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ORIGINAL RESEARCH

Bi-allelic *SHOC1* loss-of-function mutations cause meiotic arrest and non-obstructive azoospermia

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ABSTRACT

Background The genetic causes of human idiopathic non-obstructive azoospermia (NOA) with meiotic arrest remain unclear.

Methods Two Chinese families with infertility participated in the study. In family 1, two brothers were affected by idiopathic NOA. In family 2, the proband was diagnosed with idiopathic NOA, and his elder sister suffered from infertility. Whole-exome sequencing (WES) was conducted in the two patients in family 1, the proband in family 2 and 362 additional sporadic patients with idiopathic NOA. Sanger sequencing was used to verify the WES results. Periodic acid–Schiff (PAS), immunohistochemistry (IHC) and meiotic chromosomal spread analyses were carried out to evaluate the stage of spermatogenesis arrested in the affected cases.

Results We identified compound heterozygous loss of function (LoF) variants of *SHOC1* (c.C1582T:p.R528X and c.231_232del:p.L78Sfs*9, respectively) in both affected cases with NOA from family 1. In family 2, homozygous LoF variant in *SHOC1* (c.1194delA:p.L400Cfs*7) was identified in the siblings with infertility. PAS, IHC and meiotic chromosomal spread analyses demonstrated that the spermatogenesis was arrested at zygotene stage in the three patients with NOA. Consistent with the autosomal recessive mode of inheritance, all of these *SHOC1* variants were inherited from heterozygous parental carriers. Intriguingly, WES of 362 sporadic NOA cases revealed one additional NOA case with a bi-allelic *SHOC1* LoF variant (c.1464delT:p.D489Tfs*13).

Conclusion To the best of our knowledge, this is the first report identifying *SHOC1* as the causative gene for human NOA. Furthermore, our study showed an autosomal recessive mode of inheritance in the NOA caused by *SHOC1* deficiency.

INTRODUCTION

Infertility is a common reproductive disorder that affects up to 15% of couples worldwide. Male infertility-associated factors are found in half of these couples.^{1,2} Oligozoospermia is defined as <15 million spermatozoa/mL through semen analysis, whereas azoospermia refers to a complete lack of sperm in the ejaculate.^{3–5} Azoospermia affects 10% to 20% of infertile men, and is classified as obstructive azoospermia (OA) and non-obstructive azoospermia (NOA). NOA displays germ cell absence or reduction owing to the testicular atrophy. NOA is heterogeneous, with

a wide spectrum of causes including Y chromosome microdeletion, chromosome abnormalities, hypogonadism, varicocele, testicular tumour and improper drug administration. Based on testicular biopsy and subsequent pathological analysis, NOA can be classified into three types, including Sertoli cell only syndrome (SCOS), maturation arrest (MA) and hypo-spermatogenesis (HS).⁶ MA can be further delineated into early MA (spermatogonia and spermatocytes arrest) and late MA (spermatids arrest).⁷ Several studies have shown that patients with uniform MA exhibited normal testicular volume, relatively normal serum levels of follicle-stimulating hormone (FSH) and a greater frequency of genetic disorders than other types of NOA.⁸ The incidence of genetic disorders in patients with MA is as high as 45%, as shown by chromosome analysis and Y chromosome microdeletion detection.⁸

Monogenic causes of MA have been revealed in recent decades. Mutations of several genes have been identified as the causes of MA, including DNA Meiotic Recombinase 1 (*DMC1*, MIM: 602721), Stromal Antigen 3 (*STAG3*, MIM:608489), Testis Expressed 11 (*TEX11*, MIM: 300311), Synaptonemal Complex Central Element Protein 1 (*SYCE1*, MIM: 611486), Meiosis Specific With OB-Fold (*MEIOB*, MIM: 617670), Coiled-Coil Domain Containing 155 (*CCDC155*, MIM: 618125), Testis Expressed 14 (*TEX14*, MIM: 605792), Testis Expressed 15 (*TEX15*, MIM: 605795) and X-Ray Repair Cross Complementing 2 (*XRCC2*, MIM: 600375).^{9–16} However, the genetic factors associated with the majority of MA cases remain to be elucidated.

