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Generation of two iPSC lines from patient with Mucopolysaccharidosis IV B type and autosomal recessive non-syndromic hearing loss 12

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ABSTRACT

We generated two human induced pluripotency stem cell (hiPSC) lines, RCMGi011-A and 11-B, from skin fibroblast from patient with Mucopolysaccharidosis IV B type and autosomal recessive non-syndromic hearing loss 12 using non-integrating, viral CytoTune™-iPS 2.0 Sendai Reprogramming Kit. We verified variant c.808 T > G and insertion in *GLB1* gene, as well as two mutations, c.6992 T > C and c.805C > T, in *CDH23* gene which lead to autosomal recessive hearing loss type 12. We have demonstrated normal karyotype of hiPSCs and capacity for cell differentiation into three germ layers.

Resource Table.

Unique stem cell lines identifier	RCMGi011-A RCMGi011-B
Alternative name(s) of stem cell lines	P10L1, P10L2
Institution	Research Centre for Medical Genetics (RCMG)
Contact information of distributor	MD Ekaterina Kondrateva, ekaterina.kondratyeva@gmail.com
Type of cell lines	iPSC
Origin	Human
Additional origin info required	Age: 9 Sex: man Ethnicity: Caucasian
Cell Source	Skin fibroblast
Clonality	Clonal
Method of reprogramming	Sendai virus (4 transgenes: OCT3/4, SOX2, KLF4, and c-MYC)
Genetic Modification	NO
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	PCR
Associated disease	Mucopolysaccharidosis IV B type and autosomal recessive non-syndromic hearing loss 12
Gene/locus	Gene: *GLB1* Locus: 3p22.3 Mutation: NM_000404.4(GLB1):c.808 T > G (p.Tyr270Asp) *NPM1* pseudogene: NC_000003.11: g.33100046_33100061 Gene: *CDH23* Locus: 10p22.1 Mutation: NM_022124.6 (CDH23) c.6992 T > C (p.Val2331Ala) Locus: 10p22.1 Mutation: NM_022124.6 (CDH23) c.805C > T (p.Arg269Trp)
Date archived/stock date	Jun-21
Cell line repository/bank	https://hpscreg.eu/cell-line/RCMGi011-A https://hpscreg.eu/cell-line/RCMGi011-B
Ethical approval	This study was approved by FSBSI RCMG Ethics Committee (Protocol from 2015 to 5/3).

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1 panel A, scale bar 200 µm
Phenotype	Immunocytochemistry	OCT4, SSEA4, SOX2 and NANOG	Fig. 1 panel C, scale bar 200 µm
	Quantitative analysis (Immunocytochemistry counting)	**RCMGi011-A** SSEA: 100% NANOG: 100% OCT4: 100% SOX2: 99,8% **RCMGi011-B** SSEA: 99,1% NANOG: 100% OCT4: 99,2% SOX2: 99,6%	Fig. 1 panel B
Genotype	Karyotype (G-banding) and resolution	46XY, Resolution 450	Fig. 1 panel F
Identity	Microsatellite PCR (mPCR) OR	Not performed	Not performed
	STR analysis	Tested 19 loci, all matched	submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous	Fig. 1 panel G
	Southern Blot OR WGS	Not performed	Not performed
Microbiology and virology	Mycoplasma	Mycoplasma testing by end point PCR. Negative	Supplementary Fig. 2
Differentiation potential	Embryoid body formation	ectodermal marker (beta III tubulin) mesodermal marker (brachyury), endodermal marker (FOXA2)	Fig. 1 panel D, scale bar 200 µm
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	Not performed
Genotype additional info (OPTIONAL)	Blood group genotyping	Not performed	Not performed
	HLA tissue typing	Not performed	Not performed

1. Resource utility

IPSC cell lines RCMGi011-A, RCMGi011-B were obtained from fibroblast of 9-years-old boy from Russian non-consanguineous family with rare combination of disease: mucopolysaccharidosis IV B type (MBD) and autosomal recessive non-syndromic hearing loss 12. The study of this unique case could be attractive for research purpose. Table 1.

Mucopolysaccharidosis IV B type (OMIM #253010) also known as Morquio syndrome B is rare autosomal recessively disease, with prevalence 1:250.000–1:1.000.000. (Pedersen et al., 2022). Only 25 pathogenic variants in Human Gene Mutation Database (HGMD) are known. Disease caused by a deficiency of lysosomal hydrolase β-galactosidase, which causes keratin sulfate accumulation in connective tissue and leads to nodular deformities in the skeleton and joint system. MBD can also affect the respiratory and cardiovascular systems, spinal cord, eyesight, and hearing, as well as dental health. A multidisciplinary approach to diagnosis and treatment is required due to multiorgan lesion.

