Brief Report

DOI: 10.5582/irdr.2026.01023

Generation and characterization of induced pluripotent stem cells from a patient with classic Fabry disease

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SUMMARY: Fabry disease is an X-linked lysosomal storage disorder caused by pathogenic variants in the α-galactosidase A (α-Gal A, *GLA*) gene. The disease exhibits substantial clinical heterogeneity, with renal injury representing one of its most prominent manifestations. Due to the scarcity of human renal specimens and the inability of conventional animal models to recreate patient-specific pathological features, the precise mechanism underlying renal-predominant Fabry disease remains poorly understood. In this study, we successfully established and comprehensively characterized a urine-derived induced pluripotent stem cell (iPSC) line from a 35-year-old male patient with classic Fabry disease with a typical renal-dominant phenotype. The patient carried the *GLA* c.1080_1082delTGG (p.Gly361del) variant. Non-integrating episomal reprogramming was used to generate monoclonal iPSCs, which were further validated for pluripotency, trilineage differentiation ability, genomic stability, and exogenous vector clearance. The established iPSC line stably retained the patient-specific pathogenic variant, exhibited full pluripotent properties, and showed no genomic abnormality or residual episomal integration. Therefore, this well-characterized renal-phenotype-specific iPSC line provides a reliable cellular platform for investigating the mechanisms of progressive Fabry disease nephropathy and can facilitate future targeted drug screening.

Keywords: induced pluripotent stem cell, reprogramming, Fabry disease, nephropathy

1. Introduction

Fabry disease (OMIM #301500) is an X-linked lysosomal storage disorder resulting from loss-of-function variants in the *GLA* gene located on chromosome Xq22 (*1*). Clinically, Fabry disease is classified into classic and late-onset phenotypes, distinguished by residual α-galactosidase A activity and age of symptom onset. Deficient α-galactosidase A activity leads to progressive accumulation of globotriaosylceramide (GL-3/Gb3) in multiple organs, causing highly variable clinical phenotypes (*2*). Renal impairment is a major adverse event and one of the leading causes of mortality in patients with the disease (*3*).

Despite advances in disease awareness, the molecular mechanisms driving renal-specific injury and phenotypic heterogeneity have yet to be completely elucidated, hindering the development of precise therapeutic strategies (*4*). The major obstacles include limited availability of human renal biopsy samples and the poor simulation accuracy of traditional animal and cell models, which fail to recreate individual genetic backgrounds and patient-specific renal pathology (*5*).

Patient-derived induced pluripotent stem cells (iPSCs) faithfully preserve individual genetic information and possess unlimited self-renewal and multilineage differentiation capacities (*6*). Urine-derived cells (UDCs) allow non-invasive, high-compliance sampling and have become an ideal resource for rare disease modeling, especially in pediatric patients (*7*). However, iPSC resources specifically generated from patients with familial renal-predominant Fabry disease remain

extremely limited.

Here, we established and validated an iPSC line from UDCs obtained from a patient with classic Fabry disease, *i.e.*, typical childhood-onset proteinuria and end-stage renal disease, who carried the *GLA* c.1080_1082delTGG (p.Gly361del) variant. This characterized cell line will serve as a valuable tool for exploring the progressive mechanism of renal injury and developing novel therapeutic strategies for refractory Fabry nephropathy.

2. Materials and Methods

2.1. Patient and samples

This study involved a 35-year-old male patient with clinically diagnosed classic Fabry disease who presented with predominant renal manifestations and a family history of the disease. The patient initially developed proteinuria at the age of 12 as the first clinical symptom and underwent a kidney transplant to treat progressive renal failure. Genetic analysis confirmed a pathogenic in-frame deletion variant *GLA* c.1080_1082delTGG (p.Gly361del). Biochemical assays showed significantly decreased plasma α-Gal A activity (0.29 μmol/L/h; reference: 1.5–3.0 μmol/L/h) and markedly elevated plasma GL-3 levels (90.82 ng/mL; reference: < 5 ng/mL). Urine samples were collected with informed consent. This study was approved by the Ethics Committee of Shandong Biomedical Technology Center.

