Two novel biallelic mutations in *PSMC3IP* in a patient affected by premature ovarian insufficiency

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Abstract. Premature ovarian insufficiency (POI) is a heterogeneous condition occurring when a woman experiences a loss of ovarian activity before the age of 40. POI is one of the most common reproductive endocrine diseases in women of childbearing age. The present study investigated the clinical manifestations and genetic features of a Chinese patient affected by POI. Next-generation whole-exome capture sequencing with Sanger direct sequencing were applied to the proband and her clinically unaffected family members. Two novel compound heterozygous mutations were identified in *PSMC3IP*. The first was a splicing mutation (c.597+1G>T) that was inherited from her father, whereas the second mutation (c.268G>C p.D90H) was discovered in both her mother and younger sister. The two mutations were co-segregated with the disease phenotype in the family. In conclusion, the findings of the present study further support the key role of *PSMC3IP* in the etiology of POI and provide a novel insight into elucidating the mechanisms of female infertility.

Introduction

Premature ovarian insufficiency (POI) refers to the presence of ovarian atrophy and 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, with a worldwide prevalence of ~1% (2). As well as menstrual disturbance, the main symptoms of POI are decreased estradiol levels and increased plasma follicle-stimulating hormone (FSH) levels (>25 mIU/ml on two occasions, >4 weeks apart) (3,4).

The etiology of POI is highly heterogeneous and complex, including genetic, autoimmune, infectious and iatrogenic factors, among which genetic causes explain the presentation in 20-25% of patients worldwide (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 that cause POI. 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* (MIM 608665) as a novel candidate gene associated with autosomal recessive ovarian dysgenesis (8). *PSMC3IP* is important for homologous pairing and homologous recombination in meiosis (9,10), which is indicated by its yeast ortholog *HOP2*. In a previous study, female *PSMC31P*-deficient mice displayed a significantly reduction in ovarian volume and a lack of follicles (11,12), suggesting that loss of this DNA-repair protein may be associated with infertility phenotypes. To date, rare variants of *PSMC3IP* have been reported in POI (8,13,14).

The current study presented a case of an adopted Chinese woman suffering from POI. WES was performed on DNA obtained from the patient to identify potential causative genes or *PSMC3IP* mutations, which were associated with POI. Identified sequences were subjected to extensive bioinformatics analyses and screening against several databases to predict the potential effect of the mutations on protein function. Mutations were confirmed with Sanger sequencing and screened against negative control DNA from healthy female individuals.

Materials and methods

Case presentation. The proband, a 29-year-old woman from Fujian origin, was admitted to Women's and Children's

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Hospital affiliated to Xiamen University. She had primary amenorrhea, had been married for 5 years without conceiving and was diagnosed with POI (Fig. 1). She had a normal target height (160 cm) and normal weight (55 kg). Physical examination showed no dysmorphic features or breast development, and normal intellectual development. From a gynecological 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 1.23x1.00 cm, and the right ovary was 1.55x0.74 cm), and no obvious antral follicles were observed (Fig. 2). Her basic hormone levels were as follows: FSH, 62.5-78.6 mIU/l (reference range: 1.5-10 mIU/ml before ovulation; 8-20 mIU/ml ovulation period; 2-10 mIU/ml after ovulation); luteinizing hormone, 20.4-25.4 mIU/l (reference range: 5-25 mIU/ml non-ovulation period; 30-100 mIU/ml ovulation period; 4-10 mIU/ml after ovulation); estradiol, 13.0-42.5 pmol/l (reference range: 48-521 pmol/l before ovulation; 70-1835 pmol/l ovulation period; 272-793 pmol/l after ovulation). The patient had a normal 46,XX karyotype and *FMR1* repeat lengths, and a negative test for the 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. The present study fully complied with the tenets of the Declaration of Helsinki and was approved by the Ethics Board of the Women's and Children's Hospital affiliated to Xiamen University (approval number XY-2019-059). Written informed consent was obtained from all participants prior to testing.

A total of 100 unrelated ethnically matched healthy female individuals (age, 22-40 years; mean age, 28 years) were recruited as controls. The healthy controls menstruated regularly, had normal FSH levels (range, 2.5-10.1 IU/l; mean, 3.6±1.9 IU/l) and normal pelvic ultrasound imaging.

