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ORIGINAL ARTICLE

Male Infertility

A new *TEX11* mutation causes azoospermia and testicular meiotic arrest

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There are many unknown genetic factors that lead to infertility in nonobstructive azoospermia men. Here, we performed whole-exome sequencing in blood samples obtained from 40 azoospermia patients with meiotic arrest and found a novel c.151_154del (p.D51fs) frame-shift mutation in exon 3 of the testis expressed 11 (*TEX11*) gene in one patient. Sanger sequencing analysis of the patient and 288 fertile men was performed to validate the mutation. Immunohistochemical analysis showed *TEX11* expression in late-pachytene spermatocytes and in round spermatids in fertile human testes. In contrast, testes of the patient with *TEX11* mutation underwent meiotic arrest and lacked *TEX11* expression. Western blotting of human embryonic kidney (HEK293) cells transfected with a vector for the p.D51fs *TEX11* variant detected no *TEX11* expression. In conclusion, we identified a novel frame-shift mutation in the *TEX11* gene in an azoospermia patient, emphasizing that this gene should be included in genetic screening panels for the clinical evaluation of azoospermia patients.

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Keywords: male infertility; meiotic arrest; nonobstructive azoospermia; testis expressed 11

INTRODUCTION

Infertility impacts approximately 15% of couples, and about 50% of infertility is caused by male factors.^{1–4} Human spermatogenesis comprises a series of precisely regulated, continuous processes, including differentiation of spermatogonia, meiosis of spermatocytes, and sperm maturation.⁵ Factors known to deleteriously impact spermatogenesis include hormonal regulation, varicocele, cryptorchidism, immune factors, environmental chemicals, and genetic factors, among others.^{4,6} Spermatogenesis disorders caused by genetic factors represent the most commonly encountered problems, accounting for 15%–30% of male infertility, and these typically manifest as severe oligospermia or azoospermia.^{7–9}

The incidence of azoospermia is about 10%–15% in male infertility,⁸ which can be divided into obstructive azoospermia (OA; 15%–20%)¹⁰ and nonobstructive azoospermia (NOA; 80%–85%).^{6,11} The many etiologies of NOA include pretesticular factors such as endocrine abnormalities and testicular factors such as testicular torsion, genetic abnormalities, and idiopathic causes,^{12,13} whereas the posttesticular categories are considered to be the major contributors to OA.^{14,15} For OA patients, it is sometimes possible to restore reproductive function via surgery or other treatments,^{4,16} whereas NOA is understood as one of the most difficult diseases to treat in male infertility.¹⁷ At present, the etiology and mechanisms of many NOA patients remain unknown, further limiting treatment options.¹⁸ Moreover, only limited

genetic tests are currently available to help determine the etiology of infertile men, including karyotyping, Y chromosome azoospermia factor (*AZF*) microdeletion, and cystic fibrosis transmembrane conductance regulator (*CFTR*) mutation screening.^{6,8} We are unaware of any established predictive tests to indicate testicular pathology and/or the possible presence of sperm cells in the testis. In this context, research into the meiotic genetic factors in male infertility, and especially in NOA patients, is very likely to further clarify the causes and mechanisms of spermatogenesis disorders and to improve the capacity of clinicians to provide individualized diagnoses as well as genetic counseling for patients.

Testis expressed 11 (*TEX11*) is a gene located on the X chromosome that is expressed specifically in germ cells;^{1,14,19} it has high sequence identity in mouse and human.¹⁰ The incidence of *TEX11* gene mutations in male primary infertility is high, at about 1%, and *TEX11* dysregulation has been implicated in spermatocyte apoptosis, maturation disorders, and azoospermia.^{10,20,21} Work with *Tex11*^{-/-} mice has shown that the lack of *TEX11* causes meiotic arrest, and *TEX11* is known to regulate homologous chromosomal synapsis and the repair of DNA double-strand breaks.^{21,22} In the present study, a novel mutation of the *TEX11* gene was found in an azoospermia patient based on whole-exome sequencing,²⁰ and we show that this mutation is causative for azoospermia and testicular meiotic arrest.