2.2. Culture of urine-derived epithelial cells

Urine-derived epithelial cells were cultured in renal epithelial cell growth medium (REGM; Lonza, Switzerland) supplemented with 50% DMEM, 5% FBS (Gibco, USA), 1× NEAA, and 1× glutamine (Gibco, USA). Medium was refreshed every two days. Cells were passaged at a ratio of 1:3–1:4 after digestion with 0.25% trypsin-EDTA (SparkJade, China).

2.3. Reprogramming and culture of iPSCs

A total of 1.6×10^7 UDCs were nucleofected with the episomal vectors pEP4-EO2S-ET2K and pCEP4-miR302-367 using the Nucleofector 2b Device (Lonza, Switzerland). Transfected cells were seeded on 0.5% Matrigel-coated plates (Corning, USA) and cultured in mTeSR1 medium (STEMCELL Technologies, Canada). Starting on day 10 post-transfection, small molecules including CHIR99021, thiazovivin, PD0325901, and SB431542 (Selleck, China) were supplemented. Putative iPSC colonies were manually selected between days 10-20.

Established iPSCs were maintained in mTeSR1 medium at 37 °C with 5% CO_2 and passaged every three days at a 1:6 ratio using 0.5 mM EDTA-DPBS (SparkJade, China). All cellular validation experiments were performed using stable iPSCs from passage 22.

2.4. qPCR analysis of pluripotency markers

Total RNA was isolated using TRIzol (Invitrogen, USA) and reverse-transcribed with SPARKscript II RT SuperMix (SparkJade, China). qPCR was performed on a LightCycler® 480 II system (Roche, Switzerland) with SYBR Green Master Mix and custom primers (Table 1). Cycling parameters: 94 °C for 2 min, followed by 40 cycles of 94 °C for 15 s, 60 °C for 20 s, and 72 °C for 30 s.

2.5. Alkaline phosphatase (ALP) staining

ALP activity was detected using a BCIP/NBT staining kit (Beyotime Biotechnology, China) according to the manufacturer's instructions. Fixed cells were incubated in working solution in the dark and imaged under bright-field microscopy.

2.6. Immunofluorescence staining

Cells were fixed with 4% paraformaldehyde, permeabilized with 0.3% Triton X-100 (Sigma-Aldrich, USA), and blocked with goat serum (SparkJade, China). Primary antibodies against OCT4 (Immunoway, USA; #YM8272), SSEA-4 (#4755), and TRA-1-81 (#4745) (Cell Signaling Technology, USA) were incubated at 4 °C overnight. After secondary antibody incubation and DAPI counterstaining (SparkJade, China), images were acquired using an Olympus FV4000 confocal microscope (Olympus, Japan) at 20× magnification.

2.7. Single nucleotide polymorphism (SNP) array analysis

Genomic stability was assessed by SNP array analysis

Table 1. Primer sequences for pluripotency-related genes

Target genes	Size (bp)	Sequence (5′-3′)	
		Forward	Reverse
OCT4	164	CCTCACTTCACTGCACTGTA	CAGGTTTTCTTTCCCTAGCT
SOX2	151	CCCAGCAGACTTCACATGT	CCTCCCATTTCCCTCGTTTT
NANOG	237	AAGGTCCCGGTCAAGAAACAG	CTTCTGCGTCACACCATTGC
GAPDH	152	GTGGACCTGACCTGCCGTCT	GGAGGAGTGGGTGTCGCTGT

on the Illumina Asian Screening Array (ASA) (Illumina, USA) using genomic DNA extracted from iPSCs and parental UDCs (TIANGEN, China).

2.8. Mycoplasma detection

Mycoplasma contamination was ruled out using a commercial qPCR detection kit (Beyotime Biotechnology, China). Samples with Ct > 35 in the FAM channel and valid VIC amplification were considered to be negative.