Targeted exon capturing and NGS. Total genomic DNA was extracted from peripheral blood leukocytes using the magnetic bead method with the Blood Genomic DNA Mini kit (cat no: 51106; Qiagen, Inc.). The concentration of DNA (ng/μl) in each sample was analyzed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). The genomic DNA (3 μg) was fragmented into ~150 bp. In-solution hybridization-based enrichment was performed using the SureSelect Human All Exon V6 (Agilent Technologies, Inc.) according to the manufacturer's protocol. The concentrations of the library was measured by a Qubit 4.0 Fluorometer (Invitrogen; Thermo Fisher Scientific, Inc.) and the loading concentration of the final library was 16pM. The library pool was sequenced on the Illumina HiSeq 2500 sequencing platform(cat nos: PE-401-3001 and FC-401-3001,Illumina, Inc.) in high output run mode with 150 bp paired-end reads.

Bioinformatics analyses. After Illumina HiSeq sequencing, raw NGS data were imported into FastQC for assessing the quality, and high-quality reads were aligned to the human reference genome (GRCh37/hg19) using Burrows-Wheeler Aligner software (BWA 0.7.17-r1188; http://bio-bwa.sourceforge.net). Subsequently, variant calling and annotation were performed using GATK 4.0 software (https://software.broadinstitute.org/gatk). Several databases, such as the Single Nucleotide Polymorphism Database (dbSNP)138 (https://www.ncbi.nlm.nih.gov/snp), the 1000 Genome Project (http://www.internationalgenome.org), the Exome Aggregation Consortium (ExAc; http://exac.broadinstitute.org), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) and the Genome Aggregation Database (gnomAD; http://gnomad-sg.org/) were employed to select all variants with frequencies >5%. In addition, online tools such as Human Splicing Finder (http://www.umd.be/HSF3/HSF.shtml), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2), SIFT (http://sift.jcvi.org), Clustal W 2.0 (http://www.clustal.org) and Ensembl (https://asia.ensembl.org/index.html) were applied to predict the potential effect on protein function.

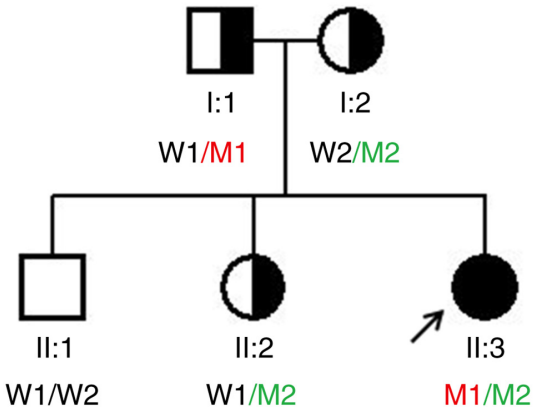

Figure 1. Pedigree of the family with premature ovarian insufficiency. W, wild type; M_1, c.597+1G>T mutation; M_2, c.268G>C mutation; the proband is identified by an arrow.

Confirmation by Sanger sequencing. Mutations of *PSMC3IP* were further confirmed by Sanger sequencing; two pair primers were designed to amplify the exon 4 and exon 7 of *PSMC3IP* (NM_016556). Exon4-F: 5'-GCCCAGCAAAGGGGTCTTAG-3'; Exon4-R: 5'-GCTGGTTCCTGAGCATATCCA-3'. Exon7-F: 5'-GCCAGTGCAAGACATCTCAC-3'; Exon7-R: 5'-CCAGATCAGCCGCTACACAAT-3'. The PCR amplifications were performed as per the following procedure: Initial denaturation of 95˚C for 5 min, 33 cycles of denaturation at 95˚C for 30 sec, annealing at 64˚C/62˚C for 30 sec, extension at 72˚C for 30 sec and a final extension of 72˚C for 10 min. The polymerase chain reaction products were sequenced on an ABI 3730xl DNA Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.). Sequencing results were analyzed using Lasergene software version 7.0 (DNASTAR, Inc.).

Results

Mutation identification by NGS and Sanger sequencing. Overall, the coverage of the target region was 99.3% with an average sequencing depth of >130X and with a variant accuracy of >99.97%. After filtering out all existing mutations with a minor allele frequency >0.05 as determined with dbSNP138, 1000 Genomes, ExAc, ClinVar and gnomAD, a total of 18 variants remained.