Herein, whole-exome sequencing (WES) was conducted to identify single-nucleotide variants (SNVs) and indels potentially responsible for male infertility in two families and 362 patients with sporadic NOA. We identified four male cases harbouring bi-allelic loss-of-function (LoF) variants in *SHOC1*, the orthologs of which have been known to be essential for crossover formation in the meiosis of yeast, *Arabidopsis*, rice and mice, while *SHOC1* mutations had not previously been reported in human NOA.

SUBJECTS AND METHODS

Study subjects

Two Chinese brothers in family 1 were diagnosed with idiopathic NOA at the Department of

Figure 1 Structure of the SHOC1 protein and the genetic context of the *SHOC1* LoF variants detected in four men diagnosed with NOA and one infertile female patient. (A) The positions of LoF mutations in *SHOC1* are shown. The conservation of the *SHOC1* mutations was analysed. (B–D) Validation of *SHOC1* variants identified by WES using Sanger sequencing in two brothers in family 1 (B), two affected patients in family 2 (C) and the sporadic patient (D).

Andrology, Urologic Medical Center, Shanghai General Hospital, Shanghai Jiao Tong University. In family 2, the proband was diagnosed with idiopathic NOA, and his sister also suffered from infertility. The family histories of the two brothers in family 1 and the proband in family 2 were collected. Furthermore, 362 patients with sporadic NOA were enrolled in this study. Reproductive congenital diseases such as Klinefelter syndrome and genomic AZF deletions, or other azoospermia-associated factors including varicocele, radiation, chemotherapy, orchitis, cryptorchidism and testicular cancer were excluded for these patients with NOA.

Whole-exome sequencing

Genomic DNA was extracted from the blood of patients with NOA using the TIANamp Blood DNA Kit (TIANGEN Biotech, Beijing, China) according to the manufacturer's instructions. DNA was fragmented through Covaris focused ultrasonication. Known exons and exon–intron boundary sequences were captured using xGen Exome Research Panel (Integrated DNA Technologies, Coralville, IA, USA), and sequencing DNA libraries were prepared following the manufacturer's instructions.

Sequencing was performed on a HiSeq X10 platform (Illumina, San Diego, CA, USA). Sequencing reads were aligned to the human genome (GRCh37/hg19) using Burrows-Wheeler Aligner (BWA). Both SNVs and indels within the captured coding exonic intervals were called using GATK, Platypus, VarScan, LoFreq, FreeBayes, SNVer, SAMtools and VarDict. The variants were filtered and annotated using the ANNOVAR software.

Genetic variants with allele frequencies higher than 1% according to the ExAC Browser and 1000 Genomes Project were excluded, and the intronic, upstream and downstream variants were removed. Nonsense, frameshift, essential splice-site and potentially deleterious missense (SIFT, PolyPhen-2 and MutationTaster) variants were kept for further analysis. Genes with two alleles with potentially deleterious missense mutations or LoF mutations were retained because autosomal recessive and X-linked inheritance were assumed for MA. Also, we compared candidate genes with known pathogenic genes for azoospermia in mice (<http://www.informatics.jax.org/mgihome/homepages/>) and testis enriched genes in the database (<http://www.proteinatlas.org/>). The aforementioned sequencing and bioinformatic analyses were conducted together with the Nuprobe company

Figure 2 PAS staining of cross-sections of testis in affected patients. (A, B) PAS staining of cross-sections of testicular biopsy in the proband (A) and the elder brother in family 1 (B). (C) PAS staining of cross-sections of seminiferous tubule in the proband in family 2 (C) and the sporadic patient (D). Scale bars=20 μ m in A–D. Asterisk indicates the spermatocytes in the testis.

(Shanghai, China). The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Sanger sequencing

PCR was performed to verify the WES results. The primers are shown in online supplementary table 1. The PCR products were bidirectionally sequenced by Sanger sequencing using a 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Immunohistochemistry (IHC)