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PATIENTS AND METHODS

Ethical approval

This study was approved by the Institutional Review Board for Reproductive Medicine of Shandong University (Jinan, China; ethical committee reference number 2019 #36), and written informed consent was obtained from each patient. All of the DNA and tissue samples were handled in accordance with the National Regulation of Clinical Sampling in China.

Patients and study population

We studied 40 NOA Chinese patients with meiotic arrest. The diagnoses in men with azoospermia were made on the basis of semen analysis, and the pathological type of meiotic arrest was confirmed by means of histologic examination of testicular biopsy specimens according to the guidelines of the World Health Organization (WHO) Laboratory Manual for the Examination and Processing of Human Semen (5th edition, 2010).²³ Any conditions that result in spermatogenic failure (*e.g.*, chromosomal abnormalities, Y-chromosomal microdeletions, orchitis, cryptorchidism, radiotherapy, or chemotherapy) were not present among the 40 meiotic arrest patients. The patients were recruited from the Center for Reproductive Medicine of the Shandong Provincial Hospital Affiliated with Shandong University (Jinan, China).

Immunohistochemical (IHC) analysis

For histology, testicular biopsy from those azoospermia men was fixed in 4% paraformaldehyde (PFA) solution (Solarbio, Beijing, China), embedded in paraffin, and cut into 5- μ m thick sections. After sectioning, the slides were rehydrated and then processed with hematoxylin and eosin staining. Testicular biopsies from a fertile patient (who underwent percutaneous testicular biopsy for oligozoospermia) and from the patient with the *TEX11* mutation underwent IHC staining. A mouse-specific HRP/DAB (ABC) Detection IHC Kit (Abcam, Hong Kong, China) was used for IHC staining. Briefly, after rehydration, sections were blocked with Hydrogen Peroxide Block Reagent (Abcam) for 10 min. The sections were then boiled for 15 min in Sodium Citrate Antigen Retrieval Solution (Solarbio). After cooling to room temperature, the rest of the procedure was conducted according to the IHC Kit product protocol. Immunostaining of *TEX11* was carried out using a primary polyclonal goat-anti-human *TEX11* antibody (1:100 diluted, Abcam), with secondary mouse-anti-goat IgG-B antibody (1:200 diluted, Santa Cruz Biotechnology, Shanghai, China).

Whole-exome sequencing (WES) analyses

We performed WES for the 40 NOA patients with meiotic arrest. Genomic DNA was extracted from peripheral blood samples using QIAamp DNA mini kits (Qiagen, Beijing, China) following the manufacturer's instructions. Then, the samples were sent to iGeneTech company (Beijing, China) for WES analysis. Briefly, DNA samples from the peripheral blood cells were used to generate sequencing libraries with Fast Library Prep Kit (iGeneTech). Then, exons of genes were captured with the AIExome Enrichment Kit (iGeneTech). Then, SAMtools (<http://www.htslib.org/>), samblaster (<https://github.com/GregoryFaust/samblaster>), and GATK (<https://gatk.broadinstitute.org/hc/en-us>) were used to sort the Binary Alignment/Map (BAM) format files and for duplicate marking, local realignment, and base quality recalibration to generate final BAM file for computation of the sequence coverage and depth. The target mean depth was 147.06, and the coverage rate of samples was 99.85%. To characterize the detected variants, dbSNP (<https://www.ncbi.nlm.nih.gov/snp/>), 1000 Genome (<https://www.internationalgenome.org/>), and other related databases were applied. For exonic variants, gene transcript annotation databases, such as Ensembl (<http://asia.ensembl.org/index.html>) and UCSC (<http://genome.ucsc.edu/>), were also used to assess amino acid alterations. Minor allele frequency (MAF) values were not previously reported or reported to have a frequency below 0.1% in four public databases, including the 1000 Genome.

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Validation of mutations via Sanger sequencing

To confirm the novel mutation identified in the *TEX11* gene, the Sanger sequencing was performed. The *TEX11* exon/intron structure was determined by alignment of the *TEX11* cDNA sequence (NM_031276) with its genomic sequence.²⁴ Exon 3 of the human *TEX11* gene was polymerase chain reaction (PCR) amplified from genomic DNA from the infertile patient harboring the *TEX11* mutation and 288 fertile men in a 25 μ l reaction using the following conditions: 95°C for 5 min, 35 cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 40 s. Forward and reverse primers were F 5'-AACCAAGTACTCCCAAAGAATGC-3' and R 5'-ACAGGTGAGAAAAGTGAAGCCTG-3', respectively. The PCR-amplified products were purified using an Avanti J-20 XP centrifuge (Beckman Coulter, Brea, CA, USA), and subsequent sequencing was completed with the use of a BigDye terminator kit with the ABI 3730-Avant Genetic Analyzer (Applied Biosystems, Shanghai, China). The results were compared with the *TEX11* gene sequence using Sequencer software, version 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA).