2.9. Short tandem repeat (STR) analysis and Sanger sequencing

Genomic DNA from iPSCs clone LJ3212 and parental UDCs line LJ3181 was subjected to STR profiling and Sanger sequencing to confirm genetic identity and variant retention.

2.10. Confirmation of exogenous vector integration

PCR was amplified using vector-specific primers (Table 2) and SparkHiFi Max Master Mix (SparkJade, China). The thermal program consisted of 98 °C for 30 s, 30 cycles of 98 °C for 10 s, gradient annealing for 10 s, 72 °C for 30 s, and a final extension at 72 °C for 5 min. Transfected UDCs served as positive controls. PCR products were separated on 2.5% agarose gels and imaged using a ChemiDoc XRS+ system (Bio-Rad, USA).

2.11. *In vitro* trilineage differentiation

iPSCs were induced into three germ layers using STEMdiff™ differentiation kits (STEMCELL Technologies, Canada). Endodermal and mesodermal differentiation lasted 4–6 days, and ectodermal differentiation lasted 7 days. Germ layer markers AFP (Immunoway, USA), α-SMA (#19245), and β3-tubulin (#5568) (Cell Signaling Technology, USA) were detected using immunofluorescence.

3. Results and Discussion

UDCs at passage 3 displayed a typical epithelioid morphology (Figure 1A). After episomal reprogramming, emerging iPSC colonies exhibited a compact morphology, clear boundaries, and a high nuclear-cytoplasmic ratio consistent with pluripotent stem

Table 2. Primer sequences for detection of episomal reprogramming vectors

Target genes	Size (bp)	Sequence (5′-3′)	
		Forward	Reverse
OCT4	657	AGTGAGAGGCAACCTGGAGA	AGGAACTGCTTCCTTCACGA
SOX2	534	CCCAGCAGACTTCACATGT	CCTCCCATTTCCCTCGTTTT
KLF4	401	AAGGTCCCGGTCAAGAAACAG	CTTCTGCGTCACACCATTGC
SV40LT	491	TGGGGAGAAGAACATGGAAG	AGGAACTGCTTCCTTCACGA
oriP	544	TTCCACGAGGGTAGTGAACC	TCGGGGGTGTTAGAGACAAC
EBNA-1	666	ATCGTCAAAGCTGCACACAG	CCCAGGAGTCCCAGTAGTCA
microRNA302/367 cluster	322	TTTCCAAAATGTCGTAATAACCCCG	CTCCCAAAGAGTCCTGTTCTGTCCT
GAPDH	152	GTGGACCTGACCTGCCGTCT	GGAGGAGTGGGTGTCGCTGT