The testicular biopsies were obtained from the patients with NOA. The testicular tissue was fixed overnight in 4% paraformaldehyde at 4°C and then embedded in warm paraffin (60°C). The tissue blocks were cut into 5- μ m-thick sections and mounted onto slides. The tissue sections were dewaxed in xylene, re-hydrated in a descending alcohol gradient and heated in sodium citrate buffer (90°C–98°C) for 15 min for antigen retrieval. After blocking with 6% normal donkey serum for 1 hour at room temperature, the sections were incubated overnight with anti-SYCP3 (dilution: 1:25, catalogue number: AF3750; R&D Systems, Minneapolis, MN, USA), anti- γ H2AX (dilution: 1:300, catalogue number: 2668445; Merck Millipore, Billerica, MA, USA), anti-DMC1 (dilution: 1:100, catalogue number: sc-373862; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and PNA (dilution: 1:400, catalogue number: L21409; Life Technologies/ThermoFisher Scientific, Waltham, MA, USA) at 4°C. The sections were washed thrice with PBS-Tween, and incubated with highly cross-adsorbed secondary antibody conjugated with Alexa Fluor 488 or Alexa Fluor 555 (dilution: 1:400, Life Technologies/ThermoFisher Scientific) for 1 hour at room temperature. After the final three washes, the sections were counterstained with 4',6-diamidino-2-phenylindole (DAPI) to label the nuclei, and the images were captured by fluorescence microscope (Leica, Wetzlar, Germany).

Periodic acid–Schiff (PAS) stain

The testicular tissues were fixed in 4% paraformaldehyde solution overnight, embedded in paraffin and sectioned at 5 μ m thickness. The sections were then stained with haematoxylin and Schiff's solution (catalogue number: ab150680; Abcam, Cambridge, UK) according to standard protocols. The images were captured by phase-contrast microscope.

Meiotic chromosomal spread analysis

Testicular tissues of patients with NOA in families 1 and 2 and patients with OA (positive control) were washed three times with aseptic Dulbecco's modified Eagle's medium (DMEM) Nutrient Mixture F-12 (DMEM/F-12; Gibco/ThermoFisher Scientific) with 2% antibiotics containing penicillin and streptomycin (Gibco/ThermoFisher Scientific). Two-step enzymatic digestion and differential plating were used to isolate germ cells from somatic cells.¹⁷ After culture for 24 hours, the suspended male germ cells were collected for meiotic chromosomal spread analysis. Cells were first suspended in hypotonic buffer (30 mM Tris–HCl, 50 mM sucrose, 17 mM trisodium citrate dehydrate, 5 mM EDTA, 0.5 mM dithiothreitol and 0.5 mM phenylmethylsulfonyl fluoride) for 30 min. Then, the cells were centrifuged, resuspended in 100 mM sucrose and spread on a glass slide in a thin layer of paraformaldehyde solution containing 0.1% Triton X-100. The slide was placed in a 37°C incubator for 2 to 3 hours for extensive drying. The slide was then blocked with 5% BSA and incubated with primary antibodies overnight at 4°C, including anti-SYCP3 (dilution: 1:25, catalogue number: AF3750; R&D Systems) and anti- γ H2AX (dilution: 1:300, catalogue number: 2668445; Merck Millipore). After extensive washes with PBS, the cells were incubated with the secondary antibody; namely, IgG conjugated with fluorescein isothiocyanate (Sigma Chemical, St. Louis, MO, USA) or rhodamine (Sigma Chemical), at a 1:500 dilution for 1 hour at room temperature. Replacement of primary antibodies with PBS was used as a negative control, and images were captured with a confocal microscope (Olympus, Tokyo, Japan).

RESULTS

Clinical data

Two Chinese families with infertility participated in this study (online supplementary figure 1A). In family 1, two brothers had a history of male infertility for 3 and 2 years, respectively. There was no family history of consanguinity or fertility problems and no chronic diseases in the medical history. Neither patient had a history of cryptorchidism, hypogonadism, cancer, drinking or smoking. They were 29 and 32 years old at phenotyping and in good physical condition (online supplementary table 2). Physical examination showed normal development of penis, epididymis, prostate, scrotum and vas deferens, and there was no varicocele in either brother. However, their testes (10 mL in both sides) were slightly smaller than reference (12–15 mL in Chinese individuals). Laboratory examination revealed that both brothers had normal serum FSH level (12.44 IU/L and 11.26 IU/L; normal range: 1.5–12.5 IU/L). Both had 46, XY karyotypes and there were no microdeletions in the Y chromosome.