Plasmid construction

The open reading frame of human *TEX11* cDNA (NM_031276) was amplified from a *TEX11* clone plasmid (GeneCopoeia, Guangzhou, China). Quantitative real-time PCR (qPCR) was performed with the PrimeSTAR system (Takara, Beijing, China) using the following conditions: 98°C for 5 min, 30 cycles of 98°C for 10 s, 60°C for 5 s and 72°C for 90 s. Forward and reverse primers were F 5'-GCCAGATCTCAAGCTATGCATCATCACCATCACCATGACA-3' and R 5'-TGAGTTTCTGCTCGACTAATCTGACTTGCTCCAGTAG-3', respectively. This pair of primers contained a 6th histone (His) tag sequence, thus a 6thHis-*TEX11* sequence with a 6thHis tag added before the *TEX11* sequence were obtained after PCR with this pair of primers. The mutant expression vector for p.D51fs was generated with a Quikchange Lightning Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA), and DNA sequencing was conducted to confirm the desired mutation. The wild-type vector and mutant vector were constructed by inserting wild-type and mutant *TEX11* cDNA directly into a PCMV6 vector (Origene, Beijing, China), respectively.

Cell culture and transfection

Human embryonic kidney 293 (HEK293) cells (National Collection of Authenticated Cell Cultures, Beijing, China) were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Beijing, China) supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin-streptomycin at 37°C in the 5% CO₂ atmosphere. For confirmatory experiments, the desired plasmids (mock vector, wild-type *TEX11*, or p.D51fs mutant plasmids) were transiently transfected into cells using X-tremeGENE HP DNA Transfection Reagent (Roche, Basel, Switzerland). HEK293 cells were seeded in a 10-cm dish for 24 h to grow to 70%–80% confluence and were then transfected with 10 μ g of the various plasmids.

Western blotting

Forty-eight hours after transfection, cells were lysed in lysis buffer (50 mmol l⁻¹ HEPES-KOH, pH 7.5, 100 mmol l⁻¹ KCl, 2 mmol l⁻¹ ethylenediaminetetraacetic acid (EDTA), 10% glycerol, 0.1% NP-40, 10 mmol l⁻¹ NaF, 0.25 mmol l⁻¹ Na₃VO₄, 50 mmol l⁻¹ β -glycerolphosphate), with protease inhibitors (Roche) for 20 min on ice, followed by centrifugation at 13 000g (Eppendorf 5424R, Shanghai, China) for 25 min

at 4°C. Equal amounts of protein (assessed via bicinchoninic acid [BCA]) were electrophoresed on 10% sodium dodecyl sulfate polyacrylamide gels and were transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked and then incubated with primary mouse monoclonal anti-His antibody (1:5000 diluted; Proteintech, Chicago, IL, USA). Immunoblots were imaged with a BIO-RAD ChemiDoc MP Imaging System (Bio-Rad, Hercules, CA, USA) and analyzed using Image Lab Software (Bio-Rad).

RESULTS

A novel azoospermia phenotype-associated mutation in the *TEX11* gene

We conducted an analysis of 40 azoospermic men using WES. All of the infertile patients of the study were prescreened for the lack of chromosomal abnormalities and Y-chromosomal microdeletions. The WES results revealed a novel deletion mutation from site 151 to 154 of the *TEX11* isoform 2 transcript (NM_031276) in one patient with meiotic arrest (Figure 1a). Table 1 lists all of the previously reported *TEX11* mutations as well as the novel mutation we identified in the present study.⁹ To verify the mutation, the infertile patient with the *TEX11* mutation and 288 fertile men were examined via Sanger sequencing. The deletion mutation in the patient was confirmed; none of the 288 control men carried the novel variant (Figure 1b). Since *TEX11* is an X-linked germ-cell specific gene, it is likely to be associated with spermatogenic failure. The frame-shift mutation in exon 3 c.151_154del (p.D51fs) was predicted to result in a premature stop codon in exon 4, resulting in a truncated protein comprising only 57 amino acids (Figure 1c).