In combination with the clinical phenotype and database analyses, two compound heterozygous mutations of *PSMC3IP*,

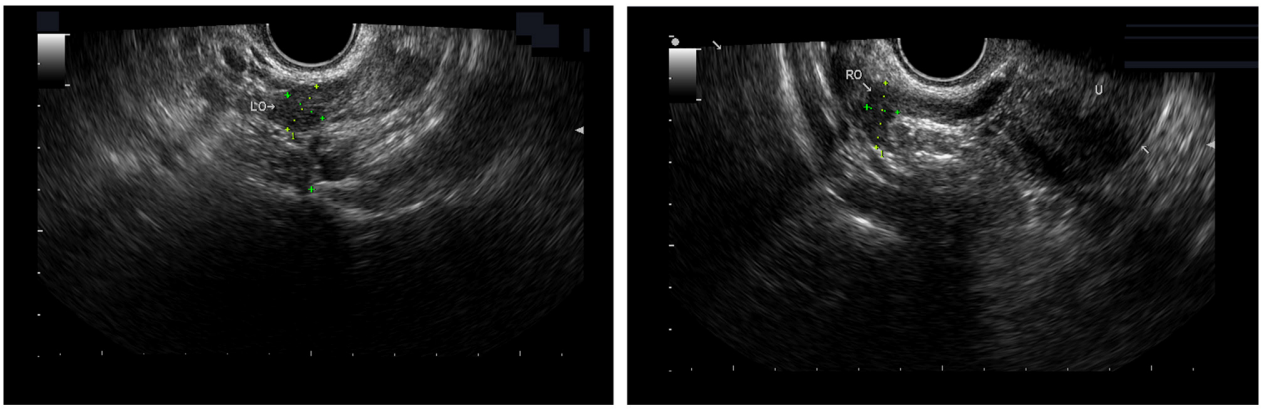

Figure 2. Transvaginal ultrasound examination of the proband showing that the bilateral ovaries are abnormally small, and no obvious antral follicles are observed. LO, left ovary; RO, right ovary.

c.597+1G>T and c.268G>C, were considered as pathogenic. Furthermore, Sanger sequencing on the DNA obtained from the family members confirmed that the c.597+1G>T mutation was inherited from the father, whereas the missense mutation (c.268G>C) was observ 3 ed in the mother and younger sister, showing complete co-segregation of the mutations with the disease phenotype (Fig. 3).

Prediction of the pathogenic significance of the mutations. According to the classification standards of American College of Medical Genetics and Genomics (15), c.597+1G>T and c.268G>C are 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 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 online software UCD Conway Institute), the species conservation analysis confirmed that the ninetieth aspartic acid residues were highly conserved among different species (Fig. 4C). The mutation was described as 'probably damaging' by the online disease prediction software, PolyPhen-2 (Fig. 4A), and it was suggested to 'affect protein function' by SIFT. Neither of the two mutations has been reported in the Human Gene Mutation Database, dbSNP138, the ExAC database, the 1000 Genomes database or in any other single-nucleotide polymorphism database. In addition, to the best of our knowledge, no relevant literature has reported on this mutation. Furthermore, 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 this family.

Discussion

The present study analyzed samples from an adopted 29-year-old Chinese woman with POI and identified two biallelic mutations, c.597+1G>T and c.268G>C in *PSMC3IP*. The two mutations carried by the patient were inherited from her biological mother (c.268G>C) and father (c.597+1G>T). *PSMC3IP* has previously been linked to hereditary breast and ovarian cancer, and has been reported to cause autosomal recessive POI (8,16,17). *PSMC3IP* defects can disrupt estrogen-driven transcription activation of PSMC3IP. Impaired estrogenic signaling can result in ovarian dysgenesis by interfering with the follicular pool and failing to counteract follicular atresia (8,18).

PSMC3IP is located at 17q21.2. The protein product consists of 217 amino acids in its monomer form, 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 (19,20). Previous studies demonstrated that, in *PSMC3IP*-knockout mice, the ovarian volume was reduced and germ cells were missing (21,22). 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 (13). The c.268G>C mutation occurs within the highly conserved leucine zipper domain (Fig. 4B). *In vitro* experiments previously revealed that a defect in the leucine zipper eliminated the dimerization of PSMC3IP (19). In the present study, 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 thereby interfering with splicing. However, the exact effects of splice site mutations on mRNA cleavage are not clear and need to be investigated further.

