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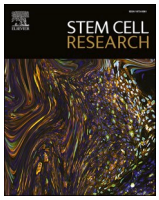

Lab Resource: Multiple Cell Lines

Generation of two iPSC lines from Mowat-Wilson syndrome patients carrying heterozygous *ZEB2* mutations

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A B S T R A C T

ZEB2 is a protein-coding gene belonging to a very restricted family of transcription factors. ZEB2 acts mainly as a transcription repressor, is expressed in various tissues and its role is fundamental for the correct development of the nervous system. The best-known clinical picture associated with *ZEB2* mutations is Mowat-Wilson syndrome, caused mostly by haploinsufficiency and characterized by possible multi-organ malformations, dysmorphic features, intellectual disability, and epilepsy. In this study we report the generation of IGGi004-A and IGGi005-A, iPSC clones from two patients carrying different heterozygous mutations in *ZEB2*, which can be used for disease modelling, pathophysiological studies and therapeutics testing.

1. Resource table

Unique stem cell lines identifier	IGGi004-A IGGi005-A
Alternative name(s) of stem cell lines	N/A
Institution	IRCCS Istituto Giannina Gaslini
Contact information of distributor	Paolo Scudieri, paolo.scudieri@unige.it
Type of cell lines	iPSC
Origin	human
Additional origin info required	IGGi004-A Age: 6 Sex: F Ethnicity: Caucasian IGGi005-A Age: 15 Sex: F Ethnicity: Caucasian
Cell Source	PBMC
Clonality	Clonal
Method of reprogramming	Sendai virus mediated delivery of OCT4, SOX2, KLF4 and c-MYC
Genetic Modification	Yes
Type of Genetic Modification	Hereditary
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	PCR
Associated disease	Mowat-Wilson syndrome
Gene/locus	*ZEB2*/2q22.3 IGGi004-A: p.Leu894Phe Fs*36 - c.2682del(A) IGGi005-A: p.R218R fsX21 - c.653-654insACCG
Date archived/stock date	2023–11-28
Cell line repository/bank	https://hpscreg.eu/user/cellline/edit/IGGi004-A https://hpscreg.eu/user/cellline/edit/IGGi005-A
Ethical approval	University of Milan, 11/23, 18–01-2023

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2. Resource utility

Mowat-Wilson syndrome is mostly caused by germline *ZEB2* haploinsufficiency and is characterized by possible multi-organ malformations, dysmorphic features, intellectual disability, and epilepsy. IGGi004-A and IGGi005-A are iPSC clones generated from PBMCs collected from two patients carrying different heterozygous mutations in *ZEB2*. These iPSC lines may lay a basis for the development of brain-

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specific or multi-organ *in vitro* models of *ZEB2* haploinsufficiency to be used for pathophysiological studies and therapeutics testing. See (Table 1).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Immunofluorescent staining	iPSC line express pluripotency markers: OCT4, SSEA4, TRA-1–60 and SOX2	Fig. 1 panel F
	Quantitative analysis	Positive for pluripotency markers > 97 % including OCT4, SOX2, SSEA4, and TRA-1–60	Fig. 1 panel E
Genotype	Whole genome array (Karyostat + Assay). Resolution 1–2 Mb	Normal karyotype 46, XX	Fig. 1 panel D
Identity	Microsatellite PCR (mPCR)	N/A	N/A
	STR analysis	10 markers tested-matched	submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sanger Sequencing	IGGi004-A: p. Leu894Phe Fs*36 - c.2682del(A) IGGi005-A: p. R218R fsX21 - c.653-654insACCG	Fig. 1 panel C
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR Negative	supplementary
Differentiation potential	Directed differentiation	Expression of specific markers: ectoderm: PAX6, NESTIN, TUBB3 Mesoderm: TBX6, Brachyury Endoderm: FOXA2, SOX17	Fig. 1 panel G and supplementary
List of recommended germ layer markers	Expression of specific germ layers markers by Immunofluorescence	Positive expression of markers of all three germ layer. Ectoderm: PAX6, NESTIN, TUBB3 Mesoderm: TBX6, Brachyury Endoderm: FOXA2, SOX17	Fig. 1 panel G and supplementary
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	
	HLA tissue typing	N/A	