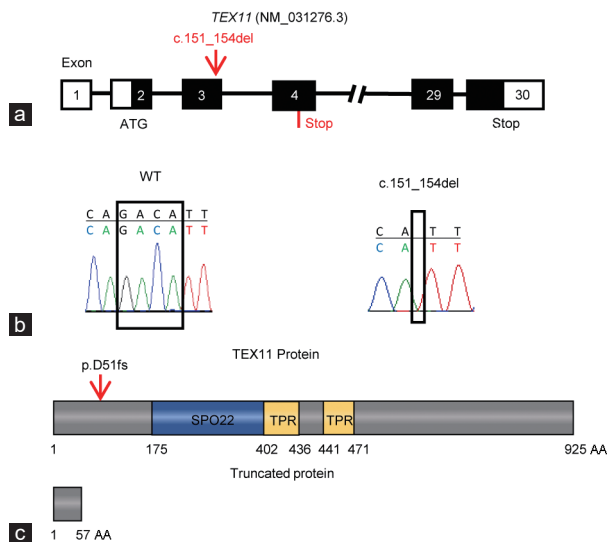


Figure 1: A novel frame-shift mutation identified in the *TEX11* gene. (a) The *TEX11* c.151_154del mutation is localized in exon 3 (red arrow), and the premature stop codon is localized in exon 4 of *TEX11* transcript isoform 2 (NM_031276.3). White rectangles represent noncoding exons, and black rectangles represent coding exons. The coding sequence of the gene begins a start codon in exon 2 and ends at a stop codon in exon 30. (b) Sanger sequencing validation of the identified deletion mutation in the patient. (c) Predicted *TEX11* domains with a SPO22 meiosis-specific motif (amino acid positions 175–402) and multiple TPR-containing regions (amino acid positions 402–436 and 441–471). The mutation in the coding region is located in the N-terminal sequence (red arrow). A predicted protein length schematic of truncated protein variant comprising 57 N-terminal amino acids is shown under the predicted protein domains for *TEX11*. *TEX11*: testis-expressed 11; SPO22: sporulation domain; TPR: tetratricopeptide repeat; WT: wild type; AA: amino acid.

Meiotic arrest in the azoospermia patient bearing the p.D51fs *TEX11* mutation

The detailed phenotypes of the azoospermic patient with the p.D51fs *TEX11* mutation were ascertained through semen analysis and histologic examination of testicular biopsy specimens using light microscopy. No sperm was found in three separate semen analyses or in testicular biopsy sections. The patient was 25 years old, with normal endocrine hormone levels (Table 2). The karyotype of the patient was normal (46,XY), and no Y chromosome microdeletions were detected (Table 2). Histological analysis of the fertile man's testis biopsy showed spermatids and different stages of male germ cells (Figure 2a–2c). In comparison, the patient's testis biopsy revealed meiotic arrest at pachytene stage in most seminiferous tubules; round spermatids were observed only rarely in tubules (arrows; Figure 2d–2f). No mature spermatozoa were observed in the seminiferous tubules, in accordance with the diagnosis of azoospermia. Postmeiotic cells were not seen in most tubules of the azoospermia patient, although we did detect a few late spermatocytes and round spermatids in rare tubules. Based on the histological analysis, the primary defect caused by the *TEX11* deletion mutation of this patient was concluded to be meiotic arrest.

