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Induced pluripotent stem cell line from a mouse model of human azoospermia with a frameshift mutation *Tex11*_1260Ins(TT)

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Abstract

Infertility is a common disease that impacts 15% of reproductive age couples worldwide, and genetic causes are implicated in about half of those cases. Non-obstructive azoospermia is a severe form of male infertility that features spermatogenic failure resulting in no sperm in the ejaculate and severely reduces the chance to have biological children. We created a *Tex11*_1260Ins(TT) (1260GATA → TTGGTA) mutant mouse that models the *Tex11_1258(TT)* mutation identified from a patient with nonobstructive azoospermia. The *Tex11*_1260Ins(TT) iPSC cells displayed characteristics of pluripotent-like morphology, expressed pluripotent protein markers, show normal karyotype, and can to differentiate into tissues of the three germ layers.

1. Resource table

Unique stem cell line identifier	*MMRIi001-A-3*
Alternative name(s) of stem cell line	*Tex11_1260Ins(TT)*
Institution	*Magee-Womens Research Institute, University of Pittsburgh School of Medicine*
Contact information of the reported cell line distributor	*Kyle E. Orwig*, orwigke@upmc.edu
Type of cell line	*iPSC*
Origin	*Mouse*
Additional origin info *(applicable for human ESC or iPSC)*	*N/A*
Cell Source	*Dermal fibroblasts*
Method of reprogramming	*Sendai virus*
Clonality	*Clonal*

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Doungkamchan and Orwig has patent #PCT/US2018/043948 pending to University of Pittsburgh.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2022.102728.

Evidence of the reprogramming transgene loss (including genomic copy if applicable)	*RT-PCR for Sendai virus*
Cell culture system used	*Feeder-dependent on mouse embryonic fibroblasts (MEFs)*
Type of Genetic Modification	*A frameshift mutation Tex11_1260Ins(TT) resulted from the change of GATG sequence to the TTGGTA sequence at the aspartic acid position 435 in exon 16*
Associated disease	*Sterility/Infertility*
Gene/locus	*Tex11 gene, Xq13.1*
Method of modification/site-specific nuclease used	*No modification*
Site-specific nuclease (SSN) delivery method	*N/A*
All genetic material introduced into the cells	*N/A*
Analysis of the nuclease-targeted allele status	*PCR amplification and Sanger sequencing for the targeted mutation Tex11_1260Ins (TT)*
Method of the off-target nuclease activity surveillance	*N/A*
Name of transgene	*N/A*
Eukaryotic selective agent resistance (including inducible/ gene expressing cell-specific)	*N/A*
Inducible/constitutive system details	*N/A*
Date archived/stock date	*November 2020*
Cell line repository/bank	*Magee-Womens Research Institute*
Ethical/GMO work approvals	*This study was approved by the ethical committee of the University of Pittsburgh (IACUC 17050289), Pittsburgh, PA, USA*
Addgene/public access repository recombinant DNA sources' disclaimers (if applicable)	*N/A*

2. Resource utility

A *TEX11_1258Ins(TT)* (1258GATA → TTGGTA; D435) mutation was identified in a patient with non-obtructive azoospermia (Yang et al., 2015). Tex11_1260Ins(TT) (1260GATA → TTGGTA; D421) male mice recapitulate the meiotic arrest phenotype observed in the azoospermic patient (Wang et al., 2021). *Tex11*_1260Ins(TT) mouse iPSCs and in vitro gametogenesis can be used to understand the infertility phenotype and develop targeted therapies.

3. Resource details

Azoospermia is defined as a complete absence of sperm in the ejaculate and affects 1% of men worldwide (Gudeloglu and Parekattil, 2013). Non-obstructive azoospermia (NOA) accounts for 80–85% of all azoospermic cases, of which, 75% are idiopathic (unexplained) and difficult to treat. The other 15–20% are obstructive azoospermia (OA, blockage in the excurrent duct system) that is easily treated by recovering sperm directly from the testes (Gudeloglu and Parekattil, 2013). Mutations in the *Tex11* gene; an X-linked gene, were found at high prevalence in two independent studies (Yang et al., 2015; Yatsenko et al., 2015). Yang and colleagues identified a frameshift mutation (GATG → TTGGTA; *TEX11_1258Ins(TT)* at the aspartic acid position 435 in exon 16 of *TEX11* in a NOA patient who was diagnosed with meiotic arrest at pachytene (Yang et al., 2015). The

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TEX11_1258Ins(TT) human mutation was modeled by mutating the homologous locus in *Tex11_1260Ins(TT)* mice that exhibit the same NOA phenotype with maturation arrest at the pachytene spermatocyte stage (Wang et al., 2021). We produced the same mouse model, confirmed the NOA phenotype and established the MWRIi001-A-3 iPSC line from skin fibroblasts.