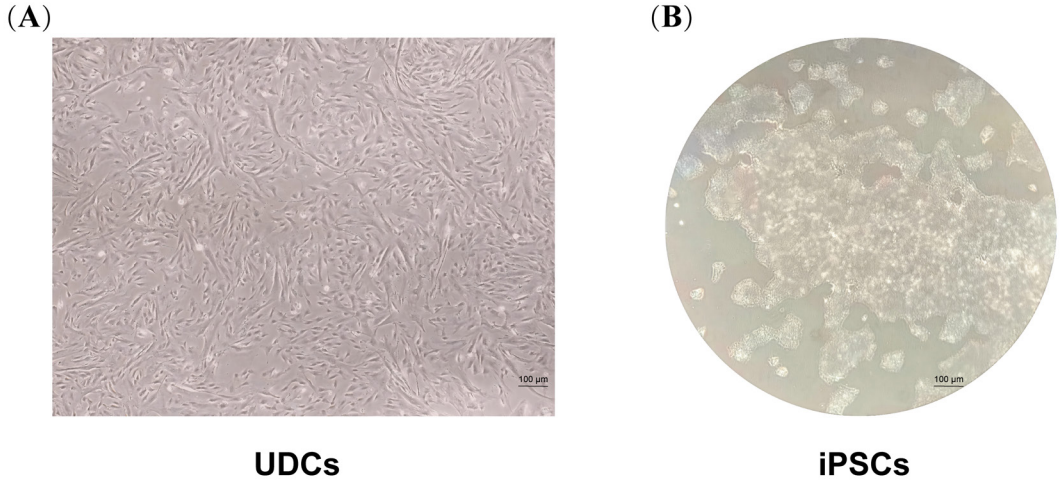

Figure 1. Morphological characterization of urine-derived cells (UDCs) and patient-derived induced pluripotent stem cells (iPSCs) derived from a patient with renal-predominant Fabry disease. (A) Phase-contrast image of UDCs at passage 3, showing a typical epithelial-like morphology. **(B)** Phase-contrast image of the established iPSCs line at passage 22, showing compact, well-defined colonies with a high nuclear-to-cytoplasmic ratio and typical human pluripotent stem cell morphology. Magnification: 4×.

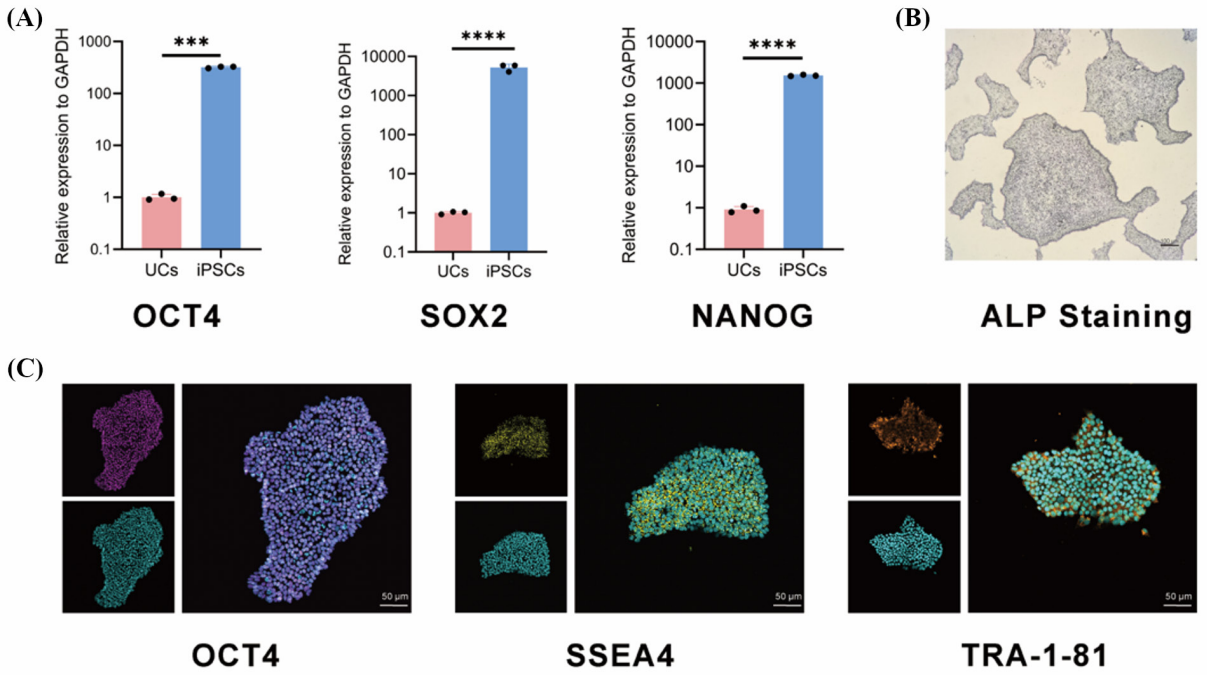

Figure 2. Pluripotency validation of patient-derived iPSCs. (A) Relative mRNA expression of pluripotency genes OCT4, SOX2, and NANOG in passage 3 UDCs and passage 22 iPSCs. Unpaired Student's *t*-test, ***$p < 0.001$; ns, non-significant. **(B)** Alkaline phosphatase (ALP) staining of passage 22 iPSCs. 4× magnification. **(C)** Immunofluorescence for pluripotency markers OCT4, SSEA-4, and TRA-1-81; nuclei counterstained with DAPI. 20× magnification.