3. Resource details

ZEB2 (Zinc Finger E-Box Binding Homeobox 2) encodes a transcription factor, mainly acting as a transcription repressor, expressed in various tissues and involved in different functions including, among others, neuro- and glio-genesis, hematopoiesis, and tissue repair (Birkhoff et al., 2021). The best-known clinical picture associated with *ZEB2* mutations is Mowat-Wilson syndrome, which is caused by *ZEB2* haploinsufficiency and characterized by possible multi-organ malformations, dysmorphic features, intellectual disability, and epilepsy. To date, most of the knowledge on *ZEB2* arise from studies carryout in mice, as, for example, the *ZEB2*-dependent modulation of Nkx2-1 during the differentiation and migration of GABA-ergic interneurons (Van den Berghe et al., 2013; Tomassy et al., 2013). However, the complete knockout of *ZEB2* is required to model the Mowat-Wilson syndrome in mice, indicating that humanized models of the disease are necessary to fully understand the underling pathological mechanisms. Accordingly, we generated the IGGi004-A and IGGi005-A iPSC lines as a basis for the development of human brain-specific or multi-organ *in vitro* models of *ZEB2* haploinsufficiency to be used for pathophysiological studies and therapeutics testing. PBMC of two patients carrying different heterozygous mutations in *ZEB2* were used: IGGi004-A (p.Leu894Phe Fs*36 – c.2682del(A)), and IGGi005-A (p.R218R fsX21 – c.653-654insACCG). The iPSC lines were generated with the non-integrating Sendai viral transduction kit CytoTune-iPS 2.0 (ThermoFisher Scientific, A1569601) delivering the Yamanaka transcription factors: OCT4, SOX2, cMYC, and KLF4. After Sendai viral transduction, the cells were cultured in feeder-free conditions using Geltrex (Thermo Fisher Scientific, A14700) and the transition from StemPro™-34 SFM (ThermoFisher Scientific, 10639011) to Essential 8™ Medium (Thermo Fisher Scientific, A1517001) started on the 7th day post-transduction. Emerging colonies were observed after 8 days post-transduction. The colonies showed typical iPSC-like morphology (Fig. 1 panel A). Loss of Sendai viral genes was confirmed by PCR at passage number 10 (Fig. 1 panel B). Heterozygous mutations of *ZEB2* were confirmed by Sanger sequencing (Fig. 1 panel C). The pluripotency was confirmed by immunostaining for cell surface markers SSEA4 and TRA-1–60, and nuclear markers OCT4 and SOX2 (Fig. 1 panel E and F) at passage number 12. STR analysis showed that these iPSCs lines had the same genetic background as the parental cell lines. We evaluated the genomic stability using the KaryoStat + Assay: no aberrations were detected (Fig. 1 panel D). To demonstrate the differentiation ability into the three germ layers we performed an *in-vitro* direct differentiation using the STEMdiff™ Trilineage Differentiation Kit (Stemcell, 052309). We found positive stainings for different markers or the three germ layers, including the ectodermal markers PAX6, NESTIN and TUBB3, the mesodermal markers TBX6 and Brachyury, and the endodermal markers FOXA2 and SOX17 (Fig. 1 panel G and supplementary). All scale bars in Fig. 1 are 50 μm. Mycoplasma contamination was examined in the culture mediums by PCR, and negative results were detected in the iPSCs.

4. Materials and methods

4.1. PBMCs recovery and culture

PBMCs for deriving IGGi004-A and IGGi005-A were processed as per the protocol of Sendai viral transduction kit CytoTune-iPS 2.0 (ThermoFisher Scientific, A1569601). Cells were thawed on day −4 and cultured in PBMC medium StemPro™-34 SFM (ThermoFisher Scientific, 10639011) supplemented with the appropriate cytokines until transfection.