Skin fibroblasts isolated from adult *Tex11*_1260Ins(TT) male mouse were used to generate MWRIi001-A-3 iPSCs using non-integrated Sendai virus (SeV) to deliver mRNAs of the Yamanaka reprograming factors Oct4, Sox2, Klf4, and c-Myc in a feeder-dependent manner. After 1 week, iPSC colonies emerged and were manually picked to be reseeded on mouse embryonic fibroblast (MEF) feeder cells. After a few passages, the MWRIi001-A-3 iPS line was established and maintained its morphology of round, dome-shaped colonies with smooth edges (Fig. 1A). PCR amplification and Sanger sequencing confirmed that the MWRIi001-A-3 iPSCs contained the Tex11_1260Ins(TT) genotype (Fig. 1B–C) and were male (Fig. 1D). After 16 passages, the MWRIi001-A-3 iPSC line was tested for absence of SeV vector by RT-PCR using specific primers (Fig. 1E). Furthermore, cells from MWRIi001-A-3 had a normal karyotype (40, XY) (Fig. 1F).

Immunofluorescence staining of putative MWRIi001-A-3 iPSCs revealed expression of pluripotency markers Alkaline Phosphatase (AP), SSEA1, OCT4, SOX2, DPPA2, NANOG (Fig. 1G–H). MWRIi001-A-3 iPSCs were also confirmed to be mycoplasma-free at passage 16 (Supplementary figure 1). MWRIi001-A-3 cells can be harvested and cryopreserved in liquid nitrogen for long-term storage.

At passage 8, the differentiation capacity of the MWRIi001-A-3 cells into three germ layers was determined by conventional teratoma assay. Histological evaluation revealed tissue differentiation to all three germ layers within the teratoma, including endoderm (gastrointestine, bone marrow), mesoderm (smooth muscle, bone) and ectoderm (neurorosette, skin) (Fig. 1I) (each indicated tissue was marked with the arrow).

Our results demonstrated that we have produced a stable iPSC line from an infertile mouse model carrying a frameshift mutation *Tex11*_1260Ins(TT) that was identified in an idiopathic NOA patient with the homologous mutation (TEX11_1258Ins(TT)). MWRIi001-A-3 cell line can be used with in vitro gametogenesis methods to understand the mechanisms of spermatogenic failure caused by this specific patient-derived mutation and to develop targeted treatments (e.g., CRISPR/Cas9 gene editing) to repair the mutant allele.

4. Materials and methods

4.1. Fibroblast isolation and reprogramming

Skin tissue pieces from an adult *TEX11_1258Ins(TT)* male mouse were used to derive fibroblasts. At passage 2, these fibroblasts were transduced with non-integrated CytoTune iPS 2.0 Sendai Reprograming Kit (ThermoFisher). Seven days post-induction with Sendai viruses, putative iPSCs (MWRIi001-A-3) were seeded on mitomycin-C treated mouse embryonic fibroblasts (MEF) in GlutaMAX DMEM/F12 (ThermoFisher), 20% Knockout Serum Replacement-Mutiple Species (ThermoFisher), 1% NEAA, 1%

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2-Methylmethacathinone (ThermoFisher), and 1ug mouse Leukemia Inhibitory Factor (Invitrogen). iPSCs were cultured in 37 °C, 5%CO2, and passaged using TrypLE (ThermoFisher) as single cells every 3–5 days.

4.2. Sanger sequencing analysis

Genomic DNA of MWRIi001-A-3 iPSCs, Tex11_1260Ins(TT) cells and wild-type mouse iPSCs cells were extracted using QIAamp Micro DNA kit (QIAGEN). DNA was mixed with Tag 2X Master Mix (BioLabs) and specific primers (Table 2) following these PCR cycle paramerters: initial denaturation at 95 °C for 5 min, denaturation at 95 °C for 30 s, annealing at 57 °C for 30 s, extension at 72 °C for 1 min, repeated for 35 cycles, followed by a final extension at 72 °C for 10 min. PCR products were analyzed by agarose gel electrophoresis, then were purified using the QIAquick PCR Purification Kit (QIAGEN) and sent to the University of Pittbsurgh Genomics Research Core for Sanger sequencing.

