

# Two novel biallelic mutations in PSMC3IP in a Chinese patient affected by primary ovarian insufficiency: case report and review of the literature

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## Research

**Keywords:** premature ovarian insufficiency, PSMC3IP, biallelic mutations, whole-exome sequencing

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# Abstract

## Background:

Premature ovarian insufficiency (POI) is a heterogeneous condition occurring when a woman experiences a loss of ovarian activity before the age of 40. It is one of the most common reproductive endocrine diseases in women of childbearing age. Here, we investigated the clinical manifestations and genetic features of a Chinese patient affected by POI.

**Methods:** We applied next-generation whole-exome capture sequencing with Sanger direct sequencing to the proband and her clinically unaffected family members.

**Results:** Two novel compound heterozygous mutations were identified in the *PSMC3IP* gene. The first is a splicing mutation (c.597+1G>T) that was inherited from her father, while the second mutation (c.268G>C p.D90H) was also discovered in her mother and younger sister. The two mutations were co-segregated with the disease phenotype in the family.

**Conclusions:** To our knowledge, this is the first report of *PSMC3IP* mutations causing POI in the Chinese population. Our findings further support the key role of the *PSMC3IP* gene in the etiology of POI. However, additional studies are required to explore the underlying molecular mechanisms involved.

## 1. Background

Premature ovarian insufficiency (POI) refers to the presence of ovarian atrophic permanent amenorrhea in women under the age of 40, characterized by hypergonadotropic hypogonadism and presenting with either primary or secondary amenorrhea [1]. In women of reproductive age, POI is one of the most commonly diagnosed endocrine diseases. The prevalence of POI is approximately 1% [2]. Apart from menstrual disturbance, the main symptoms of POI are the decrease of estradiol levels and the increase of plasma follicle-stimulating hormone levels (FSH) (>25 mIU/ml on two occasions, > 4 weeks apart) [3,4].

The etiology of POI is highly heterogeneous and complex, such as that for genetic, autoimmune, infectious, and iatrogenic factors, among which genetic causes explain presentation in approximately 20-25% of patients [5]. Over the past few years, novel methods using next-generation sequencing (NGS), particularly whole-exome sequencing (WES), have led to the identification of numerous candidate genes. These genes are mainly involved in meiosis, DNA damage repair, and homologous recombination, including X-linked genes (e.g. *FMR1*, *BMP15*, and *PGRMC1*) and autosomal genes (e.g. *FSHR*, *NOBOX*, *FIGLA*, *GDF9*, *FOXL2*, and *STAG3*) [5-7]. In 2011, WES revealed *PSMC3IP* as a novel candidate gene associated with autosomal recessive POI [8]. *PSMC3IP* is important for homologous pairing and homologous recombination in meiosis, which is indicated by its yeast ortholog *HOP2*. Female *PSMC3IP/HOP2*-deficient mice displayed a significantly reduction in ovarian volume and a lack of follicles [8,11]. To date, rare variants of the *PSMC3IP* gene were reported in POI [8-10].

Here, we present the case study of an adopted Chinese woman suffering from POI. We performed WES on the patient's DNA to identify potential causative genes or mutations of *PSMC3IP* associated with POI. Identified sequences were subjected to extensive bioinformatical analysis and screening against several databases to predict the potential effect on protein function. Mutations were confirmed with Sanger sequencing and screened against negative control DNA.

## 2. Materials And Methods

### 2.1. Clinical case report

The proband, a 29-year-old female from Fujian(**Fig 1**), China, who had primary amenorrhea, had been married for 5 years without conceiving and had been diagnosed with POI. She had a normal target height (160cm), and normal weight (55kg). Physical examination showed no dysmorphic features or breast development and normal intellectual development. From a gynaecological examination, it was clear that the patient had a sparse amount of pubic and armpit hair. A transvaginal ultrasound examination revealed that the bilateral ovaries were abnormally small, the left ovary was 1.23×1.00cm, and the right ovary was 1.55×0.74cm, but no obvious antral follicles were observed(**Fig 2**). Her basic hormone levels were as follows: FSH 62.52-78.60 IU/L, Luteinizing hormone (LH) 20.38-25.43 IU/L, estradiol (E2) 13.0-42.5 pmol/L, and anti-Müllerian hormone (AMH) 0.09 ng/ml. She had a normal 46,XX karyotype and *FMR1* repeat lengths and a negative testing of adrenal cortical antibody. The biological parents of the proband were healthy and non-consanguineous. The proband's biological mother and sister had normal menstrual histories, and the family did not report any history of systemic diseases or solid tumors. This study fully complied with the tenets of the Declaration of Helsinki and has been approved by the Ethics Board of the Women's and children's Hospital affiliated to Xiamen University, China. Informed consent was obtained from all participants before testing.

