect of the variants on *SYCP2L* proteins. This is the first report of *SYCP2L* variants associated with human POI.

## MATERIALS AND METHODS

### Patients

A total of 110 Chinese women, including 5 patients from consanguineous families, were enrolled in this study after providing informed written consent. All patients were diagnosed with POI according to previously described criteria: oligomenorrhoea/amenorrhoea for at least 4 months, and an elevated FSH level >25 IU/L on two occasions >4 weeks apart.<sup>2</sup>

All patients have a normal 46,XX karyotype and FMR1 CGG repeats in the normal polymorphic range. They did not exhibit any associated endocrinopathies or autoimmune disorders.

### WES and variant filtering

Genomic DNA from peripheral blood samples was extracted using a QIAamp DNA blood midi kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The core members of the consanguineous families, including parents and sisters, and the probands of non-consanguineous families were subjected to WES by the Beijing Genome Institute, as described previously.<sup>11</sup> Identification of candidate pathogenic variants was performed as described previously, and was detailed in online supplementary methods.<sup>6–11</sup> The pathogenicity of the variations was evaluated according to the American College of Medical Genetics and Genomics standards and guidelines for the interpretation of variations.<sup>12</sup>

The suspected variants in *SYCP2L* were confirmed by Sanger sequencing using the primer pairs shown in online supplementary table 1. Bioinformatics analysis is detailed in online supplementary methods.

### Expression of human *SYCP2L*

Total RNA was extracted from the peritumoral tissues of several patients with cancer after signing

informed written consent, including heart, liver, kidney, lung, testis and ovary. Metaphase I (MI), metaphase II (MII) and germinal vesicle (GV) oocytes were collected from couples who agreed to donate them for research after informed consent.

Expression of the human candidate gene (*SYCP2L*) was evaluated by reverse transcription PCR (RT-PCR) with a specific primer (online supplementary table 1).

### Western blot analysis and immunofluorescence analysis

Plasmid construction are described in online supplementary methods. Chinese hamster ovary (CHO) cells were transiently transfected with expression vectors containing wild-type or mutated *SYCP2L* using Lipofectamine 3000 (Thermo Fisher Scientific, Carlsbad, California, USA) according to the manufacturer's instructions. Cells transfected with an empty vector (without *SYCP2L*) served as the negative control.

Cells were harvested 48 hours after transfection and western blot analysis is detailed in online supplementary methods, and anti-V5 antibody (Cell Signaling Technology, Danvers, Massachusetts, USA) was used as primary antibody.

Immunofluorescence analysis was performed on the transfected CHO cells grown on coverslips with anti-V5 antibody (1:800 dilution) and anticentromere (1:500, Immunovision, Springdale, Arkansas, USA) primary antibodies. Fluorescent images were captured by confocal microscopy (Olympus FV1000, Tokyo, Japan).

## RESULTS

### Identification of *SYCP2L* variants

The raw data collected by WES had a mean depth of 110.24-fold to 151.46-fold for the target regions, indicating the high sequencing quality. The WES dates of family P0001 and family P0005 are shown in online supplementary table 2. In two consanguineous families (family P0001 and family P0005), the proband, parents and sisters were subjected to WES. According to the filtering criteria, a homozygous frameshift variant of *SYCP2L* (NM\_001040274: c.150\_151del, p.Ser52Profs\*7) was identified in the proband of family P0001, and a homozygous missense variant (NM\_001040274: c.999A>G, p.Ile333Met) was found to be present in the proband of family P0005 (online supplementary table 3). Their parents and mothered sisters were carriers (figure 1A). In addition, there is no rare variant of *SYCP2L* identified in the other patients.

### Expression of human *SYCP2L*

RT-PCR showed that human *SYCP2L* was specifically expressed in the testis, ovary and oocytes (GV, MI and MII oocytes; figure 2A).

### Phenotype of patients with the *SYCP2L* variant

The proband (IV-2) from family P0001, a woman aged 31 years, was admitted to the clinic because of primary infertility (figure 1A). Her parents were first cousins. She was diagnosed with primary infertility after >3 years of marriage. She exhibited normal growth and development but was diagnosed with POI at 30 years of age with clinical symptoms of secondary amenorrhoea. Transvaginal ultrasound examination showed that she had a normal uterus and small bilateral ovaries, with no detectable antral follicle. Her hormonal profile showed high FSH and luteinising hormone levels as well as low serum anti-Müllerian hormone (AMH) and E2 levels (online supplementary table 4).