4.3. Reverse transcription PCR analysis of SeV vectors

After passage 16, total RNA was extracted from MWRIi001-A-3 iPSCs using RNeasy MiniKit (QIAGEN). RNA from transduced cells at passage 3 was used as the positive control. RT-PCRs for the detection of Sendai transgenes were performed using the GoScript Reverse Transcriptase cDNA synthesis kit (Promega). SeV specific primers were used to assess the presence of remaining Sendai virus (SeV) vectors (Table 2).

4.4. Karyotype analysis

Metaphase chromosomes were prepared from MWRIi001-A-3 iPSCs at passage 4. Twenty metaphase spreads were analyzed and karyotyped by the Cytogenetics and Molecular Pathology Laboratory at Washington University, St. Louis.

4.5. Immunofluorescence staining for pluripotency markers

At passage 5, the pluripotent status of putative MWRIi001-A-3 iPSCs was evaluated by immunofluorescent staining for four six markers (AP, SSEA1, SOX2, OCT4, DPPA2, NANOG) according to instructions in the Flourescent Mouse ES/iPS Cell Characterization Kit (Millipore) (Table 1).

4.6. Flow cytometry analysis for pluripotent markers

At passage 17, MWRIi001-A-3 iPSCs were harvested by TryLE and incubated for 45 min at 4 °C with conjugated surface marker anti-SSEA1 antibody (Table 2). For nuclear marker staining, cells were first fixed and permealized using CytoFix/CytoPerm (Fisher Scientific) before incubation with conjugated anti-SOX2 or anti-OCT4 antibody (Table 2). A published mouse iPSC line (ALSTEM) was used as the control. FCS Express 6 software was used for the flow cytometry analysis.

4.7. Teratoma assay

MWRIi001-A-3 iPSCs were harvested using TryPLE, and 1×10^6 cells in 8 μl mPSC medium was injected into the interstitial space of 6-week-old NOD/SCID mouse testes. After 2–4

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months, tumors were harvested, fixed in Bouins, histologically processed and stained with hematoxilin/eosin for teratoma analysis.

4.8. Short tandem repeat (STR) analysis

STR anlysis was performed by the ATCC Cell Line Authentication Service.

4.9. Mycoplasma test

The LookOut Mycoplasma PCR Detection Kit (Sigma) was used to detect mycoplasma contamination in iPSCs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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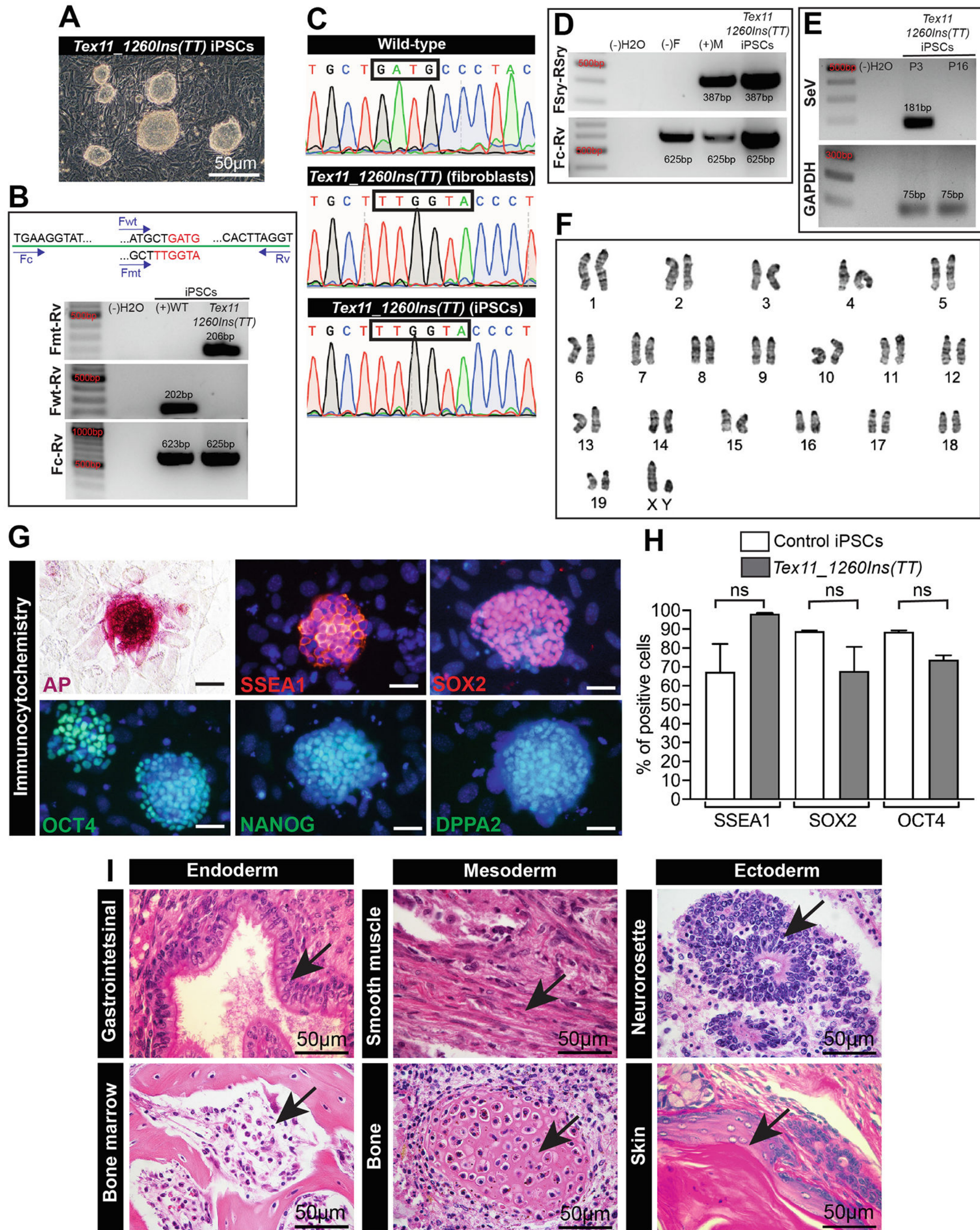