One hundred unrelated ethnically matched healthy female individuals (aged between 22 and 40, average age of 28) were recruited as controls, They were known to be menstruating regularly, had normal FSH levels(range, 2.5-10.1IU/L ; mean, 3.6± 1.9 IU/L) and normal pelvic ultrasound imaging.

### 2.2 Targeted exon capturing and next-generation sequencing

Total genomic DNA was extracted from peripheral blood leukocytes using the Blood Genomic DNA Mini Kit (Qiagen, Valencia, CA, USA). The concentration of DNA samples was analyzed using a NanoDrop 2000 spectrophotometer (Thermo Fischer Scientific, Waltham, MA, USA). The exomes were captured with SureSelect Human All Exon V6 (Agilent Technologies, Santa Clara, CA, USA) and sequenced on an average depth of 150x with the use of the Illumina HiSeq platform (Illumina, San Diego, CA, USA) according to the manufacturer's protocol.

### 2.3 Bioinformatic analysis

After Illumina HiSeq sequencing, raw NGS data were imported into FastQC software for assessing the quality and the high-quality reads were aligned to the human reference genome (GRCh37/hg19) using Burrows-Wheeler Aligner (BWA) software. Then, variant calling and annotation were performed using GATK software. Several databases, such as the Single Nucleotide Polymorphism Database (dbSNP)138, the 1000 Genome Project, the Exome Aggregation Consortium (ExAc), ClinVar, and the Genome Aggregation Database (gnomAD) were employed to select all variants with frequencies higher than 5%. In addition, online tools such as Human Splicing Finder<sup>1</sup> and PolyPhen-2<sup>2</sup> and SIFT were applied to predict the potential effect on protein function.

### 2.4 Confirmation by Sanger sequencing

The mutations of the *PSMC3IP* gene were further confirmed by Sanger sequencing. The PCR products were sequenced on an ABI 3730xl DNA Analyzer (Applied Biosystems, Thermo Fisher Scientific). Sequencing results were analyzed using the DNASTAR Lasergene software (DNASTAR, Madison, WI, USA). Subsequently, the novel identified mutations were verified and screened in 100 unrelated healthy women (aged between 22 and 40, average age of 28) with DNA sequencing.

## 3. Results

### 3.1 Mutation identification by next-generation sequencing and Sanger sequencing

Overall, the coverage of the target region was 99.3% with an average sequencing depth of more than 130x and with a variant accuracy of more than 99.97%. After filtering out all the existing mutations with a minor allele frequency (MAF) greater than 0.05 as determined with dbSNP138, 1000 Genomes, ExAc, ClinVar, and gnomAD, a total of 18 variants were remaining (Supplementary Table 1).

In combination with the clinical phenotype and database analyses, two compound heterozygous mutations of *PSMC3IP*, c.597+1G>T and c.268G>C, were considered as pathogenic compound heterozygous mutations. Furthermore, Sanger sequencing on the family members' DNA confirmed that the c.597+1G>T mutation was inherited from the father while the missense mutations (c.268G>C) was also observed in her mother and younger sister, showing complete co-segregation of the mutations with the disease phenotype (Fig 3).

### 3.2. Prediction of the pathogenic significance of the mutations

According to the classification standards of American College of Medical Genetics and Genomics (ACMG), c.597+1g>T and c.268g>C were classified as suspected pathogenic mutations. The splicing mutation c.4106+2T>C was predicted to alter the splice donor site, most likely influenced by splicing, according to Human Splicing Finder (<http://www.umd.be/HSF3/HSF.shtml/>). The c.268G>C mutation is a missense mutation and results in a substitution of aspartate with histidine at amino acid position 90 (p.D90H). According to Clustal W/Ensembl software (UCD Conway Institute, Dublin, Ireland), species conservation analysis confirmed that the ninetieth aspartic acid residues were highly conserved among different species (Fig 4 b). The mutation was described as "possible damage" by using online disease prediction software PolyPhen-2 (Fig 4 a), and "Affect Protein Function" by SIFT. Neither of the two mutations has been reported in the Human Gene Mutation Database (HGMD), dbSNP138, the ExAC database, the 1000 Genomes database, or in any other single-nucleotide polymorphism database. In addition, no relevant literature has been reported. Moreover, neither of the heterozygous mutations were found in 100 unrelated control individuals from the same ethnic origin (data not shown). Taken together, these results powerfully support that *PSMC3IP* mutations are disease causing mutations in the family.