The proband (IV-2) of family P0005 (figure 1A), a woman aged 31 years, was the daughter of a consanguineous marriage

between first cousins. She experienced highly irregular menstrual cycles (some cycles >90 days) at 21 and developed oligomenorrhoea at 26 years of age. At 30, she had a pregnancy that ended very early (39 days). She had elevated FSH levels and low serum AMH levels (online supplementary table 4). Transvaginal ultrasound examination revealed that the uterus and ovaries were of normal size, with three small follicles visualised in total.

Routine semen analyses of their husbands revealed normal fertility. The results of infertility-related examinations from the two patients are shown in online supplementary table 4.

### Impact of *SYCP2L* variant

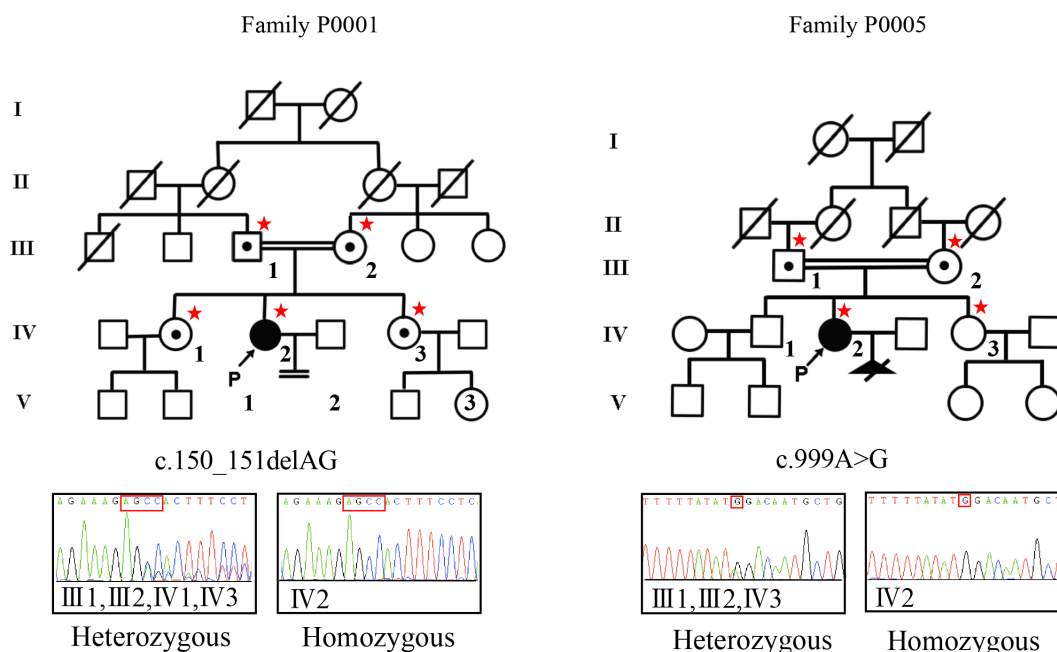
The two variants (c.150\_151del and c.999A>G) were predicted as pathogenic by three different tools, except that c.999A>G was predicted as a polymorphism by MutationTaster (online supplementary table 3). Multiple sequence alignment showed that amino acid p.Ile333 (c.999A) is highly conserved across species, from *Xenopus tropicalis* to *Homo sapiens* (figure 1B). A three-dimensional structure of the N-terminal structures of *SYCP2L* (20–403 amino acid residues) was modelled with good confidence based on the 41.18% identical N-terminal structures of *SYCP2* (Siwz.1.A). We analysed the impact of p.Ser52Profs\*7 and p.Ile333Met in *SYCP2L*. SWISS-MODEL revealed that the frameshift variant causes the formation of a truncated protein, with loss of N-terminal structures, and the p.Ile333Met variant changes the shape of the protein in residues 88–95 and 205–207 (figure 1C). Meanwhile, a redundant hydrogen bond was formed between Asp334 and Ala311 when Ile333 was substituted with Met (figure 1C).