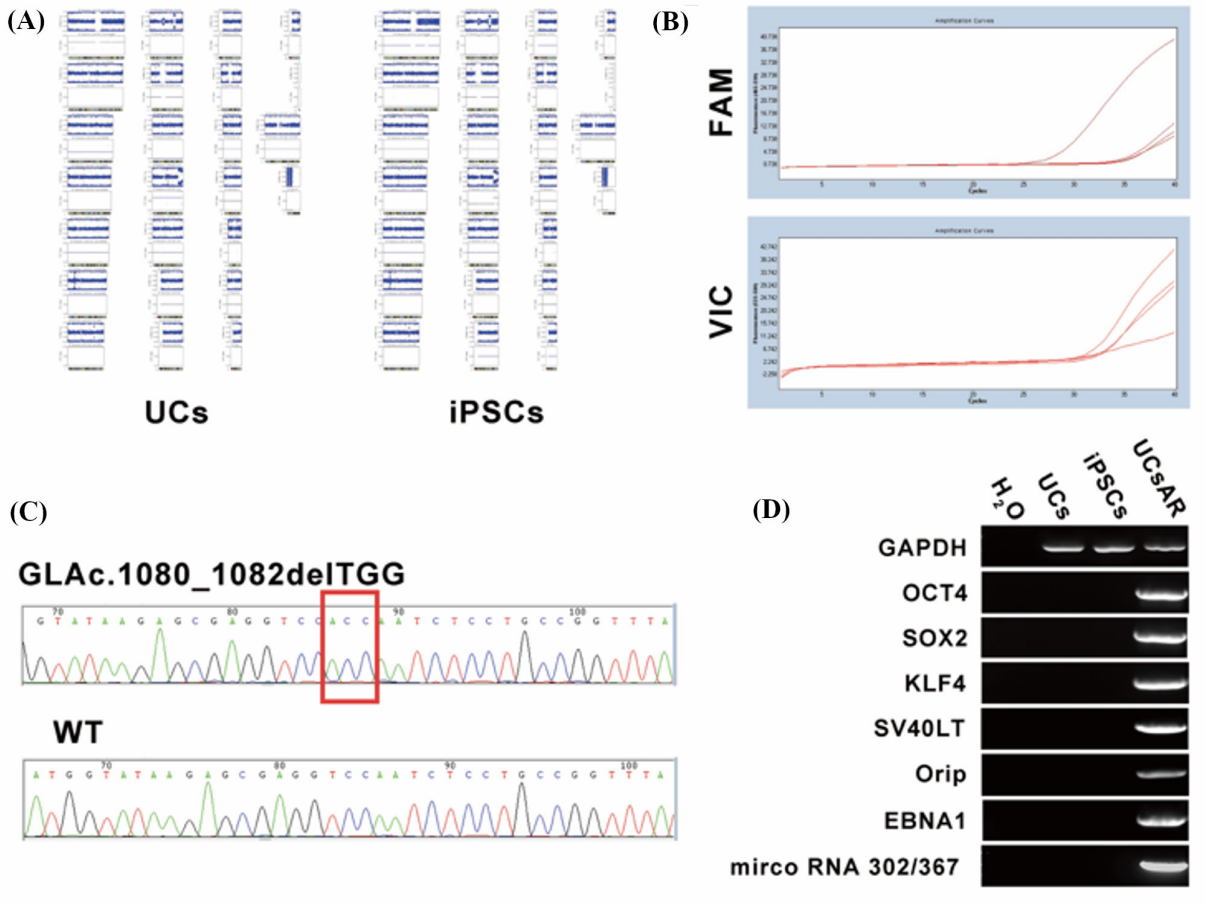

Figure 3. Genetic characterization and quality control of the established iPSC line. (A) SNP typing profiles of parental UDCs and the established iPSCs line. **(B)** qPCR detection of mycoplasma in iPSCs. **(C)** Comparison of Sanger sequencing chromatograms of the patient-derived iPSC line and WT sequences. **(D)** PCR analysis of exogenous episomal vector clearance.