Autosomal recessive non-syndromic hearing loss 12 (OMIM #601386) has a prevalence of 1:1000 worldwide. Disease associated with missense mutations in cadherine-23 gene CDH23, which functions as calcium-dependent cell–cell adhesion glycoproteins Kniffin, (2012, March 19).

Clinical features include sensorineural hearing loss, so cochlear implant was placed to patient in 8 months about birth. Disease manifested in 6 years and 10 months with gait disturbance after an acute upper respiratory infection episode. At the time of examination clinical feature of «pure» MBD were observed and decrease activity β-Galactosidase (0,4 ng/ml/h (ref. 2–30 ng/ml/h)).

Firstly, to establish the molecular genetic diagnosis whole-exome sequencing was performed, which revealed two variants in the *CDH23* gene (c.6992T>C (p.Val2331Ala) and c.805C>T (p.Arg269Trp)) associated with sensorineural hearing loss and the missense variant in the *GLB1* gene (c.808T>G (p.Tyr270Asp)). To identify the second causative variant in *GLB1*, whole genome sequencing was performed and the insertion of a mobile genetic element from the class of retrotransposons in the *GLB1* intron 5 was revealed. The insertion represents the processed pseudogene*NPM1*, and according to ACMG criteria, is probably pathogenic (Bychkov et al., 2022). All mentioned mutations were confirmed by Sanger sequencing in obtained cell lines (Fig. 1 panel E ang G).

2. Resource details

The patient's skin fibroblasts were reprogrammed into RCMGi011-A and 11-B by using non-integrating viral CytoTune™-iPS 2.0 Sendai Reprogramming Kit. The RCMGi011-A and 11-B cells correspond the normal iPSCs morphology (Fig. 1A). G-banding analysis exhibited a normal karyotype (46,XY) in the cell lines (Fig. 1F). The pluripotency of the generated hiPSC lines was verified by immunocytochemical staining with pluripotency markers OCT4, SSEA4, NANOG, and SOX2 (Fig. 1B, C). The RCMGi011-A and 11-B hiPSC lines have the same heterozygous compound state NM_000404.4(GLB1):c.808 T > G (p.Tyr270Asp) and insertion NC_000003.11:g.33100046_33100061 inherited from proband's parents and confirmed in the patient's samples (Fig. 1E and G). Embryonic body formation assessment demonstrated that the hiPSC lines expressed all three germ layer markers, ectodermal marker (beta III tubulin), mesodermal marker (brachyury) and endodermal marker (FOXA2), by immunocytochemical staining (Fig. 1D). Lines RCMGi011-A at passage 18 and RCMGi011-B at passage 39 were mycoplasma free (Evrogen, Russia) (Supplementary Fig 2). PCR for confirmation of reprogramming factors elimination in the RCMGi011-A and 11-B hiPSC lines at passages 34 and 29, respectively, was performed. Short tandem repeat (STR) analysis showed that allele loci of RCMGi011-A and 11-B were consistent with fibroblasts (Direct PCR Amplification Kit (Applied Biosystems)). Detailed information on STR analysis is presented in the supplementary data. Cell lines were obtained and collected in Moscow Branch of Biobank «All-Russian Collection of biological Samples of Hereditary Diseases».

3. Materials and methods

3.1. Reprogramming and hiPSC maintenance

Human iPSCs were generated by reprogramming fibroblasts using a CytotuneiPS™-iPS 2.0 Sendai Reprogramming Kit (Life Technologies) according to the manufacture's recommendation. Generated iPSC lines were cultured onto Matrigel solution (Corning) coated plates with TeSR™-E8™ medium (Stemcell Technologies). The culture medium was changed daily.

3.2. Karyotyping

The cultured cells at 12 passage and confluency ~ 70% were treated by 0,1 µg/mL demecolcine (Sigma) and then harvest by trypsinization,

