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Establishment of a homozygous LMNA knock-out human induced pluripotent stem cell line using CRISPR/Cas9 system

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A B S T R A C T

The *LMNA* gene encodes lamin A/C, essential components of the nuclear envelope that play crucial roles in maintaining nuclear architecture, mechanotransduction, and gene regulation. *LMNA* mutations are linked to laminopathies, affecting multiple organ systems, including muscle, adipose tissue, and the cardiovascular system. To investigate *LMNA*-related disorders, we generated a human-induced pluripotent stem cell (hiPSC) line with a homozygous *LMNA* frameshift mutation (c.351_352insA) using CRISPR/Cas9 genome editing. The edited hiPSCs retained normal colony morphology and expressed key pluripotency markers. This *LMNA* knockout hiPSC line provides a valuable model for studying lamin A/C functions in nuclear integrity, cellular homeostasis, and disease pathogenesis.

1. Resource Table

Unique stem cell line identifier	YCMi019-A-10
Alternative name(s) of stem cell line	
Institution	Yonsei University College of Medicine
Contact information of the reported cell line distributor	Seung-Hyun Lee, tiger815@yuhs.ac
Type of cell line	iPSC
Origin	Human
Additional origin info *(applicable for human ESC or iPSC)*	Age: N/A Sex: Male
Cell Source	Cord blood cell
Method of reprogramming	Sendai virus
Clonality	Clonal
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	N/A
The cell culture system used	TeSR-E8 medium on matrigel-coated plate
Type of the Genetic Modification	CRISPR/Cas9-mediated knock-out
Associated disease	Dilated cardiomyopathy
Gene/locus modified in the reported transgenic line	Homozygous mutation in *LMNA* gene (NM_001257374.3)/c.351_352 ins A, p. Ala118Serfs*9
Method of modification/user-customisable nucleases (UCN) used, the resource used for design optimisation	Plasmid-mediated CRISPR/Cas9 genome editing

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(*continued*)

Unique stem cell line identifier	YCMi019-A-10
User-customisable nuclease (UCN) delivery method	Electroporation
All double-stranded DNA genetic material molecules introduced into the cells	N/A
Evidence of the absence of random integration of any plasmids or DS DNA introduced into the cells.	PCR
Analysis of the nuclease-targeted allele status	Sanger sequencing
Homozygous allele status validation	Sanger sequencing
Method of the off-target nuclease activity prediction and surveillance	http://www.rgenome.net/cas-offinder/Targeted PCR and Sanger sequencing
Descriptive name of the transgene	N/A
Eukaryotic selective agent resistance cassettes (including inducible, gene/cell type-specific)	Puromycin
Inducible/constitutive expression system details	N/A
Date archived/stock creation date	2023
Cell line repository/bank	https://hpscreg.eu/cell-line/YCMi019-A-10
Ethical/GMO work approvals	Ethical committee: Yonsei University Health System, Severance Hospital, Institutional review board approval number: 4-2020-0112
Addgene/public access repository recombinant DNA sources' disclaimers (if applicable)	Addgene plasmid # 62,988

2. Resource utility

Human-induced pluripotent stem cell (hiPSC) models with *LMNA* knockout serve as essential tools for studying lamin A/C function in nuclear integrity, mechanotransduction, and cellular homeostasis. These models enable the investigation of disease mechanisms, identification of novel molecular pathways, and development of targeted therapies for laminopathies.

3. Resource details

The Lamin A/C (*LMNA*) gene is located on chromosome 1q21.2-q21.3 and has 12 exons (Lin and Worman, 1993; Wydner et al., 1996). It produces the intermediate filament proteins lamin A and lamin C, which comprise the nuclear lamina. Lamins are distinct tetrameric 3.5 nm thick filaments different from the cytoskeletal filaments found in eukaryotes (Turgay et al., 2017). Functionally, the lamins provide mechanical stability of the nucleus, maintain chromatin organization, regulate gene expression, and promote cellular homeostasis (Stuurman et al., 1998; Dechat et al., 2010). The mutations in the *LMNA* gene cause various diseases such as Emery-Dreifuss muscular dystrophy (EDMD), lipodystrophies, dilated cardiomyopathy, and Hutchinson-Gilford Progeria. These diseases can cause severe complications in humans, such as muscular dystrophy, an abnormal reduction of adipose tissue, heart failure, and accelerated premature aging (Fatkin et al., 1999; Sullivan et al., 1999; Shackleton et al., 2000; Gonzalo et al., 2017).