cell characteristics (Figure 1B). Approximately 18 colonies were obtained from 1.6×10^7 UDCs. qPCR results confirmed significantly elevated expression of the endogenous pluripotency genes OCT4, SOX2, and NANOG in iPSCs compared to parental UDCs (Figure 2A). Positive ALP staining (Figure 2B) and immunoreactivity for OCT4, SSEA-4, and TRA-1-81 further verified pluripotency (Figure 2C). STR analysis confirmed full genetic matching between iPSCs and donor UDCs (Supplementary Table S1, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=309*). SNP array results demonstrated genomic stability without pathogenic copy number variations or chromosomal aberrations (Figure 3A). Mycoplasma testing was negative (Figure 3B). Sanger sequencing confirmed faithful retention of the *GLA* c.1080_1082delTGG variant in iPSCs (Figure 3C). No residual integration of an episomal vector was detected (Figure 3D). Moreover, the iPSC line exhibited a robust trilineage differentiation capacity, confirmed by specific expression of AFP, α-SMA, and β3-tubulin (Figure 4A-C).

The established iPSC line satisfies all standard criteria for a qualified human pluripotent stem cell resource. Renal injury is the most critical prognostic factor in Fabry disease, and progressive proteinuria and end-stage renal failure are the leading causes of severe clinical outcomes in affected patients (*8*). Fabry disease presents with prominent genotype-phenotype heterogeneity, and rare in-frame deletion variants of the *GLA* gene are closely associated with progressive renal pathological damage (*9*). Based on our previously unpublished data from a clinical cohort, the *GLA* c.1080_1082delTGG (p.Gly361del) variant is extremely rare in the Chinese population, with the vast majority of identified cases being sporadic. Notably, the patient in this study harbors