## 4. Discussion

We evaluated an adopted 29-year-old Chinese woman with POI and identified two biallelic mutations, c.597+1G>T and c.268G>C, in the *PSMC3IP* gene. The two mutations carried by the patient were inherited respectively from her biological mother and father. *PSMC3IP* has previously been linked to hereditary breast and ovarian cancer, as well as causing autosomal recessive POI [8,12,13]. *PSMC3IP* defects can disrupt estrogen-driven transcription activation of *PSMC3IP*. Impaired estrogenic signaling can result in ovarian dysgenesis by interfering the follicular pool and against counteracting follicular atresia [8,14].

The *PSMC3IP* gene is located at 17q21.2, consist of 217 amino acids in its monomer, encoding a nuclear, tissue-specific protein with multiple functions, including a role in meiotic recombination and acting as a coactivator of ligand-dependent transcription mediated by nuclear hormone receptors, which is conserved in evolution [15,16]. Previous studies proved that in the *PSMC3IP* knockout model of mice, the ovarian volume is reduced and the germ cells are missing [17,18]. *PSMC3IP* is a DNA-binding protein dimer, characterized by the presence of three domains including a leucine zipper domain, a DNA-binding domain, and a RAD51/DMC1 interaction domain [9]. The c.268G>C mutation occurs within the highly conserved leucine zipper domains (Fig 4 c). *In vitro* experiments showed that a

defect of the leucine zipper eliminated the dimerization of *PSMC3IP* [15]. The c.268G>C mutation was detected in the proband's mother and sister with normal ovarian function. The splicing mutation c.597+1G>T is predicted to alter the splice donor site so that most likely to interfere with splicing. However, the exact effects of splice site mutations on mRNA cleavage are not clear and need to be investigated further.

To date, only four POI families with *PSMC3IP* mutations have been described worldwide, including one reported in this paper (Table 1). A total of six pathogenic *PSMC3IP* mutations have been identified, comprising three frameshift mutations, one nonsense mutation, one missense mutation, and a splicing mutation. In 2011, Zangen *et al.* [8] first identified a homozygous 3 bp in-frame deletion in the exon 8 of *PSMC3IP* gene in a large consanguineous Arab Palestinian pedigree with XX-female gonadal dysgenesis, leading to the deletion of glu201. Also, in a consanguineous Yemeni family of a brother with azoospermia and four sisters with ovarian dysgenesis, Abdulmoein *et al.* [9] identified a homozygous stop mutation (c.489C>G, p.Tyr163Ter) in *PSMC3IP*, suggesting an important role of PSMC3IP in the development of male and female germ cells. In our study, the proband's father carried the heterozygous splice site mutation c.597+1G>T, but he didn't show spermatogenesis dysfunction. Recently, two compound heterozygous mutations of *PSMC3IP* (c.430\_431insGA, p.L144\*; c.496\_497delCT, p.R166Afs) were found in a 28-year-old French female who presented with POI [10]. However, Norling A *et al.* [19] reported that *PSMC3IP* gene mutations are not common causes of POI in this Swedish cohort. The pathogenesis of POI caused by *PSMC3IP* needs to be further verified in the future. Since the limited number of cases of *PSMC3IP* mutations associated with POI, we are not able to make a clear correlation between this genotype and phenotype.

Table 1

Family	Age at diagnosis	Ethnic origin	CS	karyotype	Nucleotide change	Amino acid change	Status	Reference
1	21	Palestinian	Yes	46,XX	c.[600_602del]	p.Glu201del	ho	Zangen <i>et al.</i> , (2011)
2	28	French	No	46,XX	c.[496_497delCT]+ [430_431insGA]	p.[R166Afs]+ [L144X]	He	Xiang <i>et al.</i> , (2018)
3	27	Yemen	Yes	46,XX	c.[489 C > G]	p.[Tyr163Ter]	ho	Al-Agha <i>et al.</i> , (2018)
4	29	Chinese	No	46,XX	c.[597 + 1G > T]+ [268G > C]	P[splicing]+ [p.D90H]	He	This study
CS, consanguineous; he, heterozygous; and ho, homozygous.								