To better understand the effects of lamin A/C in human biology, we generated an *LMNA* knockout hiPSC line using CRISPR-Cas9 to achieve a homozygous *LMNA* frameshift mutation, c.351_352insA (p.Ala118-Serfs*9). We designed our single guide RNA (sgRNA) sequence to be a 20-nucleotide sequence located directly upstream of a 5′-NGG protospacer adjacent motif (PAM). As the sgRNA guides the Cas9 to a specific DNA target site, the PAM sequence allows Cas9 to initiate enzymatic cleavage. A previous study (Lee et al., 2019) used patient-derived hiPSC-CMs with *LMNA* mutations to study cardiac laminopathy and performed CRISPR/Cas9-mediated correction to generate isogenic controls. Building on this, we introduced the *LMNA* mutation in the wild-type (WT) hiPSC line, CMC-hiPSC-011, using the same guide RNA (Fig. 1A). This allows a unique perspective of *LMNA* mutation in a uniform genetic background. An adenine insertion at the target site caused a frameshift at amino acid position 118, replacing alanine (Ala) with serine (Ser), introducing a premature stop codon in exon 2, and resulting in a homozygous *LMNA* knockout confirmed by sequencing (Fig. 1B). Then, we subsequently screened for individual single-cell colonies with *LMNA* deletion and successfully isolated an *LMNA* knockout hiPSC with normal morphology (Fig. 1C).

We then tested for pluripotency using immunohistochemistry and flow cytometry, which confirmed robust expression of OCT4, SOX2, SSEA4, and TRA1-60 (Fig. 1D-E). Karyotype plot and G-banding analysis revealed a gain-of-function mutation at chromosome 20q11.21 but otherwise showed a normal karyotype (Fig. 1F-G). Off-target analysis of our sgRNAs showed no unintended genome editing, and no random plasmid integration was detected (Fig. S1). Additionally, mycoplasma testing confirmed no presence of mycoplasma bacteria in our *LMNA* KO cell line (Fig. 1H).

We confirmed that the *LMNA* knockout stem cell line can differentiate into all three germ layers with immunofluorescent staining for TUBB3 (ectoderm), TBXT (mesoderm), and SOX17 (Fig. 1I). Finally, qPCR confirmed reduced *LMNA* mRNA expression, and western blot confirmed the absence of lamin A/C protein expression. (Fig. 1J-K). These contents are summarized in Table 1. These findings demonstrate that the *LMNA* knockout stem cell line serve as a valuable resource for understanding the molecular mechanisms of *LMNA* in laminopathies.

4. Materials and methods

4.1. Cell culture

Human induced pluripotent stem cells (hiPSCs) were maintained at 37 °C with 5 % CO_2 in TeSR-E8 medium (StemCell Technologies) on matrigel −coated plate. The culture medium was replenished daily, and cell colonies were subcultured every 4–5 days at a 1:12 ratio using ReLeSR (StemCell Technologies) in the presence of 10 μM Y-27632 (Tocris).

4.2. CRISPR/Cas9-mediated gene editing

The hiPSCs were dissociated into single cells using the Gentle Cell Dissociation Reagent (StemCell Technologies). sgRNA target sequences for *LMNA* knock-out were designed with the GeneArt CRISPR gRNA Design Tool (Thermo Fisher Scientific). sgRNA was cloned into the pSpCas9-2A-Puro (PX459) vector and delivered into the cells via electroporation using the Neon Electroporation System (850 V, 30 ms, 2 pulses), followed by puromycin selection (1 μg/μL) for 1 day. Purified cells were plated in matrigel-coated 96-well plates with mTeSR1 and 10 μM Y-27632. Colonies were selected for PCR and sequencing.

4.3. Flow cytometry

Cells were rinsed with phosphate-buffered saline (PBS) and dissociated into a single-cell suspension using the Gentle Cell Dissociation Reagent (StemCell Technologies). After counting, they were fixed and permeabilized for 15 min with a Fixation/Permeabilization Solution Kit (BD Bioscience). The hiPSCs at passage 7 were then labeled with stemness markers, including SOX2, TRA-1–60, SSEA4, and OCT3/4. Stained cells were analyzed using an LSRII flow cytometer (BD Bioscience) and processed with FLOWJO v10.0.7 software (Tree Star).