TEX11 protein expression in a healthy control and the azoospermia patient

IHC analysis of testis sections from the fertile control man showed that *TEX11* was mainly expressed in late-pachytene spermatocytes and in round spermatids; *TEX11* was completely absent in surrounding somatic cells including Sertoli cells and interstitial cells (Figure 3a). However, no *TEX11* expression was detected in the azoospermia patient with the *TEX11* mutation (Figure 3b). We also conducted experiments wherein Western blotting was used to assess *TEX11* protein accumulation in HEK293 cells, which were transiently transfected with expression vectors including an empty control (PCMV6), a fusion construct of the wild-type *TEX11* coding sequence (CDS) with a terminal His tag (Figure 3c), and a fusion construct of the CDS for the p.D51fs mutant allele with a His tag. Normal His-*TEX11* was detected as expected (approximately 107 kDa) from

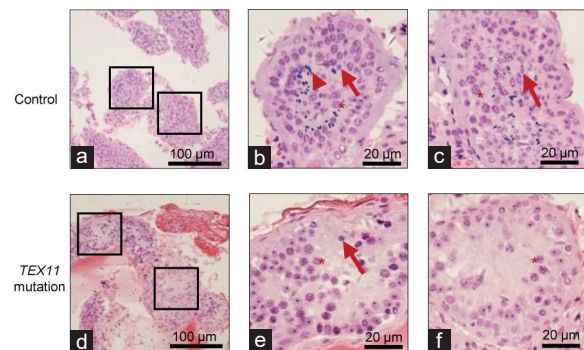


Figure 2: Hematoxylin and eosin staining of the testicular biopsy of the patient and fertile control. (a) Testis sections from a fertile control man and expanded view of one seminiferous tubule in (b) the left square and (c) the right square, with marked mature sperm (arrowhead) and different stages of developing male germ cells (including pachytene spermatocytes [asterisk] and round sperm cells [arrow]). (d) The azoospermia patient and expanded view of one seminiferous tubule in (e) the left square and (f) the right square, with meiosis arrested at the pachytene stage (asterisk) in most of the seminiferous tubules; round spermatids (arrow) were observed only rarely. *TEX11*: testis-expressed 11.

Table 1: Mutations of *TEX11* reported for azoospermia patients in published literature and our data^a

| Position | Nucleotide change | Protein/RNA change | Testicular sperm extraction | Patients (n) | Reference |
|------------|------------------------|---|-----------------------------|--------------|------------|
| Exon 6 | 405C→T | Silent mutation, A135spl d ^b | Few sperm | 1 | 20 |
| Exon 7 | 466A→G | Missense mutation, M156V | No sperm | 1 | 20 |
| Exons 9–11 | 607del237bp | 203del79aa | Few sperm | 2 | 20 |
| Intron 10 | 748+1G→A ^c | L249spl d ^b | No sperm | 1 | 20 |
| Intron 21 | 1793+1G→C ^c | R597spl d ^b | No sperm | 1 | 20 |
| Exon 24 | 2047G→A | Missense mutation, A683T | Few sperm | 1 | 20 |
| Exon 6 | 349T→A | Missense mutation, W117R | No sperm | 1 | 10 |
| Exon 6 | 405C→T | Silent mutation | No sperm | 1 | 10 |
| Exon 7 | 424G→A | Missense mutation, V142I | No sperm | 1 | 10 |
| Exon 7 | 515A→G | Missense mutation, Q172R | No sperm | 1 | 10 |
| Exon 10 | 731C→T | Missense mutation, T244I | No sperm | 1 | 10 |
| Exon 16 | 1258Ins (TT) | Frameshift mutation, 1258GATG→TTGGTA | No sperm | 1 | 10 |
| Exon 26 | 2243T→C | Missense mutation, V748A | No sperm | 1 | 10 |
| Exon 27 | 2319T→C | Silent mutation | No sperm | 1 | 10 |
| Intron 3 | -17T→C ^c | Intronic alteration | No sperm | 1 | 10 |
| Intron 5 | -48G→A ^c | Intronic alteration | No sperm | 1 | 10 |
| Intron 10 | +42C→A ^c | Intronic alteration | No sperm | 1 | 10 |
| Intron 12 | -28T→C ^c | Intronic alteration | No sperm | 1 | 10 |
| Intron 15 | -64G→A ^c | Intronic alteration | No sperm | 1 | 10 |
| Intron 21 | -1G→A ^c | Alteration of splicing acceptor site | No sperm | 1 | 10 |
| Intron 22 | -37A→G ^c | Intronic alteration | No sperm | 1 | 10 |
| Intron 24 | +119G→A ^c | Intronic alteration | No sperm | 1 | 10 |
| Intron 27 | -55A→C ^c | Intronic alteration | No sperm | 1 | 10 |
| Intron 28 | -44A→G ^c | Intronic alteration | No sperm | 1 | 10 |
| Exon 29 | 2568G→T | Missense mutation, W856C | No sperm | 2 | 9 |
| Exon 3 | 151_154del | D51 frame-shift mutation | No sperm | 1 | This study |