Fig. 1.

Characterization of the *Tex11_1260Ins(TT)* induced pluripotent stem cell (iPSC) line derived from an infertile male mouse model. (A) Phase contrast image showing normal morphology of *Tex11_1260Ins(TT)* iPSCs growing on a feeder-coated plate (scale bar = 50 μm). (B) Genotyping PCR products of *Tex11_1260Ins(TT)* iPSCs to confirm their origin from *Tex11_1260Ins(TT)* mouse fibroblasts. Wild-type control iPSCs (labeled as (+) WT) were used as a control. (C) Sanger sequencing of PCR products for wild type iPSCs, *Tex11_1260Ins(TT)* fibroblasts*, and Tex11_1260Ins(TT)* iPSCs to confirm their genotype as wild-type (GATG) or mutant (TTGGTA). (D) PCR results confirming the sex

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of *Tex11_1260Ins(TT)* iPSCs as male using F_{Sry}-R_{Sry} primer pair specific for the Sry gene. Cells of male (M) origin or female (F) origin were used as controls. Fc-Rv primer pair was used to confirm presence of DNA in all cell samples. (E) RT-PCR result showing absence of sendai virus (SeV) in *Tex11_1260Ins(TT)* iPSCs at passage 16. *Tex11_1260Ins(TT)* iPSCs at passage 3 were used as positive control for SeV. GAPDH housekeeping gene was used as a control for RT-PCR analysis. (F) *Tex11_1260Ins(TT)* iPSCs showing normal karyotype of 40 chromosomes (mouse). (G) Immunofluorescent staining of *Tex11_1260Ins(TT)* iPSCs showing positive staining for pluripotent markers Alkaline phosphatase (AP), SSEA1, SOX2, OCT4, NANOG, DPPA2. (H) Flow cytometry analysis showing no difference in percentage of SSEA1+, SOX2+, or OCT4 + *Tex11_1260Ins(TT)* iPSCs compared to control iPSCs. (I) Teratoma assay analysis confirming tri-lineage (endoderm, mesoderm, ectoderm) differentiation potential of transplanted *Tex11_1260Ins(TT)* iPSCs.

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Table 1

Characterization and validation.