## 5. Conclusion

In conclusion, we identified two novel variants in the *PSMC3IP* gene in a Chinese female with POI. This is the first time that *PSMC3IP* mutation causing POI has been reported in Chinese population. Our findings expands the genotypic spectrum of the *PSMC3IP* gene in POI patients and supports the application of NGS in the genetic diagnosis of POI. Moreover, this finding sheds new light on genetic counseling, diagnosis, and therapy in POI.

# Declarations

## Acknowledgments

We thank the participating family members for their assistance and support.

## Authors' contributions

Libin Mei and Lingling Huang carried out research design, experiments, analyzed data, explained the results, and drafted manuscripts. Yanru Huang participated in the design and interpretation of the research results. XueMei He was responsible for ultrasound examination and interpretation of the results. Xiaoling Wu and Huang He were involved in the experiment. Zhiying Su and Ping Li participated in the supervision, revising manuscript and its critical review.

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## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Ethics approval and consent to participate

This study fully complied with the tenets of the Declaration of Helsinki and has been approved by the Ethics Board of the Women's and children's Hospital affiliated to Xiamen University, China. Informed consent was obtained from all participants before testing.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no conflict of interest.

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## Figures

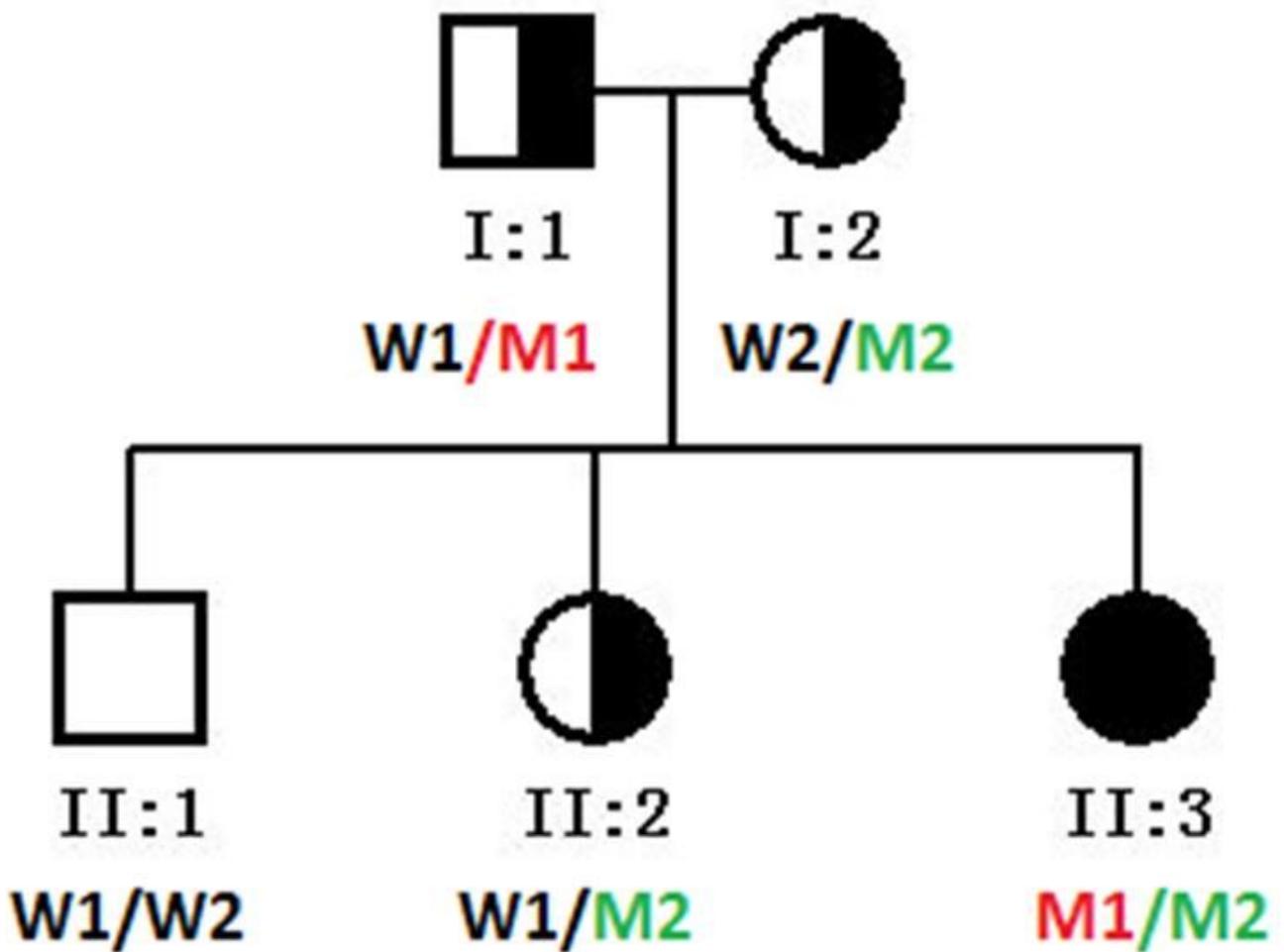


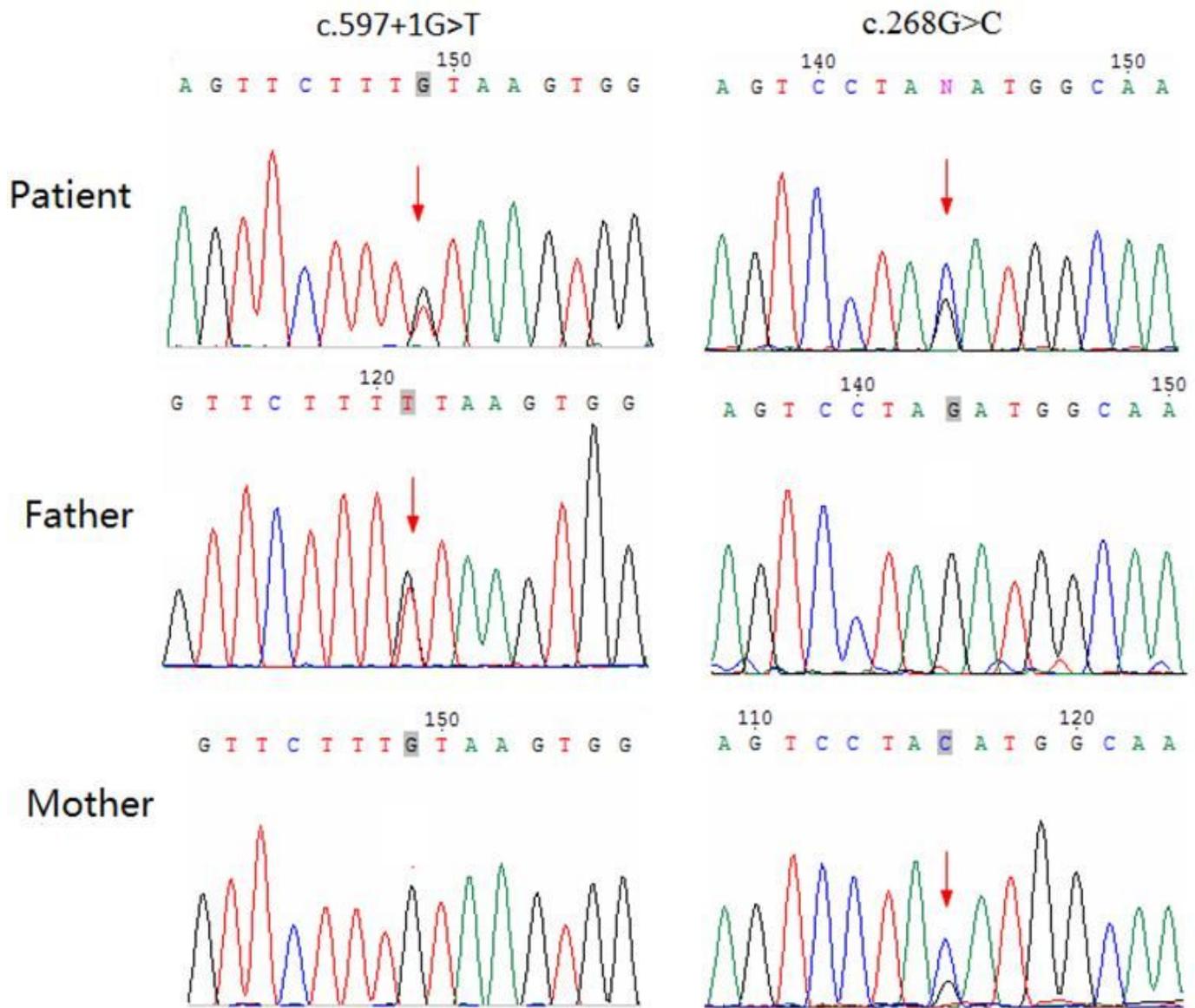
Figure 1

A. Pedigree of the family with POI. W, wild type; M1, c.597+1G>T mutation; M2, c.268G>C mutation; the proband is identified by an arrow