Western blot analysis showed that the mutant protein with missense variant p.Ile333Met was expressed in CHO cells after transfection with the expression construct (figure 2B). Although immunofluorescence analysis showed that the *SYCP2L* truncated protein was expressed in cells after transfection with the expression construct, western blot analysis showed that expected size of the truncated protein (p.Ser52Profs\*7; 57 amino acids) was not observed (figure 2B,C). It is likely that the truncated protein has reduced stability leading to its degradation and failed detection by western blot analysis. Given the variant occurs in a central exon and causes a premature stop codon, it is likely that in the in vivo setting, the transcript is degraded by nonsense-mediated decay and little of this alternative protein is encoded. As expected, immunofluorescence analysis showed that wild-type *SYCP2L* localised to the centromeres; however, cells transfected with the frameshift variant construct displayed cytoplasmic mislocalisation, and those transfected with the missense variant construct were not specifically localised to the centromeres, with several mutant proteins not co-localising with the centromeres (figure 2C). These results demonstrate that the mutant *SYCP2L* proteins have altered functions. The frame-shifted protein is unlikely to be expressed in patient cells due to nonsense-mediated decay, however, any residual transcript will encode a protein with impaired stability and/or function. The two variants in *SYCP2L* were classified as pathogenic variants.

## DISCUSSION

In this study, we performed WES to reveal the genetic causes of POI in 110 patients, identifying two homozygous *SYCP2L* variants in two probands. Immunofluorescence revealed that the mutant *SYCP2L* proteins induced marked mislocalisation in vitro. To the best of our knowledge, this is the first study to report that *SYCP2L* is involved in POI in humans.

A



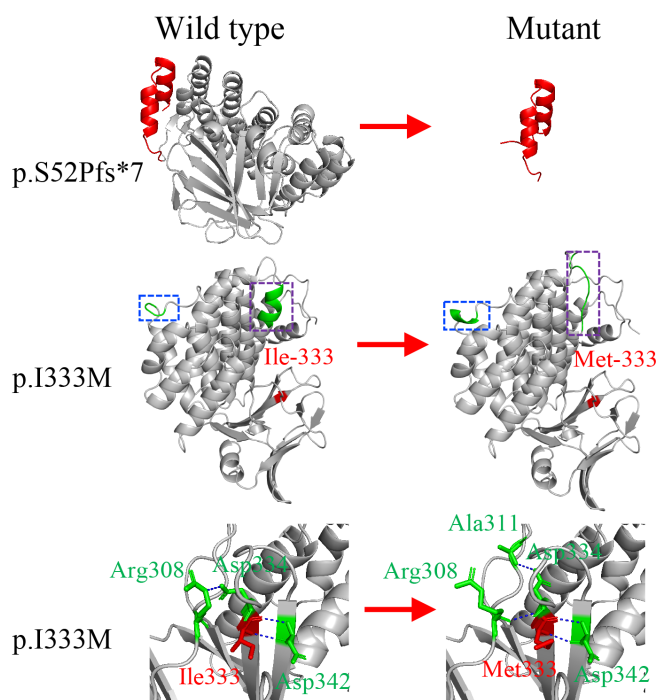
B

Mutated	SVTFYMDNAEN
H. Sapiens	SVTFYIDNAEN
P. Troglodytes	SVTFYIDNAEN
M. Mulatta	SVTFYIDNAEN
C. Lupus	SITFYINNTKS
B. Taurus	SVTFYCINNPES
R. Norvegicus	SVTFYIDNAES
G. Gallus	SVTFYIDSIEG
X. Tropicalis	CVSFYIHNKEG

