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Lab resource: Stem Cell Line

Generation of a human iPSC line, INMi002-A, carrying the most prevalent USH2A variant associated with Usher syndrome type 2

Carla Sanjurjo-Soriano, Nejla Erkiliç, Gaël Manes, Gregor Dubois, Christian P. Hamel, Isabelle Meunier, Vasiliki Kalatzis

Abstract

We generated an induced pluripotent stem cell (iPSC) line using dermal fibroblasts from a patient with Usher syndrome type 2 (USH2). This individual was homozygous for the most prevalent variant reported in the USH2A gene, c.2299delG localized in exon 13. Reprogramming was performed using the non-integrative Sendai virus reprogramming method and the human OSKM transcription factor cocktail under feeder-free culture conditions. This iPSC line will be an invaluable tool for studying the pathophysiology of USH2 and for testing the efficacy of novel treatments.

Resource table

| Unique stem cell line identifier | INMi002-A |
| Alternative name(s) of stem cell line | USH2A-USHiPSC |
| Institution | Institute for Neurosciences of Montpellier, Montpellier, France |
| Contact information of distributor | Vasiliki Kalatzis, vasiliki.kalatzis@inserm.fr |
| Type of cell line | iPSC |
| Origin | Human |
| Age: 59 years old | Sex: Female |
| Ethnicity: Caucasian | |
| Cell Source | Dermal fibroblasts |
| Clonality | Clonal |
| Method of reprogramming | Non-integrative Sendai virus vectors |
| Genetic modification | Yes |
| Type of modification | Congenital mutation |
| Associated disease | Usher Syndrome type II |
| Gene/locus | USH2A, 1p41 |
| Name of transgene or resistance | N/A |
| Inducible/constitutive system | N/A |
| Date archived/stock date | December 2017 |
| Cell line repository/bank | https://hpscreg.eu/cell-line/INMi002-A |
| Ethical approval | Regional committee: CPP Southern Mediterranean I (2014-A00549-38) National committee: ANSM (140549-62) |

Resource utility

We established an iPSC line from a patient with Usher syndrome type 2 (USH2), characterized by retinitis pigmentosa and hearing loss, homozygous for the recurrent USH2A variant, c.2299delG. This line will allow modelling of the USH2 retinal and inner ear defects, and the development of novel gene and cell therapies.

Resource details

Mutations in USH2A cause a syndromic inherited retinal dystrophy (IRD) known as Usher syndrome type 2 (USH2), which is characterized by progressive hearing and vision loss. In addition, mutations in USH2A also cause a wide majority of autosomal recessive retinitis pigmentosa (RP) cases (Kremer et al., 2006). In the present study, we have generated an iPSC line from a patient with USH2 carrying a homozygous variant in exon 13 of the USH2A gene, c.2299delG; p.Glu767Serfs*21. This is a recurrent mutation originating from a common ancestor. Therefore, c.2299delG is observed more frequently in the population compared to the > 600 USH2A mutations identified (Lenassi et al., 2015), conferring a great deal of interest from a clinical perspective.

Human dermal fibroblasts were isolated and cultured from a patient skin biopsy sample and reprogrammed using an integration-free method, the CytoTune™-iPS 2.0 Sendai Reprogramming Kit. This method is based on transient overexpression of the four Yamanaka factors: OCT3/4, SOX2, KLF4 and c-MYC (Takahashi et al., 2007). The morphology of the iPSC line generated displayed a typical colony.
Fig. 1. Characterization of the INM002-A (USH2A-USH-iPSC) line.
appearance comprised of tightly packed cells (Fig. 1A). The absence of the Sendai reprogramming vectors in the INMi002-A clones was confirmed using reverse transcription (RT)-PCR. As a negative control, non-transduced patient fibroblasts (Fibro) did not carry the Sendai vectors. By contrast, the transduced fibroblasts (Fibro + SeV) expressed all three vectors that were used for reprogramming. Due to their non-integrative nature, the INMi002-A cell line had lost these vectors by passage 12 (P12) (Fig. 1B). The iPSC line generated showed a normal 46,XX karyotype at P12, which excluded major chromosomal abnormalities as a result of the reprogramming process (Fig. 1C).