4.2. iPSC reprogramming and culture conditions

On the day of transfection PBMCs were counted, and vectors were administered with MOI 5:5:3 (KOS, hc-Myc, hKlf4). After transduction,

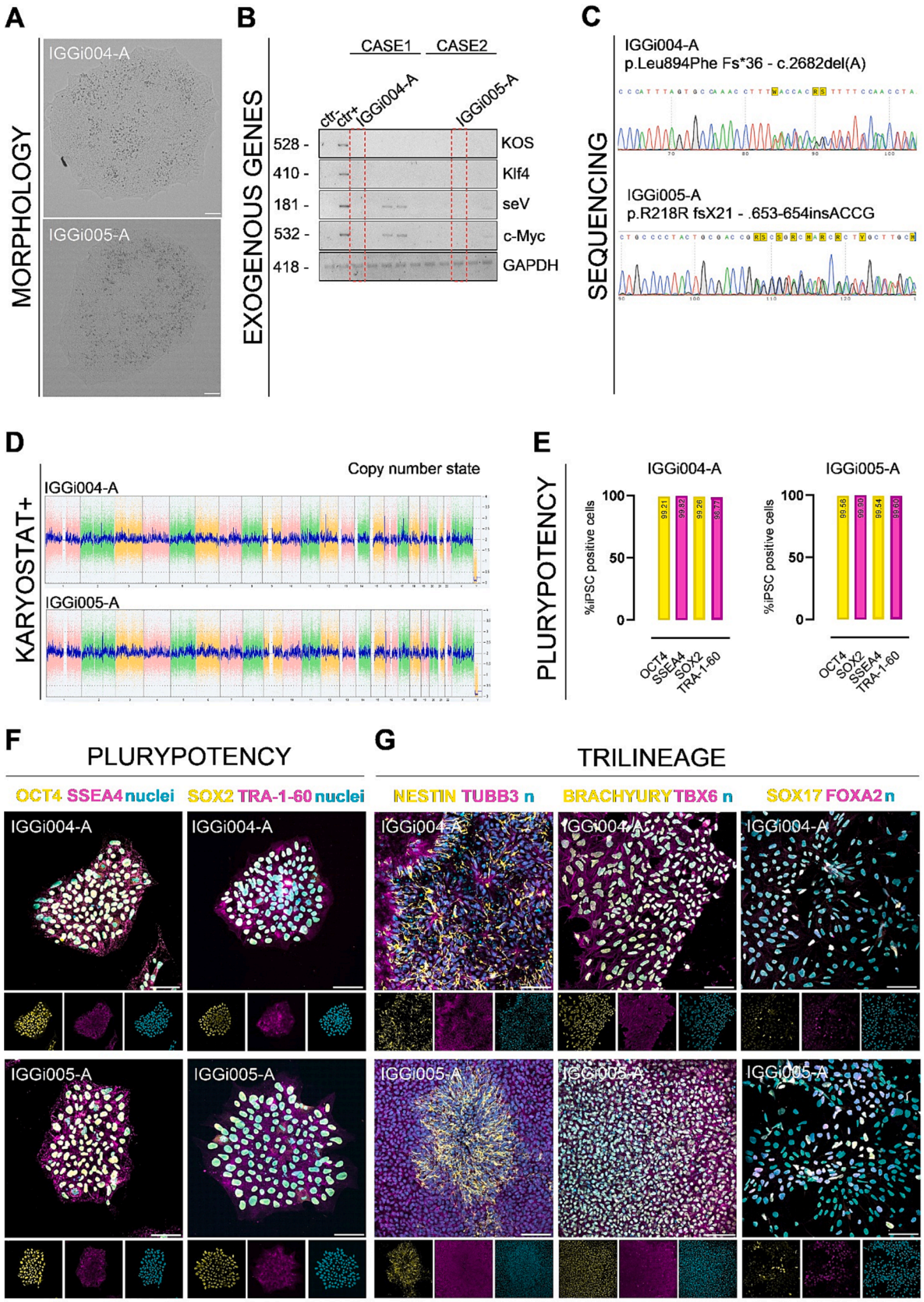

Fig. 1. Characterization of iPSC lines IGGi004-A and IGGi005-A.