To date, four studies have described *PSMC3IP* variants unique to patients with ovarian dysgenesis, including the one reported in the present study (Table I). 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 exon 8 of *PSMC3IP* in a large consanguineous Arab Palestinian pedigree with XX-female gonadal dysgenesis, leading to the deletion of Glu201. Furthermore, in a consanguineous Yemeni family of one brother with azoospermia and four sisters with ovarian dysgenesis, Al-Agha *et al* (13) identified a homozygous C-terminal nonsense mutation (c.489C>G, p.Tyr163Ter) in *PSMC3IP*, suggesting an important role of

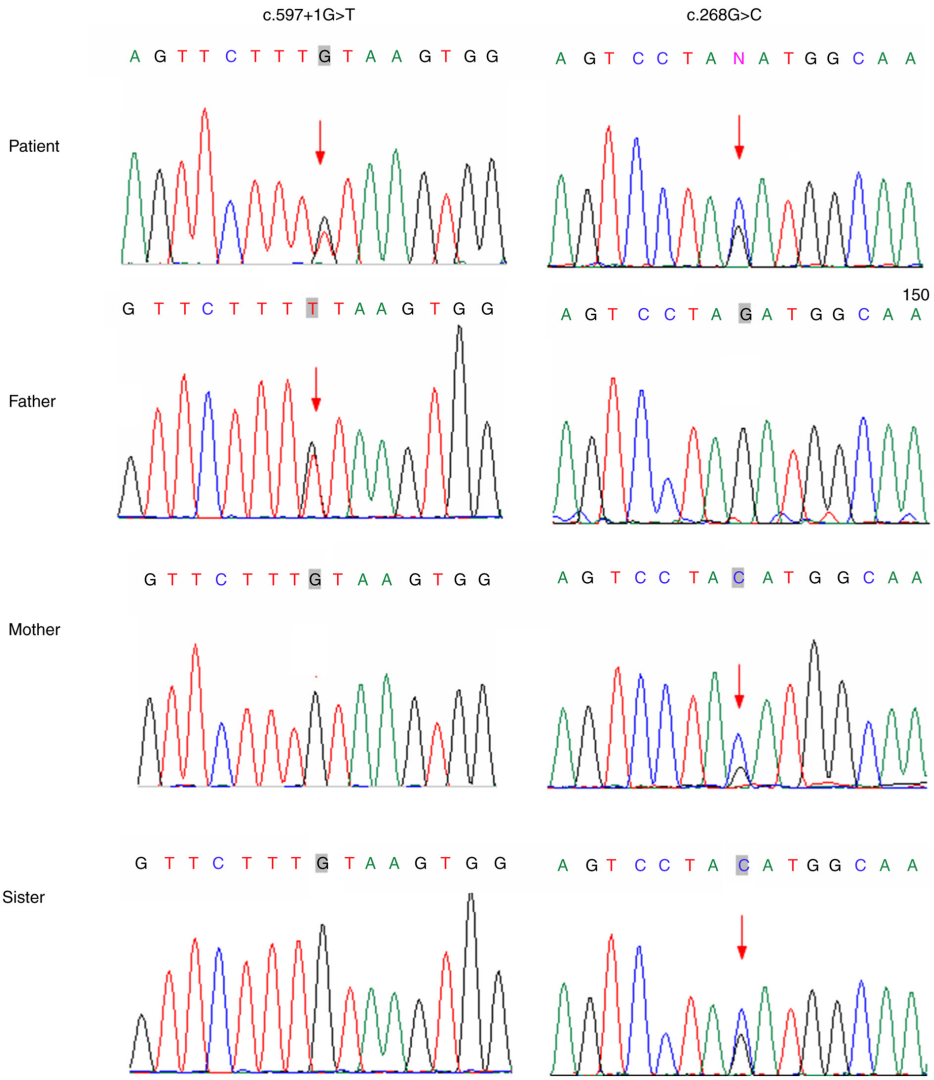

Figure 3. Identifying mutations in *PSMC3IP*. Electropherogram analysis of *PSMC3IP* in the proband shows compound heterozygous c.597+1G>T and c.268G>C mutations of *PSMC3IP*. The father (I1) carried a c.597+1G>T mutation, whereas the mother (I2) carried a c.268G>C mutation.