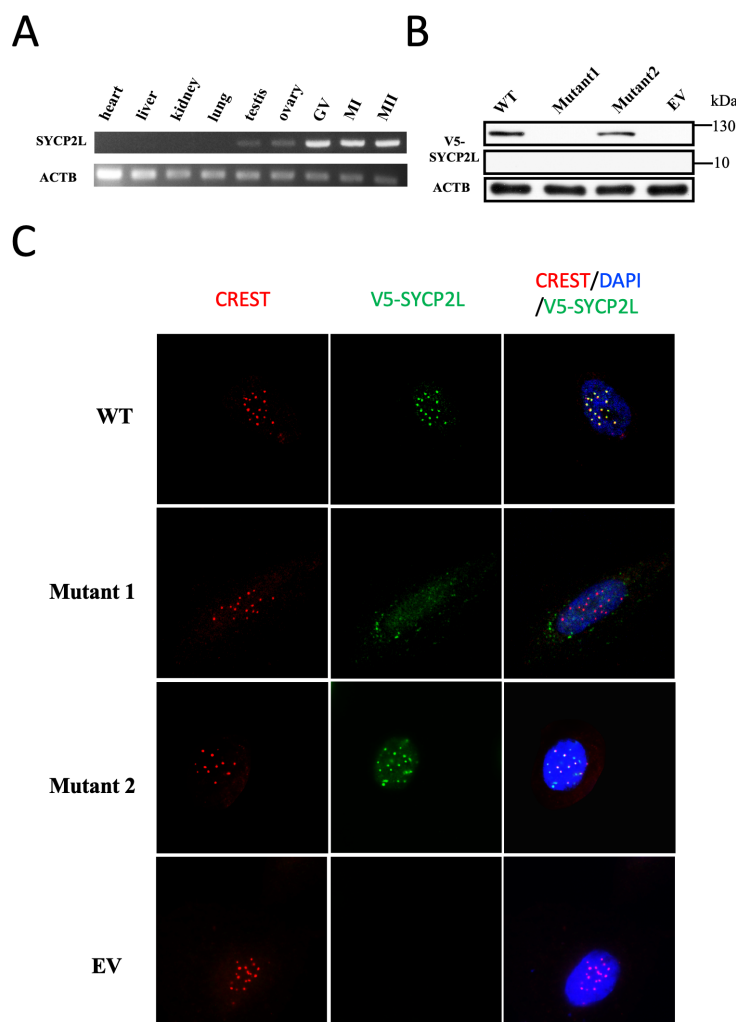
↑

p.I333M

C



**Figure 1** Pedigree and bioinformatics analysis. Affected members are indicated with filled symbols; unaffected relatives are indicated by open symbols; heterozygous carriers are indicated with a dot in the middle of the symbol. Arrows indicate the proband. Numbers are allotted to the family members whose DNA samples were used in this study; asterisks are marked on those members whose DNA was used for the whole-exome sequencing. Panel A: Sanger sequencing confirmed that in family P0001, the proband's unaffected parents (III-1 and III-2) and two mothered sisters (IV-1 and IV-3) are heterozygous carriers of the SYCP2L variant c.150\_151del, whereas the proband (IV-2) is homozygous. In family P0005, the affected woman (IV-2) is homozygous for the missense SYCP2L variant c.999A>G; however, her parents (III-1 and III-2) and sister (IV-3) are heterozygous. Panel B: isoleucine at position 333 is highly conserved in different animal species from *Xenopus tropicalis* to *Homo sapiens*. Panel C: the molecular structures of the wild-type and mutant SYCP2L proteins in the N-terminal region were modelled using SWISS-MODEL software, which revealed that the frameshift variant (c.150\_151del) causes the formation of a truncated protein and p.Ile333Met variant (c.999A>G) changes the shape of the protein at residues 88–95 (purple dashed box) and residues 205–207 (blue dashed box). Additionally, a redundant hydrogen bond was formed between Asp334 and Ala311 when Ile333 was substituted by Met.



**Figure 2** Human *SYCP2L* expression and in vitro functional analysis. Panel A: human *SYCP2L* is expressed in the testis, ovary and oocytes (GV, MI and MII) of humans. Panel B: western blot analysis showing stable expression of the missense variant but no detectable truncated protein in Chinese hamster ovary (CHO) cells after transfection. Panel C: immunofluorescence analysis demonstrating expression of both constructs. The WT *SYCP2L* localised to the centromeres; however, the truncated *SYCP2L* and missense mutant *SYCP2L* (p.Ile333Met) displayed cytoplasmic mislocalisation and were not specifically localised to the centromeres, respectively. EV, empty vector (without *SYCP2L*); GV, germinal vesicle oocytes; MI, metaphase I oocytes; MII, metaphase II oocytes; mutant 1, c.150\_151del of *SYCP2L* (p.Ser52Profs\*7); mutant 2, c.999A>G (p.Ile333Met) of *SYCP2L*; WT, wild-type *SYCP2L*.