In family 2, the proband was 28 years old at phenotyping, and he had a history of male infertility for 2 years with no family history of consanguinity or fertility problems. As in family 1, physical examination showed normal development of penis, epididymis, prostate, scrotum and vas deferens. The volumes of his testes were 15 mL (left) and 20 mL (right). Routine semen analyses (three times) revealed complete azoospermia, and the

Figure 3 Expression of recombination proteins DMC1 and acrosomal marker PNA in the testis of all affected patients and a patient with obstructive azoospermia as a positive control. Immunohistochemical staining showed the expression of DMC1 (green) and PNA (red) in the testis of the proband (A), the elder brother in family 1 (B), the proband of family 2 (C), the sporadic patient (D) and in the testis of the patient with OA (positive control) (E). Scale bars=20 μ m in A–D. Arrow indicates the acrosome of spermatids in the testis.

semen volume was normal. Sex hormone levels were comparable with the reference values (online supplementary table 2). The proband's karyotype was 46, XY and there were no Y chromosome microdeletions in the proband. The proband's older sister had a history of infertility for 3 years. After failure of several assisted reproduction technology trials, the couple adopted a child. The history was described by the proband. Unfortunately, the sister's basal hormone levels at phenotyping and ovary volume were unknown (online supplementary figure 1B) and unavailable to us.

The probands in families 1 and 2 underwent the microsurgical testicular sperm extraction (mTESE) procedure at Urologic Medical Center of Shanghai General Hospital. Histopathological analysis revealed MA in the probands. Furthermore, the

older brother in family 1 underwent a testicular biopsy, which confirmed MA.

WES revealed bi-allelic *SHOC1* LoF mutations in the affected patients of families 1 and 2

After the genetic analyses pipeline, compound heterozygous LoF mutations in *SHOC1* (NM_173521:c.C1582T:p.R528X and c.231_232del:p.L78Sfs*9, respectively) and a homozygous *SHOC1* LoF mutation (NM_173521:c.1194delA:p.L400Cfs*7) were assumed as the most likely causes of meiosis defect in the two brothers of family 1 and the proband in family 2, respectively. *SHOC1* protein has a highly conserved domain (aa 937–1105) among metazoans, plants, mice and human, which was termed as 'the *SHOC1* homolog region'. This region has a

Figure 4 Expression of SYCP3 and γ H2AX in the testis of all affected patients and a patient with obstructive azoospermia (OA; positive control). Immunohistochemical staining showed the expression of SYCP3 (green) and γ H2AX (red) in the testis of the proband (A), the elder brother in family 1 (B), the proband of family 2 (C), the sporadic patient (D) and in the testis of the patient with OA (positive control) (E). Scale bars=20 μ m in A–D. The arrow indicates the XY body in the spermatocytes in the testis.

conserved XPF endonuclease-like central domain and a helix-hairpin-helix (HhH2) domain in the C-terminal.¹⁸ *SHOC1* LoF mutations could result in the truncated *SHOC1* proteins without the expression of the *SHOC1* homolog region in families 1 and 2 (figure 1A).

Sanger sequencing confirmed bi-allelic LoF mutations in *SHOC1* in the siblings of families 1 and 2. In family 1, compound heterozygous LoF mutations in *SHOC1* were confirmed in the two brothers (figure 1B). A paternally inherited nonsense variant (NM_173521:c.C1582T:p.R528X) resulted in a premature STOP codon and a maternally inherited frame-shift variant (NM_173521:c.231_232del:p.L78Sfs*9) led to a truncated *SHOC1* protein. In family 2, the homozygous LoF mutation in *SHOC1* (NM_173521:c.1194delA:p.L400Cfs*7)

was confirmed in the proband and his elder sister (figure 1C). Consistent with the autosomal recessive mode of inheritance, the unaffected parents were heterozygous carriers of this same *SHOC1* variant. Collectively, bi-allelic *SHOC1* LoF mutations were identified in the siblings in families 1 and 2.

MA phenotypes in the cases with bi-allelic mutations in *SHOC1*

MA phenotypes in the affected male patients with bi-allelic *SHOC1* LoF mutations were testified by PAS, IHC and meiotic chromosomal spread analysis. The PAS assay revealed decreased number of spermatocytes and absence of spermatozoa and spermatids in the seminiferous tubules of the patients in family 1

Figure 5 Immunostaining of SYCP3 and γ H2AX in the probands of families 1 and 2 and the positive control. (A, B) Meiotic chromosome spread assay showed the expression of SYCP3 (red) and γ H2AX (green) in the testis of the proband in family 1 (A) and family 2 (B). Only scattered γ H2AX signal (zygotene stage) was detected in germ cells in these patients. Meiotic chromosome spread analysis showed the expression of SYCP3 (red) and γ H2AX (green) in the testis of a patient with obstructive azoospermia (positive control) (C). The γ H2AX signal was only detected in the sex body of control germ cells at pachytene. Scale bars=20 μ m in A–C. Asterisk indicates the XY body in the spermatocytes at pachytene stage in the testis.