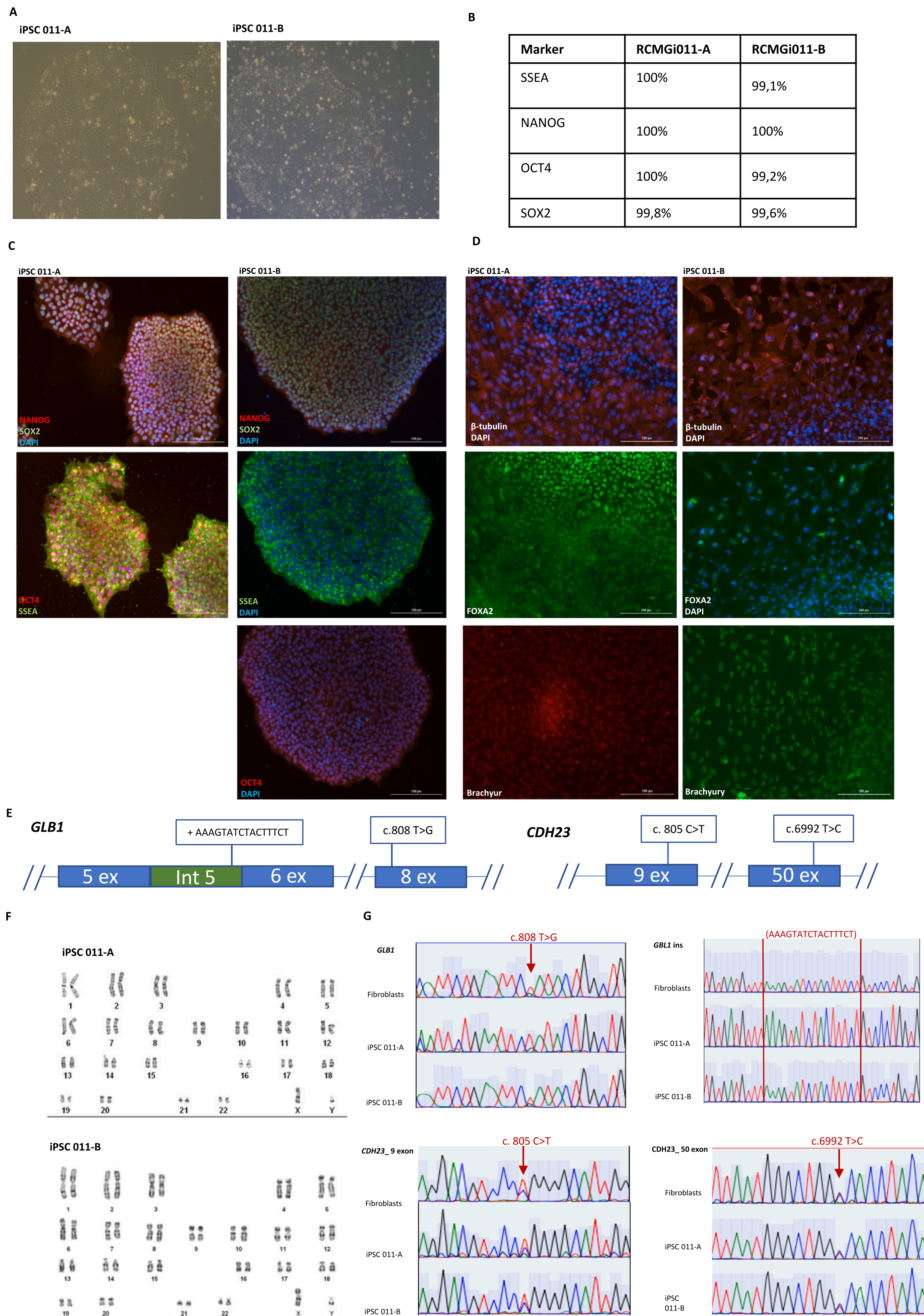

Fig. 1. Characterization of RCMGi011-A and 11-B cell lines.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	SSEA4 Monoclonal Antibody (MC813-70), Mouse	1:100	Thermo Fisher Scientific, Cat #: 41–4000	RRID:AB_2533506
	Anti-Oct4 antibody, Rabbit	1:100	Abcam, Cat #: ab18976	RRID:AB_444714
	Nanog Polyclonal Antibody, Rabbit	1:100	Thermo Fisher Scientific, Cat #: PA1-097X	RRID:AB_2539868
	Anti-SOX2 antibody [9–9–3], Mouse	1:200	Abcam, Cat #: ab79351	RRID:AB_10710406
Differentiation Markers	Brachyury Monoclonal Antibody (X1AO2), Mouse	1:100	eBioscience™, Cat#:2028029	RRID: AB_2573016
	Anti-beta III Tubulin antibody [2G10], Mouse	1:100	Abcam, Cat #: 78,078	RRID:AB_2256751
	Anti-FOXA2 antibody [EPR4465], Rabbit	1:400	Abcam, Cat #: ab108396	RRID:AB_10863255
Secondary antibodies	Goat anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	1:300	Abcam, Cat #: A11037	RRID:AB_2534095
	Goat anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:150	Thermo Fisher Scientific, Cat# A-11032	RRID:AB_2534091
	Goat Anti-Mouse IgG (H + L) Highly Cross-adsorbed Antibody, Alexa Fluor 594	1:200	Invitrogen, #A11002	RRID:AB_2534091
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Targeted mutation analysis	*GLB1*:c.808 T > G	244 bp	GGCATTGGAAGGTCATAAGTCGAACACGGTACTTCACTGTGA	
	NPM1 pseudogene	285 bp	GTCTGTGTAAATCTAGACGGCTGTCTCATGTCCATGTCCATCGAATC	
	CDH23: c.805C > T	650 bp	GGTCAGGTCAATCCTCAGGCCCTCCTTCTTGGCCTTCCTG	
	CDH23: c.6992 T > C	651 bp	ATCTGTTCTTCCGTGGTGGTCCTCAGACCTTGGACAGCGG	
Primers for Sendai virus elimination	SeV	181 bp	GGATCACTAGGTGATATCGAGCACCAGACAAGAGTTTAAGAGATATGTATC	
	KOS	528 bp	ATGCACCGCTACGACGTGAGCGCACCTTGACAATCCTGATGTGG	
	Klf4	410 bp	TTCCTGCATGCCAGAGGAGCCCAATGTATCGAAGGTGCTCAA	
	c-Myc	532 bp	TAACTGACTAGCAGGCTTGTCGTCCACATACAGTCCTGGATGATGATG	