**Figure 2**

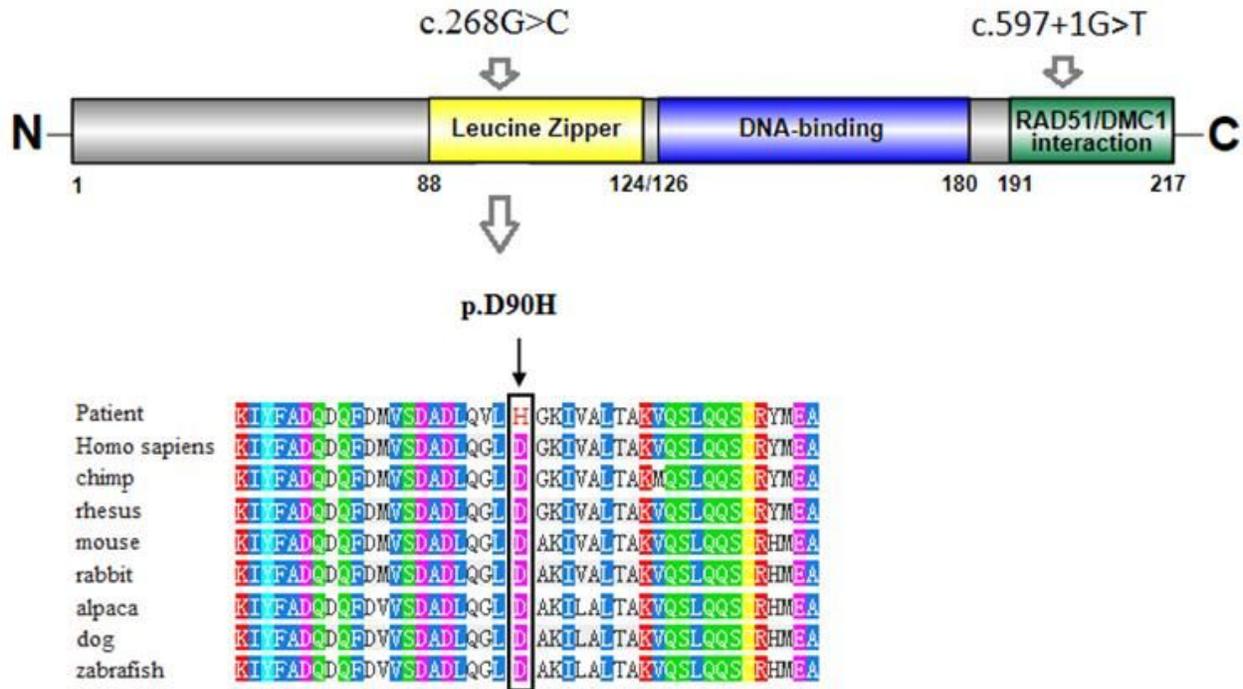
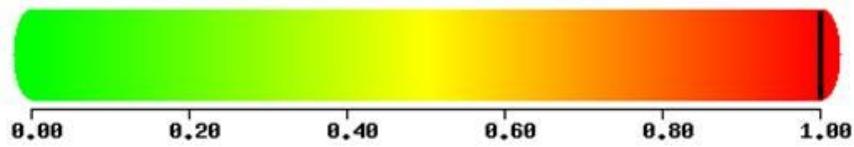
Transvaginal ultrasound examination showed that the bilateral ovaries were abnormally small, and no obvious antral follicles were observed.



**Figure 3**

Identifying mutations in PSMC3IP. Electropherogram analysis of PSMC3IP in the proband showing compound heterozygous c.597+1G>T and c.268G>C mutations of PSMC3IP. The father (I1) carried c.597+1G>T, while the mother (I2) carried c.268G>C

This mutation is predicted to be **PROBABLY DAMAGING** with a score of 1.00 (sensitivity: 0.00; specificity: 1.00)



**Figure 4**

a) PolyPhen-2 reports for the pathogenicity of the amino acid substitution p.D90H in PSMC3IP. b) Diagram of PSMC3IP with predicted locations of protein sequence changes. c) Protein alignment showed that PSMC3IP p.D90H residues were conserved across multiple species, thus the two mutations occurred at an evolutionarily conserved amino acid

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTable1.pdf](#)