(figure 2A, B) and in family 2 (figure 2C). However, the number of spermatogonia and Sertoli cells at the basement membrane within the tubules remained unchanged. IHC revealed the expression of DMC1 (indicating double-strand break repair) in the patients' seminiferous tubules. However, no signal of PNA, an acrosomal marker and therefore a marker of spermatids and spermatozoa, was observed (figure 3A–C). Moreover, there were positive expressions of SYCP3 and γ H2AX foci in the patients with NOA (figure 3E). SYCP3 is used to label components of the axial/lateral element (AE and LE), and γ H2AX foci is a DNA double-stranded break marker that is expressed in preleptotene to zygotene spermatocytes. Nevertheless, the XY body, a visibly distinct domain in the nucleus of pachytene spermatocytes also indicated by γ H2AX staining, was not expressed in seminiferous tubules of these patients (figure 4A–C). In contrast, positive expression of SYCP3, γ H2AX, DMC1 and PNA was observed in testis from the patient with OA (figure 4E). Together, these results indicated arrest at the spermatocyte stage for all three patients with NOA.

To evaluate the specific stage of spermatocytes arrested, meiotic chromosomal spread analysis was performed. SYCP3 is a marker of AE and LE. During the leptotene and zygotene, AE forms and extends, and in the pachytene stage, complete synapses form. It is identified that there were leptotene and zygotene spermatocytes in the seminiferous tubules in the probands of families 1 and 2 according to SYCP3 and γ H2AX staining. However, there was no signal of SC complex and XY body (indicated by SYCP3

and γ H2AX staining) in these two patients, suggesting that the spermatogenesis arrested at zygotene (meiosis I prophase I) (figure 5A,B). In contrast, large numbers of pachytene spermatocytes were present in the positive control (OA testis) (figure 5C). In summary, it is verified that the spermatogenesis was arrested at the zygotene stage in the probands with bi-allelic *SHOC1* LoF mutations.

Sporadic MA-affected subject with a bi-allelic *SHOC1* LoF mutation

We also conducted WES in the 362 patients with NOA. Intriguingly, a sporadic MA individual with a bi-allelic mutation in *SHOC1* was identified (NM_173521:c.1464delT:p.D489Tfs*13) (figure 1A) that was verified by Sanger sequencing (figure 1D).

This patient was 25 years old when he was first referred to our hospital because of male infertility. Physical examination showed that the volume of the testes (10 mL in both sides) was slightly less than the reference value. The patient's FSH and testosterone levels were normal (4.47 mIU/mL and 27.24 nmol/L, respectively). He had a 46, XY karyotype and no Y chromosome microdeletion (online supplementary table 2). The patient underwent mTESE at our centre in 2017, and PAS assay showed maturation arrest in this case (figure 2D). The IHC results revealed positive expression of DMC1, SYCP3 and γ H2AX, but no expression of PNA or XY body, suggesting MA at spermatocytes (figures 3D and 4D). Thus, we identified an additional case with a bi-allelic LoF mutation in *SHOC1*, which was the most likely primary cause of azoospermia in this case.

DISCUSSION

In the current study, bi-allelic *SHOC1* LoF mutations were reported in patients with NOA from two families and a sporadic NOA case. All these variants could lead to truncated *SHOC1* proteins. Spermatogenesis was arrested at zygotene stage in the four patients. Thus, our study revealed that *SHOC1* was a novel causative gene for NOA.

Bi-allelic LoF variants in *SHOC1* were very rare in human populations according to the gnomAD database. The allele frequencies of *SHOC1* variants (rs1004968910 and rs777595871) found in family 1 were 1.8×10^{-5} and 2.8×10^{-5} , respectively. The allele frequency of *SHOC1* variant (rs1432616103) found in family 2 was 8.0×10^{-6} . No homozygotes have been reported for these *SHOC1* LoF variants. There are no previous reports of the *SHOC1* variant (NM_173521:c.1464delT:p.D489Tfs*13) in the sporadic patient (online supplementary table 3). These four variants resulted in truncated *SHOC1* proteins without the expression of *SHOC1* homolog region (figure 1A). According to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) guidelines, all four *SHOC1* variants were assessed as deleterious, including PS3 (well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect on the gene or gene product), PM2 (for recessive disorders, this mutation is at extremely low frequency in Exome Sequencing Project, 1000 Genomes Project or Exome Aggregation Consortium) and PM3-Strong (for recessive disorders, detected in *trans* with a pathogenic variant).¹⁹