Using real time polymerase chain reaction (qPCR), we showed expression of the endogenous pluripotency markers, NANOG, OCT3/4 and LIN28a in the INMi002-A cell line when compared to non-transduced (Fibro) or transduced (Fibro + SeV; Fig. 1D) fibroblasts. The pluripotency state was further confirmed by immunofluorescence staining of NANOG (Fig. 1E), OCT3/4 (Fig. 1F) and SOX2 (Fig. 1G). The ability of the INMi002-A cell line to give rise to the three embryonic cell layers was determined by spontaneous differentiation of the iPSC into embryoid bodies (EBs). EBs were plated onto Matrigel-coated dishes and the expression of the three germ layer markers, Glial Fibrillary Acidic Protein (GFAP; ectoderm; Fig. 1H), Smooth Muscle Actin (SMA; mesoderm; Fig. 1I) and α-Fetoprotein (AFP; endoderm; Fig. 1J) was verified by immunofluorescence staining. We verified the presence of the homozygous causative mutation in exon 13 of USH2A (c.2299delG) in the INMi002-A iPSC, as compared to wild type iPSC, by Sanger sequencing (Fig. 1K). The identity of the patient iPSC line was confirmed by microsatellite PCR analysis in comparison to fibroblasts of the same individual and wild type iPSC (available with the authors). Lastly, the generated INMi002-A cell line was confirmed to be free of mycoplasma contamination (Supplementary File 1).

### Materials and methods

#### Human dermal fibroblast cell culture

Dermal fibroblasts derived from a skin biopsy were cultured in AmnioMAX C100 basal media with GlutaMAX (Gibco) containing 10% decomplemented foetal calf serum (FCS; Lonza), 1% penicillin-streptomycin-amphotericin B (Lonza) and 2% AmnioMax-C100 supplement (Gibco).

#### iPSC generation

Fibroblasts were seeded and reprogrammed using the integration-free CytoTune™-iPS 2.0 Sendai Reprogramming Kit as previously described (Torriano et al., 2017). iPSC colonies were dissociated with Accutase (Stemcell Technologies) and seeded on ultra-low attachment dishes for 2 days in E8 containing 0.2μg/ml bisBenzimide (Sigma-Aldrich). Cells were observed with a Zeiss ApoTome 2 Upright wide-field microscope.

### In vitro differentiation assay

iPSC were dissociated with Accutase (Stemcell Technologies) and seeded on ultra-low attachment dishes for 2 days in E8 containing...
Y27632 StemMACS. At day 3, the medium was changed to DMEM/F12 (Gibco) supplemented with 20% Knockout serum replacement (Gibco), 1% penicillin-streptomycin (Gibco), 1% GlutaMax (Gibco), and 55mM β-mercaptoethanol (Gibco). At day 7, embryoid bodies were seeded onto Matrigel-coated wells and cultured in the same medium for a further 10 days before staining.

**Mutation analysis**

Genomic DNA was isolated using the DNeasy Blood & Tissue Kit (Qiagen) and PCR-amplified using USH2A-specific primers (Table 2). The dNTPs were removed using the ExoSAP-IT PCR Clean-up kit (GE Healthcare) and the amplicon sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction kit V3.1 on an Applied Biosystems 3130xL Genetic Analyzer.

**Microsatellite PCR analysis**

Genomic DNA was amplified using primers for informative markers (Table 1). The PCR products were mixed with Genescan 400HD ROX size standard and subsequently analyzed on an Applied Biosystems 3130xl Genetic Analyzer.

**Mycoplasma analysis**

Mycoplasma detection was performed on cell culture supernatant using the MycoAlert Mycoplasma Detection Kit (Lonza), according to the manufacturer's instructions, and a CLARIOstar microplate reader (BMG Labtech).

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.11.007.

**References**