4.4. Immunocytochemistry

Cells at passage 9 were fixed with 4 % paraformaldehyde for 20 min at room temperature (RT). Blocking and permeabilization were

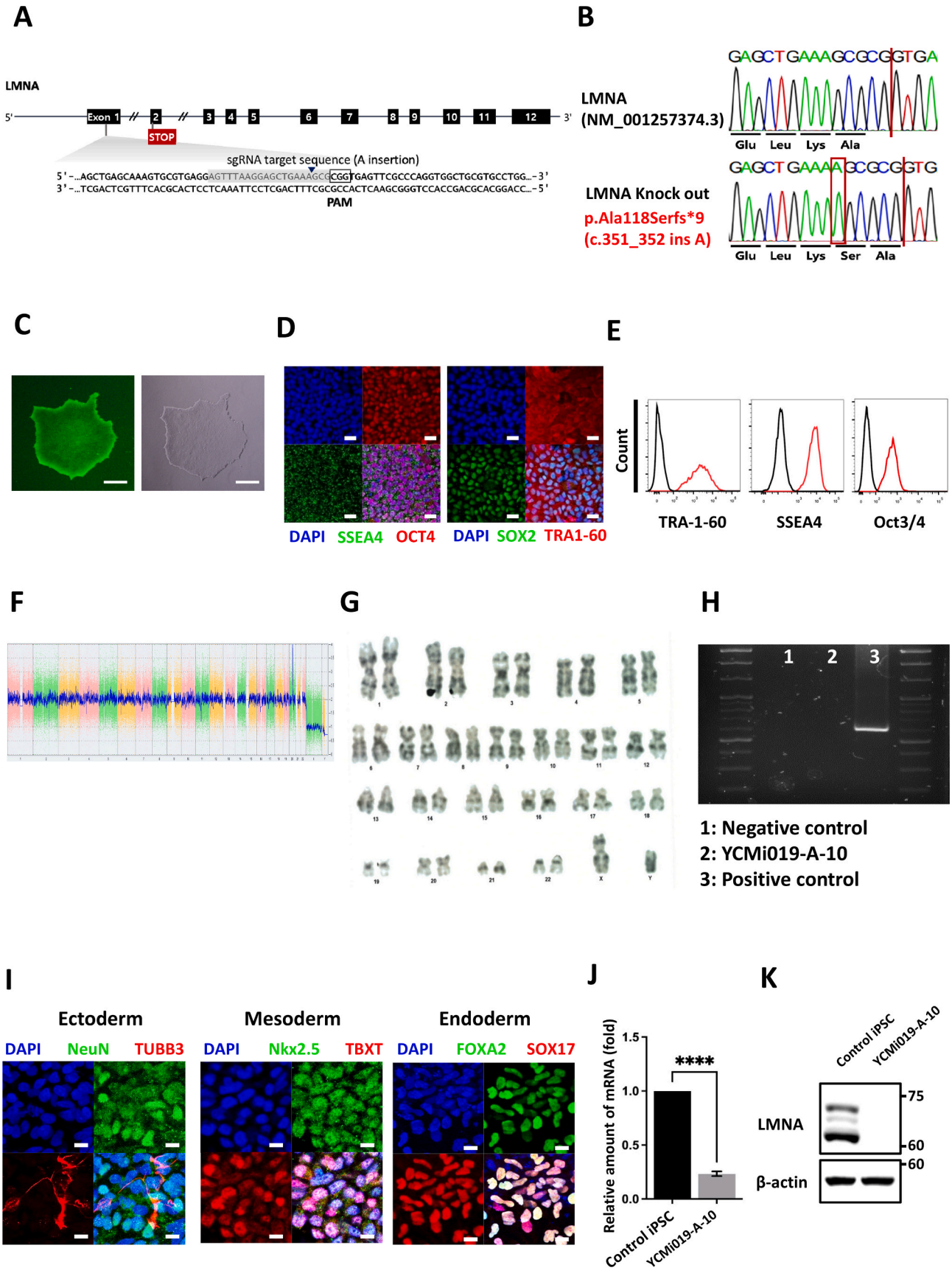

Fig. 1. Characterization for iPSC line YCMi019-A-10.