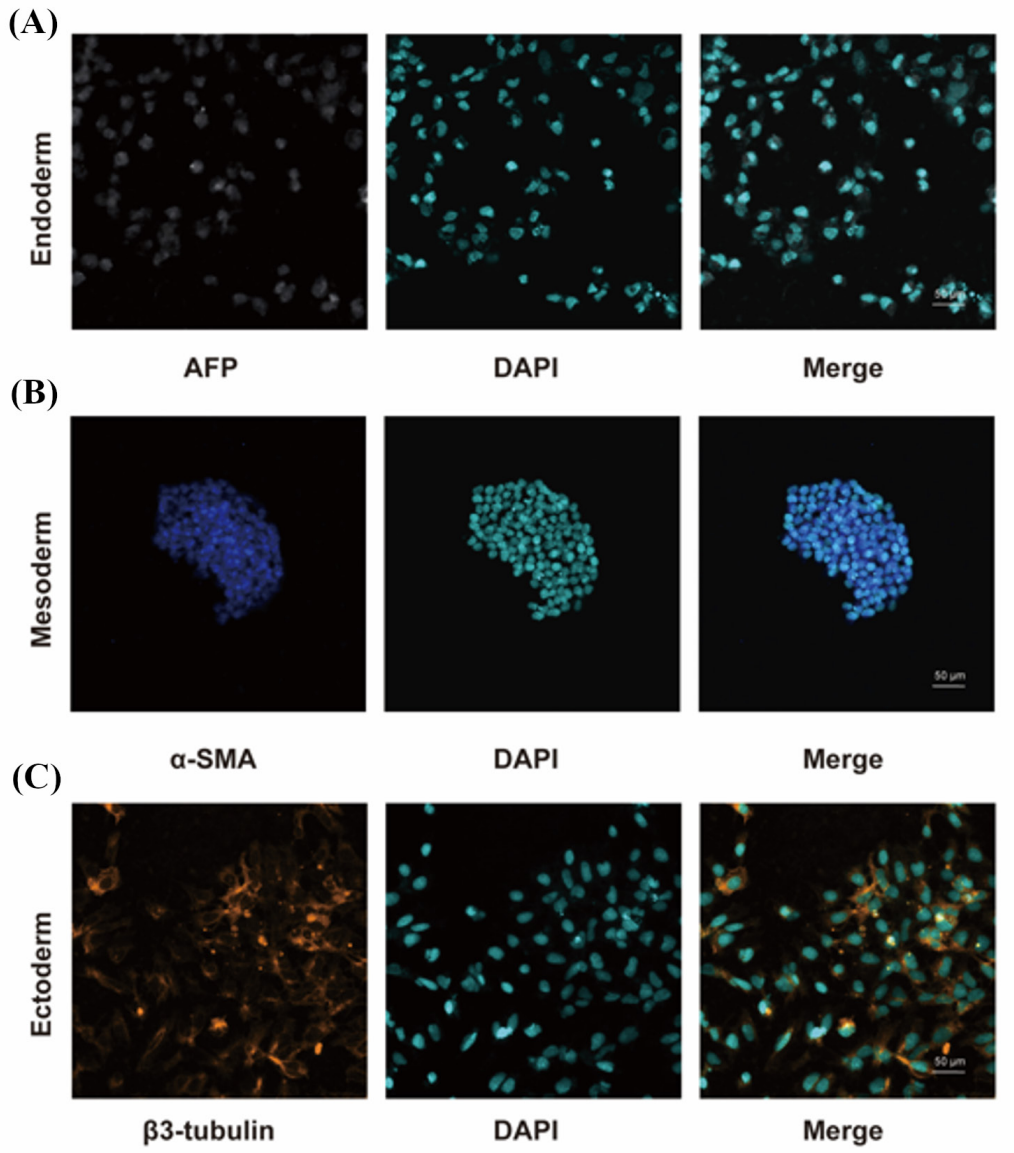

Figure 4. Immunofluorescence analysis of trilineage differentiation markers in iPSCs. (A) Endodermal marker AFP (white). **(B)** Mesodermal marker α-SMA. **(C)** Ectodermal marker β3-tubulin. Nuclei were counterstained with DAPI.

this rare variant and manifests a typical familial renal-dominant Fabry phenotype characterized by childhood-onset proteinuria and progressive renal failure requiring transplantation. This represents an exceedingly rare clinical subtype in Chinese Fabry cohorts. More interestingly, we previously created another patient-derived iPSC line harboring an in-frame deletion in the same *GLA* codon region, it mainly exhibited neuropathic pain (*10*). Therefore, the unique patient-derived iPSC line that was established in this study fills the gap in cell resources for rare familial renal Fabry disease among the Chinese population. That line provides a specific and valuable cellular tool for exploring the molecular mechanism of progressive renal injury induced by the p.Gly361del variant, it elucidates the genotype-renal phenotype correlation of this rare mutation, and it facilitates subsequent mechanistic research and targeted therapeutic screening for intractable Fabry nephropathy.

This study is limited to cell line construction and basic characterization; future studies need to examine further renal lineage differentiation and perform functional phenotyping. In conclusion, this well-established iPSC line enriches the limited bank of patient-specific cell resources for Fabry disease and it provides a powerful platform for future mechanistic studies and therapeutic screening for familial renal-predominant Fabry disease.

Acknowledgements

Thanks to the patient and his family for their participation and support in this study. Thanks to the Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China, for the plasmids offered in this work. We acknowledge all researchers contributing to experimental procedures and data analysis.

Funding: This research was supported by the Academic Promotion Program of Shandong First Medical University (LJ001).

Conflict of Interest: The authors have no conflicts of interest to disclose.

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Received March 7, 2026; Revised June 21, 2026; Accepted June 24, 2026.

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Released online in J-STAGE as advance publication July 8, 2026.