The SC is required to promote synapsis and recombination during meiosis; thus, mice without SCs exhibit infertility.<sup>13 14</sup> *SYCP2L* localises to the SC lateral elements in late diplotene oocytes.<sup>10</sup> Female *Sycp2l*-KO mice presented with subfertility.<sup>10</sup> Recent genome-wide association studies reported that SNPs of *SYCP2L* (c.337–756G>A; rs2153157) may be associated with age at menarche and natural menopause in East Asian women.<sup>15</sup> We identified two homozygous *SYCP2L* variants in two patients with POI presenting with secondary amenorrhoea or oligomenorrhoea and low AMH levels. The clinical features observed in our patients were similar to the phenotypes of *Sycp2l*-KO mice, indirectly evidencing that *SYCP2L* is involved in meiotic synapsis, recombination and regulating ovarian function in humans.

*SYCP2L* is a sequence homologue of the SC protein *SYCP2*, with their N-terminals (amino acids 27–406) exhibiting 41% identity and showing expression at the nuclear SC in oocytes beginning in the late diplotene stage, as well as localisation to centromeres in late diplotene stage oocytes.<sup>10</sup> In vitro, ectopically expressed human wild-type *SYCP2L* localised to centromeres in transfected somatic cells, whereas the human truncated *SYCP2L* mutants were not specifically localised to the centromeres but

distributed diffusely throughout the nucleus.<sup>10</sup> In our study, the *SYCP2L* frameshift variant (c.150\_151delAG) resulted in low expression of truncated protein in cells after transfection, likely due to its reduced stability. In vivo, it is likely the frameshift variant results in a remarkably reduced level of *SYCP2L* transcript due to nonsense-mediated decay, and a subsequent deficiency of *SYCP2L* protein in patient cells. Our experiments demonstrate that if residual transcript encodes truncated protein, it is likely unstable and/or has impaired function. The region in which the missense variant of family P0005 (c.999A>G, p.Ile333Met) is located and the amino acid itself are highly conserved, indicating that it might have an essential function. Furthermore, in vitro analysis showed that wild-type *SYCP2L* specifically localised to the centromeres, whereas the mutant *SYCP2L* proteins did not, suggesting that mutant *SYCP2L* proteins cause mislocalisation and ultimately altered function. Therefore, the two *SYCP2L* variants identified are deleterious and disease-associated variants.

In *Sycp2l*-KO mice, the mutant female mice completely lost *Sycp2l* function and its primordial follicles were progressively lost with increasing age, leading to subfertility.<sup>10</sup> In our study, the patient from family P0001 developed secondary amenorrhoea



at age 30 years, whereas the patient from family P0005 experienced oligomenorrhoea and had a pregnancy at the age of 30 years. The severity of clinical symptoms of the two patients can be explained by the fact that the frameshift variant in family P0001 was a complete loss-of-function variant, but the missense variant caused partial loss of SYCP2L function, as confirmed by in vitro analysis.

We showed that human SYCP2L was expressed in both the testis and ovaries, suggesting that SYCP2L deficiency might affect both male and female fertility. Since no males in our study were homozygous for the SYCP2L variant, the effect of SYCP2L variants on human male infertility remains unclear.

In conclusion, we identified two homozygous POI-causing SYCP2L variants in humans that could act as new molecular biomarkers for POI in clinical settings. Our findings improve the understanding of the genetic basis of female infertility.

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**Contributors** Y-QT, JD designed the research; W-BH analysed the data and wrote the paper; W-BH, Y-XZ, L-LM and CT performed the research; FG, G-XL and GL performed the clinical work.

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**Competing interests** None declared.

**Patient consent for publication** Not required.

**Ethics approval** This study was approved by the Ethics Committee of the Reproductive and Genetic Hospital of CITIC-Xiangya of Central South University.

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