hypotonized 13 min in 0,075 M KCl at 37 °C and fixed using standard cytogenetic procedures. After that followed by the preparation of metaphase slides stained with DAPI and contrasted with 0,3 μg/mL Actinomycin (Serva). Finally, 15–20 metaphase images were analyzed according to ISCN 2020 nomenclature.

3.3. Mutation sequencing

Sanger sequencing was used for confirm mutations and duplication in samples. DNA from the original fibroblasts and the resulting iPSCs was isolate using ExtractDNA Blood & Cells kit (Evrogen) according to manufacturer's protocol. The loci of *GLB1* and *CDH23* genes with mutations were amplified using original primers for PCR (Table 2). Amplifications using Fusion DNA polymerase (NEB) for detection of duplication in *GLB1* gene and by Taq DNA polymerase (Evrogen) for verification mutations in *GBL1* and *CDH23* genes were performed in ProFlex PCR system (Applied Biosystems). PCR conditions were as follows: 95 °C for 3 min; 35 cycles of 95 °C for 30 sec, 60 °C for 50 sec, and 72 °C for 60 sec; 72 C for 5 min. ABI Prism 3130XL Genetic Analyzer (Applied Biosystems) was used for Sanger sequencing. Cycle parameters were as follows: 95 °C for 3 min; 25 cycles of 95 C for 15 sec, 50 C for 15 sec, and 60 C for 2 min.

3.4. Immunocytochemistry

IPSCs were fixed with 4% paraformaldehyde solution and blocking with 0.25% Triton X-100 in PBS and 1% bovine serum albumin (BSA) (Gibco), respectively. Fixed cells were incubated with primary antibodies at room temperature (RT) one hour and then with secondary antibodies at RT for 1 h in the dark, diluted in 1% BSA, respectively. Nuclei was stained with DAPI (Abcam) at RT for 10 min (Table 2). Fluorescent images were captures with Lionheart FX Automated Microscope (BioTek). Amount of positive cell were analyzed by open-source software CellProfiler 3.0.0 (Fig. 1, B).

3.5. EB-formation

For embryoid body formation the iPSCs after the passage 10 were detached using Versen solution, then dissociated into single cell suspension and transferred to ultra-low attachment plate with E8 medium with 5 mkM Y27632. On day 4, the medium was changed with new medium consisting of 1:1 of TeSR-E8 and ES medium (Advanced DMEM/F12 (Gibco), 20% KOSR (Gibco), 1% GlutaMAX, 1% MEM NEAA, 1% penicillin/streptomycin). On day 7, the medium was changed with EB medium consisting of 1⁄2 vol of ES medium and 1⁄2 vol of fibroblasts medium without FBS. Thereafter, the medium was renewed every 2–3 days with a stepwise increase in the percentage of FBS from 1 to 10%. On day 28, EBs were transferred onto gelatin-coated plates in EB medium with 10% FBS. On next step, EBs were cultured during 14 days, and after all the expression of markers was verified by immunocytochemical staining (Table 2).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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.2023.103183.

References

Bychkov, I., Kuznetsova, A., Baydakova, G., Gorobets, L., Kenis, V., Dimitrieva, A., Filatova, A., Tabakov, V., Skoblov, M., Zakharova, E., 2022. Processed pseudogene insertion in GLB1 causes Morquio B disease by altering intronic splicing regulatory landscape. Npj Genomic Medicine 7 (1), 44. https://doi.org/10.1038/s41525-022-00315-y.

Cassandra L Kniffin. (2012, March 19). *DEAFNESS, AUTOSOMAL RECESSIVE 12.* Online Mendelian Inheritance in Man, OMIM. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD) 04/01/2023. World Wide Web URL: https://www.Omim.org/entry/601386.

Pedersen, J.J., Duno, M., Wibrand, F., Hammer, C., Krag, T., Vissing, J., 2022. β-Galactosidase deficiency in the GLB1 spectrum of lysosomal storage disease can present with severe muscle weakness and atrophy. JIMD Reports 63 (6), 540–545. https://doi.org/10.1002/jmd2.12324.