the spent medium was changed every day with StemPro™-34 SFM (ThermoFisher Scientific, 10639011) until day 7 and then transitioned to Essential 8™ Medium (Thermo Fisher Scientific, A1517001) until iPSC colonies appeared. iPSCs were manually picked and cultured on Geltrex (Thermo Fisher Scientific, A14700) plates in Essential 8™ Medium (Thermo Fisher Scientific, A1517001) and subsequentially Essential 8™ Flex Medium (Thermo Fisher Scientific, A2858501) at 37 °C, 5 % CO_2. Cells were passaged at a ratio of 1:5 – 1:10 every 2–3 days using Versene solution (Thermo Fisher Scientific, 15040066). At passaging and thawing steps, the medium was supplemented with RevitaCell™ Supplement 100X (Thermo Fisher Scientific, A2644501) overnight. Freezing was performed with Bambanker (NIPPON Genetics EUROPE, BB01). RT-PCR was performed to check if the iPSCs were exogenous factor genes free (primers in Table 2).

4.3. End-point PCR

Total RNA was extracted using Rneasy Micro Kit (Quiagen, 74004) at passage 10. cDNA was obtained by using the iScript™ cDNA Synthesis Kit (Biorad, 1708891). RT-PCR was performed using the AmpliTaq Gold™ 360 Master Mix (Thermo Fisher Scientific, 4398881). Primers are listed in Table 2.

4.4. Immunocytochemistry

Pluripotency of iPSC was examined at passage 12 with the Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit (Thermo Fisher Scientific, A24881). Briefly, cells were fixed with 10 % neutral buffered formalin (Bio-Optica, 05-01005Q), permeabilized with 0.3 % Triton X-100 in PBS for 5 min, and blocked with 1 % bovine serum albumin (BSA) in PBS for 2 h. Primary antibodies for hPSC markers (OCT4, SOX2, SSEA4, and/or TRA-1–60) were diluted in PBS containing 1 % BSA and incubated overnight at 4 °C. Secondary antibodies were then incubated for 1 h at RT in PBS containing 1 % BSA (more information in Table 2). Nuclei were stained by the histology mounting medium Fluoroshield™ with DAPI (Merck, F6057). The 3-germ layer differentiation was examined at passage 14 with the above-mentioned protocol and antibody specified in Table 2. Images were acquired using Leica SP8 confocal microscope.

4.5. Genomic stability and DNA fingerprint

KaryoStat+ (Thermo Fisher Scientific, A52986) and STR analysis (internal service) of 10 polymorphic markers in donor PBMCs and iPSC lines were performed at passage 14.

4.6. Three-germ layer differentiation

The three-germ layer differentiation was performed at passage 12 using STEMdiff™ Trilineage Differentiation Kit (STEMCELL Technologies, 05230).

4.7. Mycoplasma detection

EZ-PCR Mycoplasma Test Kit (Biological Industries, 20–700-20) was performed at passage 10.