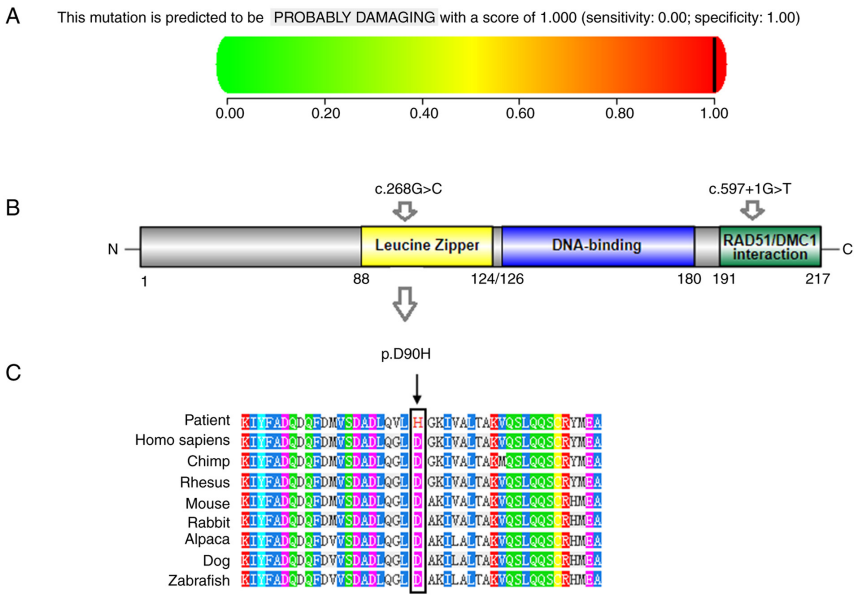

Figure 4. (A) PolyPhen-2 reported the pathogenicity of the amino acid substitution, p.D90H, in *PSMC3IP*. (B) Diagram of *PSMC3IP* with predicted locations of protein sequence changes. (C) Protein alignment showing that *PSMC3IP* p.D90H residues are conserved across multiple species; thus, the two mutations occurred at an evolutionarily conserved amino acid.

Table I. *PSMC3IP* mutations identified in patients with primary ovarian insufficiency.

First author, year	Age at diagnosis, years	Ethnic origin	CS	Karyotype	Nucleotide change	Amino acid change	Status	(Refs.)
Zangen *et al*, 2011	21	Palestinian	Yes	46,XX	c.[600_602del]	p.Glu201del	Ho	(8)
Yang *et al*, 2019	28	French	No	46,XX	c.[496_497delCT] + [430_431insGA]	p.[R166Afs] + [L144X]	He	(14)
Al-Agha *et al*, 2018	27	Yemeni	Yes	46,XX	c.[489 C>G]	p.[Tyr163Ter]	Ho	(13)
Present study	29	Chinese	No	46,XX	c.[597+1G>T] + [268G>C]	P.[splicing] + [p.D90H]	He	-

CS, consanguineous; He, heterozygous; Ho, homozygous.

PSMC3IP in the development of both male and female germ cells. In the present study, the proband's father carried the heterozygous splice site mutation, c.597+1G>T, but he did not show spermatogenesis dysfunction. Previously, two compound heterozygous mutations of *PSMC3IP* (c.430_431insGA, p.L144*; c.496_497delCT, p.R166Afs) were found in a 28-year-old French woman who presented with POI (14). By contrast, a cohort of 50 Swedish women with POI did not exhibit any pathogenic variants of *PSMC3IP* (23). Our study also screened 112 Chinese women suffering from POI using WES and identified pathogenic mutations in known genes (*ERCC6*, *FIGLA* and *NOBOX*), but no pathogenic mutation in *PSMC3IP* gene was detected (unpublished data), highlighting the genetic complexities that give rise to this syndrome. The pathogenesis of POI caused by PSMC3IP is unknown. Because of the limited number of cases of *PSMC3IP* mutations associated with POI, we are not able to make a clear association between this genotype and phenotype. Further functional studies are required to evaluate the genotype and phenotype associations in large cohorts containing patients of various ethnicities.

In conclusion, the present study identified two novel variants in *PSMC3IP* in a Chinese female patient with POI. The present findings provide further evidence that the *PSMC3IP* gene serves a role in the pathogenesis of POI and support the application of NGS in the genetic diagnosis of female infertility. Moreover, the findings extend the context of genotype and phenotype in the POI patients and have important implications for genetic counseling for the family.

Acknowledgements

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Availability of data and materials

All data generated or analyzed during this study are included in this published article, except for next-generation sequencing. The next-generation sequencing datasets generated and/or analyzed during the current study are not publicly available, as the patient did not consent to the public release of these data.

Authors' contributions

PL and LM designed the research protocols. LM and LH performed the experiments. XW and HH acquired the clinical data. LM, YH, XH and ZS analyzed and interpreted the study data. LM and LH wrote the manuscript. PL participated in the supervision and critically reviewed the manuscript. LH and PL confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study fully complied with the tenets of the Declaration of Helsinki and was approved by the Ethics Board of the Women's and Children's Hospital affiliated to Xiamen University, China (approval number XY-2019-059). Written informed consent was obtained from all participants before testing.

Patient consent for publication

Enrolled study participants and the patient provided written informed consent for the publication of this manuscript; however, the patients did not consent to having their data shared out of concern for their privacy.

Competing interests

The authors declare that they have no competing interests.

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