^a*TEX11* mutations are mapped to isoform 2 (GenBank accession number, NM_031276); ^bThe term spl d represents the splicing donor site; +1 refers to the first base of a given intron, while -1 denotes the last base. *TEX11*: testis expressed 11; del: deletion; bp: base pair; Ins: insertion

Table 2: Clinical data of the analyzed *TEX11* mutation-bearing azoospermic patient

| Variable | Patient | Normal range |
|--|--------------------------------|------------------------|
| Testosterone (ng dl ⁻¹) | 354.3 | 280–800 |
| Luteinizing hormone (IU l ⁻¹) | 2.74 | 1.7–8.6 |
| Follicle stimulating hormone (IU l ⁻¹) | 3.25 | 1.5–12.4 |
| Prolactin (ng ml ⁻¹) | 6.81 | 4.04–15.2 |
| Testis (ml) | | |
| Left | 12 | 12–25 |
| Right | 12 | 12–25 |
| Y chromosome microdeletion | No deletion | No deletion |
| Karyotype | 46,XY | 46,XY |
| Histology | Spermatocyte maturation arrest | Normal spermatogenesis |

TEX11: testis expressed 11

cells expressing wild-type *TEX11*; while no such band was detected from cells transfected with the empty control or the p.D51fs mutant sequence (**Figure 3d**).

DISCUSSION

The *TEX11* protein is required for chromosomal synapsis, repair of DNA double-strand breaks, meiotic crossover, and recombination.^{21,22,25} Specifically, previous work has shown that *TEX11* forms distinct foci on homologous chromosomes that synapse with each other, apparently forming components of meiotic nodules that are needed for recombination: *Tex11*-null mice with exon 3–29 knocked out exhibit meiotic arrest and spermatocytes deletion,²² and this phenotype is

consistent with our study. However, Adelman and Petrini²¹ figured out that exon 3 knockout male mice were fertile with the production of normal litter sizes.²¹ As was stated above, the phenotypes of *Tex11*-null mice were not in accordance in different kinds of *Tex11*-knockout mice, and we suppose this may result from knockout of a different exon.

The well-studied *TEX11* protein contains a meiosis-specific sporulation (SPO22) domain (175–429 AA) and numerous tetratricopeptide repeat (TPR) containing regions (402–436 AA, 441–471 AA) that have been shown to mediate protein–protein interactions.^{19,20} In *Saccharomyces cerevisiae* and *Arabidopsis thaliana*, zinc transporter 4 precursor (Zip4), an ortholog of *TEX11*, physically interacts with ZMM (abbreviation for Zip1/2/3/4, Msh4/5, and Mer3) proteins and with the axial element of the synaptonemal complex (SC), providing a direct physical link between crossover-designated recombination intermediates and SC assembly.^{21,26} Previous studies have reported that full-length mouse *TEX11* interacts with synaptonemal complex protein 2 (SYCP2),²² and the C-terminal portion of human *TEX11* is known to interact with both shortage in chiasmata 1 (SHOC1, also known as ZIP2) and nijmegen breakage syndrome 1 (NBS1)^{4,21} that are involved in meiotic synapsis, repair, and crossover formation. However, we are unaware of any studies reporting any dysfunction related to the N-terminal region of *TEX11*. In addition, mutations previously reported in human were either single base replacement or bases deletion and insertion from exon 6 to exon 29.^{9,10,20} Therefore, the *TEX11* c.151_154del mutation in exon 3 found in our study was the first reported mutation on the N-terminal region of human *TEX11*.