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Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	Rabbit anti-OCT4	1:200	Thermo Fisher Cat# A24867	RRID: AB_2650999
	Mouse anti-SSEA4	1:100	Thermo Fisher Cat# A24866	RRID: AB_2651001
	Mouse anti-TRA-1–60	1:100	Thermo Fisher Cat# A24868	RRID: AB_2651002
	Rat anti-SOX2	1:100	Thermo Fisher Cat# A24759	RRID: AB_2651000
Secondary antibodies	Alexa Fluor™ 488 goat anti-mouse IgG3	1:250	Thermo Fisher Scientific Cat# A24877	RRID: AB_2651008
	Alexa Fluor™ 488 donkey anti-rat	1:250	Thermo Fisher Scientific Cat# A24876	RRID: AB_2651007
	Alexa Fluor™ 555 goat anti-mouse IgM	1:250	Thermo Fisher Scientific Cat# A24871	RRID: AB_2651009
	Alexa Fluor™ 555 donkey anti-rabbit	1:250	Thermo Fisher Scientific Cat# A24869	RRID: AB_2651006
Differentiation Markers	Mouse anti- NESTIN	1:500	Thermo Fisher Scientific Cat# MA1-5840	RRID: AB_1077111
	Mouse anti-PAX6 IgG1	1:200	Thermo Fisher Scientific Cat# MA1-109	RRID: AB_2536820
	Guinea pig anti-TUBB3	1:200	Synaptic Systems Cat# 302 304	RRID: AB_10805138
	Mouse anti-TBX6 IgG2a	1:14	Abnova Cat# H00006911-M06	RRID: AB_626501
	Mouse anti-Brachyury IgG2b	1:100	Thermo Fisher Scientific Cat# 14–9770-82	RRID: AB_2573016
	Rabbit anti-FOXA2	1:100	Thermo Fisher Scientific Cat# 701,698	RRID: AB_2573016
	Mouse anti-SOX17 IgG1	1:50	Thermo Fisher Scientific Cat# MA5-24885	RRID: AB_2725396
Secondary antibodies	Alexa Fluor™ 488 goat anti-rabbit	1:500	Thermo Fisher Scientific Cat# A-11008	RRID: AB_143165
	Alexa Fluor™ 555 goat anti-mouse IgG1	1:500	Thermo Fisher Scientific Cat# A-21127	RRID: AB_2535769
	Alexa Fluor™ 647 goat anti- guinea pig	1:500	Thermo Fisher Scientific Cat# A-21450	RRID: AB_2735091
	Alexa Fluor™ 488 goat anti-Mouse IgG2a	1:500	Thermo Fisher Scientific Cat# A-21131	RRID: AB_2535771
	Alexa Fluor™ 647 goat anti-Mouse IgG2b	1:500	Thermo Fisher Scientific Cat# A-21242	RRID: AB_2535811
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Sendai viral vector (RT-PCR)	SeV	181 bp	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC	
	KOS	528 bp	ATGCACCGCTACGACGTGAGCGC/ACCTTGACAATCCTGATGTGG	
	Klf4	410 bp	TTCCTGCATGCCAGAGGAGCCC/AATGTATCGAAGGTGCTCA	
	c-Myc	532 bp	TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG	
House-Keeping Genes (RT-PCR)	GAPDH	139 bp	GTCAACGGATTTGGTCGTATTG/CATGGGTGGAATCATATTGGAA	
Targeted mutation analysis/sequencing	ZEB2 - p.Leu894Phe Fs*36 - c.2682del(A)	234 bp	AGATGAGCCTCTGAACTTGACT/TGTGGTAGGAAGCTCATCTG	
	ZEB2 - p.R218R fsX21 - c.653-654insACCG	235 bp	GCCCATTAGCTGCCATTGAT/CGGTAGGCAAACGTGTAGC	

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CRediT authorship contribution statement

Giulia Gorrieri: Writing – original draft, Visualization, Validation, Resources, Methodology, Data curation. **Serena Tamburro:** Methodology, Data curation. **Simona Baldassari:** Methodology, Data curation. **Sara Guerrisi:** Methodology, Data curation. **Federico Zara:** Visualization, Validation. **Emilia Ricci:** Visualization, Validation, Resources, Funding acquisition. **Duccio Maria Cordelli:** Visualization, Validation. **Paolo Scudieri:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Ilaria Musante:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Conceptualization.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2024.103333.

References

Birkhoff, J.C., Huylebroeck, D., Conidi, A., 2021. ZEB2, the Mowat-Wilson syndrome transcription factor: confirmations, novel functions, and continuing surprises. Genes 12 (7), 1037. https://doi.org/10.3390/genes12071037.

Tomassy, G.S., Lodato, S., Arlotta, P., 2013. A sip of GABA for the cerebral cortex. Neuron 77 (1), 1–3. https://doi.org/10.1016/j.neuron.2012.12.026.

Van den Berghe, V., Stappers, E., Vandesande, B., Dimidschstein, J., Kroes, R., Francis, A., Conidi, A., Lesage, F., Dries, R., Cazzola, S., Berx, G., Kessaris, N., Vanderhaeghen, P., van Ijcken, W., Grosveld, F.G., Goossens, S., Haigh, J.J., Fishell, G., Goffinet, A., Aerts, S., Huylebroeck, D., Seuntjens, E., 2013. Directed migration of cortical interneurons depends on the cell-autonomous action of Sip1. Neuron 77, 70–82. https://doi.org/10.1016/j.neuron.2012.11.009.