Classification (optional italicized)	Test	Result	Data
Morphology	Photography	*Typical round, dome-shaped colonies with clear edges*	Fig. 1A
Pluripotency status evidence for the described cell line	Qualitative analysis (Immunocytochemistry)	*Positive staining for pluripotency markers (Alkaline Phosphatase (AP), SSEA1, SOX2, OCT4, DAPP2, NANOG)*	Fig. 1G
	Quantitative analysis	*Flow cytometry (SSEA1 98.2%, SOX2 73.1%, OCT4 79.1%)*	Fig. 1H
Karyotype	Karyotype (G-banding)	*40XY, 300–850 band resolution*	Fig. 1F
Genotyping for the desired genomic alteration/allelic status of the gene of interest	Mutant-specific PCR and Sanger sequencing	*The* Tex11_1260Ins(TT) mutation in MWRIi001-A-3 iPSCs *was confirmed by PCR amplification with mutant primers (Fmt-Rv), but not by the wild-type primers (Fwt-Rv). Positive control primers (Fc-Rv) detected PCR product in both mutant and wild-type samples. Negative control (H2O) showed no PCR amplification.*	Fig. 1B
		Sanger sequencing analysis verified the Tex11_1260Ins(TT) mutation in fibroblasts and iPSCs derived from Tex11_1260Ins(TT) mice but not in wild-type mouse iPSCs.	Fig. 1C
		The male genotype of MWRIi001-A-3 iPSCs was confirmed *by PCR for Sry, a Y chromosome-specific gene using FSry-RSry primers. Tail snip DNA from male mice was used as a positive control. Tail snip DNA from female mice and no DNA (H2O) were negative controls.*	Fig. 1D
	Transgene-specific PCR	*Not performed*	*N/A*
Verification of the absence of random plasmid integration events	PCR/Southern	The MWRIi001-A-3 iPSC line was tested for absence of SeV vector by RT-PCR using specific primers	Fig. 1E
Parental and modified cell line genetic identity evidence	Microsatelite PCR (mPCR) STR analysis	*Not performed*	*N/A*
		18 mouse short tandem repeat (STR) loci were analyzed. The result showed the derived iPSC line was 95% matched with that of the parental fibroblast cells	*Submitted in archive with journal*
Mutagenesis / genetic modification outcome analysis	Sequencing (genomic DNA PCR and Sanger sequencing)	X-linked hemizygous Tex11_1260Ins(TT) mutation	Fig. 1C
	PCR-based analyses	Tex11_1260Ins(TT) mutation	Fig. 1B
	Immunohistochemistry	*Not performed*	*N/A*
Off-target nuclease analysis-	PCR across top 5/10 predicted top likely off-target sites, whole genome/exome sequencing	*Not performed*	*N/A*
Specific pathogen-free status	Mycoplasma	*No mycoplasma contamination*	Supplementary Fig. 1
Multilineage differentiation potential	Teratoma formation	*Endoderm: gastrointestinal, bone marrow*	Fig. 1I
		Mesoderm: smooth muscle, bone	
		Ectoderm: neurorosette, skin	
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	*Not performed*	*N/A*

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Classification (optional italicized)	Test	Result	Data
Genotype - additional histocompatibility info	Blood group genotyping	*Not performed*	*N/A*
	HLA tissue typing	*Not performed*	*N/A*

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Table 2

Reagents details.

Antibodies and stains used for immunocytochemistry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency Markers	*Alkaline phosphatase*	*1:2:1*	*Millipore (Cat# SCR077)*
	Mouse anti-SSEA1	*1:100*	*RRID: AB_2904506*
	Mouse anti-SOX2	*1:100*	
	Mouse anti-OCT4	*1:100*	
	Mouse anti-NANOG	*1:100*	
	Mouse anti-DPPA2	*1:100*	
Nuclear stain	*DAPI*	*1:1000*	*Millipore (Cat# SCR077)*
			RRID: AB_2904506
Site-specific nuclease			
Nuclease information	*N/A*		
Delivery method	*N/A*		
Selection/enrichment strategy	*N/A*		

Primers and Oligonucleotides used in this study		
Genotyping (PCR)	**Target**	**Forward/Reverse primer (5′ –3′)**
Tex11 common sequence	*Fc-Rv (625 bp)*	F: TGAAGGTATCTCCACTAGCATGG R: ACCTAAGTGCCACAGCAAAGAAC
Tex11 wild-type	*Fwt-Rv (202 bp)*	F: GGTCCAAAAATATGCTGATG R: ACCTAAGTGCCACAGCAAAGAAC
*Tex11*_1260Ins(TT) *mutant*	*Fmt-Rv (206 bp)*	F: GGTCCAAAAATATGCTTTGGTA R: ACCTAAGTGCCACAGCAAAGAAC
Male genotype (Sry-positive)	*FSry- RSry (387 bp)*	F: TGGTCTGGACCCAAACGCTGTCCACA R: GAGTACAGGTGTGCAGCTCT
Sendai virus checking (RT-PCR)	**Target**	**Forward/Reverse primer (5′-3′)**
Sendai virus vector	*SeV (181 bp)*	F: GGATCACTAGGTGATATCGAGC R: ACCAGACAAGAGTTTAAGAGATATGTATC
House-keeping gene	*GAPDH (75 bp)*	F: GCACCGTCAAGGCTGAGAAC R: AGGGATCTCGCTCCTGGAA