Yang *et al.*¹⁰ reported that the frequency of rare *TEX11* mutations is significantly elevated in azoospermic men, suggesting that *TEX11*



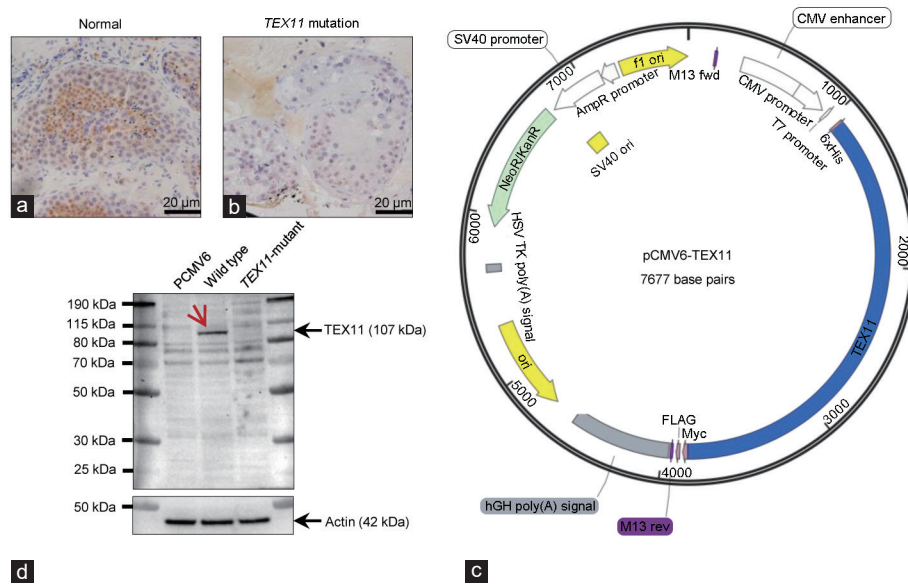


Figure 3: Expression of wild-type *TEX11* and the p.D51fs truncation mutant variant in human testis and HEK293 cells. (a) Immunohistochemical detection of *TEX11* in testicular tissue sections using a polyclonal anti-*TEX11* antibody. In a control with normal spermatogenesis, *TEX11* was highly expressed in late-pachytene spermatocytes and round spermatids. (b) In the patient, germ cells exhibited no staining for *TEX11*. (c) Schematic for the pCMV6-His-*TEX11* vector used for cell transfection as wild-type *TEX11*-His. (d) Western blotting results stained for anti-His antibody with HEK293 cells transiently transfected with mock vector PCMV6, wild-type *TEX11*-His, or *TEX11*-mutant (p.D51fs)-His vectors. Actin served as a loading control, and molecular weight markers are shown on the left. SV40: simian virus 40; CMV: cytomegalovirus; f1: F1 phage; ori: origin; AmpR: ampicillin resistance gene; M13 fwd: enterobacteria phage M13 forward; T7: promoter for bacteriophage T7 RNA polymerase; His: histone; *TEX11*: testis-expressed 11; FLAG: DYKDDDDK amino acid sequence tag; Myc: EQKLISEEDL amino acid sequence tag; M13 rev: enterobacteria phage M13 reverse; hGH poly(A): human growth hormone polyadenylation; HSV TK poly(A): herpes simplex virus-thymidine kinase polyadenylation; Neor/KanR: neomycin/kanamycin resistance gene; pCMV6: plasmid CMV6.

may function in human spermatogenesis.¹⁰ Mutations in this single X-linked gene cause infertility in 1% of azoospermic men.^{10,20,27} Our study identified a *TEX11* mutation in infertile patients with meiotic arrest, representing a 2.5% incidence. Our study therefore supports previously published data that it would be beneficial to include the *TEX11* gene in genetic screening panels for the clinical evaluation of azoospermia patients. One deficiency of the present study is that we were unable to explore the inheritance of the mutation, because the proband's family declined to participate in the study. It will be helpful to further explore the relative incidence of this *TEX11* mutation in azoospermia in larger and more diverse cohorts. In conclusion, we identified a novel (p.D51fs) mutation in the *TEX11* gene of an azoospermia patient and demonstrated that the genetic disruption of *TEX11* is causative for azoospermia.

AUTHOR CONTRIBUTIONS

MJL, HBL, and HBZ carried out the genetic studies and participated in the WES analysis. XCY, FFC, and SJY carried out the histological experiment. XCY and MJL carried out *in vitro* protein expression experiment. XCY, HBL, and HBZ drafted the manuscript. MJL, HBL, and HBZ conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

ACKNOWLEDGMENTS

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