In the sporadic patient, we identified a bi-allelic *SHOC1* LoF mutation. Although this could be a homozygous frameshift mutation in *SHOC1* (NM_173521:c.1464delT:p.D489Tfs*13), it is also possible that the individual carries a heterozygous D489Tfs*13 mutation on the one allele and a heterozygous deletion in *SHOC1* on the other allele. However, we also employed

the WES for CNV analysis in this case according to the protocol as described previously,^{20–22} and no evidence of *SHOC1* deletion has been obtained in this study.

SHOC1, a meiosis-specific gene located at 9p13 in humans, has 26 exons encoding a 1444-aa protein. The protein is essential for crossover formation in meiosis prophase I. During the meiosis prophase I, homologous recombination results in crossover formation in the context of the synaptonemal complex (SC). In yeast, mutations of *zip2*, an ortholog of *SHOC1*, can result in 40% to 50% reduction in crossover formation.²³ In *Arabidopsis* and rice, knock-out of *SHOC1* can lead to a striking reduction in the number of meiotic crossovers.^{24–26} In mice, it is demonstrated that *Mzip2* knock-out male and female mice are sterile. Spermatogenesis in *Mzip2*^{−/−} mice arrests at zygotene stage, and there is no SC assembly and expression of MLH1 loci in the *Mzip2* deficiency spermatocytes.²⁷ *SHOC1* orthologs are involved in meiosis prophase I via the formation of ‘ZZS’ complex in yeast, *Arabidopsis*, rice and mice. In yeast, the ZZS complex is composed of Zip2 (the ortholog of *SHOC1*), Zip4 and Spo16.²⁸ Zip2–Spo16 interaction can form an XPF-ERCC1-like complex via the XPF-like domain in Zip2, and is essential for crossover formation by binding meiotic recombination intermediates.²⁹ The Zip2–Zip4 complex can interact with Msh4/Msh5 to stabilise crossover-specific DNA intermediates.²⁹ In the present study, our results showed that bi-allelic *SHOC1* LoF mutations caused meiotic arrest at zygotene stage, suggesting that *SHOC1* deficiency could result in defects of both crossover formation and SC assembly in humans. However, the mechanism of *SHOC1* in crossover formation and SC assembly needs further investigation in humans.

In conclusion, we found bi-allelic *SHOC1* LoF mutations in two Chinese families and one sporadic NOA case. The meiotic arrest phenotype was confirmed in all of our male patients. Further studies are required to uncover the roles of *SHOC1* in meiosis in human spermatogenesis and whether bi-allelic *SHOC1* mutations are associated with female infertility.

Web resources

- dbSNP, <https://www.ncbi.nlm.nih.gov/projects/SNP/>.
- ENCODE, <https://www.encodeproject.org>
- Exome Aggregation Consortium (ExAC) Browser, <http://exac.broadinstitute.org>
- FANTOM, <http://fantom.gsc.riken.jp>
- GenBank, <https://www.ncbi.nlm.nih.gov/genbank/>
- gnomAD, <http://gnomad.broadinstitute.org>
- GTEx, <http://www.gtexportal.org>
- Human Protein Atlas, <https://www.proteinatlas.org/>
- MGI, <http://www.informatics.jax.org/mgihome/homepages/>
- OMIM, <https://www.omim.org/>
- PolyPhen-2, <http://genetics.bwh.harvard.edu/pph2>
- SIFT, <http://sift.jcvi.org>

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Contributors ZL, ZZ and FZ designed the research; CCY, CY and LYZ performed the research; PL, RHT, HXC, YG, YHH, ELZ and JZ performed the bioinformatics analysis; HFS, JXZ, YH and LZ analysed the data; CCY, CY, ZYJ and LYZ wrote and revised the paper.

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Competing interests None declared.

Patient consent for publication Obtained.

Ethics approval This study was approved by the Institutional Ethical Review Committee of Shanghai General Hospital, Shanghai Jiao Tong University (Permit Number 2018KY052), and an informed consent of clinical data and testicular tissues for research was obtained from the donors.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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