Table 1
Characterization and validation.

Classification	Output type	Result	Data
Schematic of a transgene/genetic modification	Schematic illustrating the structure and location of the introduced genetic modification		Fig. 1 panel A
Morphology	Photography	normal	Fig. 1 panel C
Pluripotency status evidence for the described cell line	Qualitative analysis Immunocytochemistry	Positive for pluripotency markers including OCT4, SOX2, SSEA4, and TRA-1-60	Fig. 1 panel D
	Quantitative analysis Flow cytometry	TRA 1–60: 90.3 %SSEA-4: 98.7 % Oct3/4: 92.9 %	Fig. 1 panel E
Karyotype	Karyotype (G-banding) and resolution (KaryoStat)	46XY, dup(20) (q11.21)Resolution: > 1 Mb for chromosomal gains; > 1 Mb for chromosomal losses; ~5Mb for telomere ends and centromeres	Fig. 1 panel F and G
Genotyping for the desired genomic alteration/allelic status of the gene of interest	PCR across the edited site or targeted allele-specific PCR	Homozygous LMNA gene 0c.351_352 ins A	Fig. 1 panel B
	Evaluation of the − (homo-/hetero-/hemi-) zygous status of introduced genomic alteration(s)	Homozygous LMNA gene 0c.351_352 ins A	Fig. 1 panel B
	Transgene-specific PCR (when applicable)	N/A	N/A
Verification of the absence of random plasmid integration events	PCR	pSpCas9(BB)-2A-Puro (PX459) V2.0	Supplementary
Parental and modified cell line genetic identity evidence	STR analysis	18 independent loci matched	Submitted in archive with journal
Mutagenesis/genetic modification outcome analysis	Sequencing (genomic DNA PCR or RT-PCR product)	Homozygous LMNA gene 0c.351_352 ins A	Fig. 1 panel B
	PCR-based analyses	N/A	N/A
	Southern Blot or WGS; western blotting (for knock-outs, KOs)	N/A	N/A
Off-target nuclease activity analysis	PCR across top 5/10 predicted top likely off-target sites, whole genome/exome sequencing	N/A	N/A
Specific pathogen-free status	Mycoplasma	PCR negative	Fig. 1 panel H
Multilineage differentiation potential	Directed differentiation into three germ layers	Ectoderm: TUBB3, NeuN Endoderm: SOX17, FOXA2 Mesoderm: TBXT, NKX2.5	Fig. 1 panel I
Outcomes of gene editing experiment (OPTIONAL)	Brief description of the outcomes in terms of clones generated/establishment approach/screening outcomes	N/A	N/A
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype − additional histocompatibility info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

performed using 3 % bovine serum albumin (BSA, LPS Solution) and 0.3 % Triton-X-100 (USB) for 30 min at RT. Primary antibodies were diluted in the blocking buffer and incubated overnight at 4 °C, followed by incubation with secondary antibodies for 3 h at RT (Table 2). Nuclei were stained with NucBlue Fixed Cell ReadyProbes Reagent (DAPI) (Thermo Fisher Scientific) for 1 min.

4.5. In vitro differentiation

YCMi019-A-10 cells at passage 7 were differentiated in vitro using the STEMdiff Trilineage Differentiation Kit (StemCell Technologies) following the manufacturer's protocol. After seven days, cells were fixed with 4 % PFA and stained for ectodermal (NeuN, TUBB3), mesodermal (NKX2.5, TBXT), and endodermal (FOXA2, SOX17) markers (Table 2).

4.6. Confirmation of homozygous LMNA c.351_352 insA

Genomic DNA was extracted from hiPSCs at passage 8 using the G-spin Genomic DNA Extraction Kit (iNtRON Biotechnology) and PCR amplification (GeneAmp PCR 9700, Thermo Fisher Scientific) of exon 1 of the *LMNA* gene. We performed PCR using 26 cycles with the following thermocycling conditions: 98 °C, 62 °C, and 72 °C. Specific primers were prepared and the mutation site was identified through Sanger Sequencing (Bionics).

4.7. Mycoplasma screening

Mycoplasma was tested with passage 7 cells using the TaKaRa PCR Detection Set (Takara, 6601), and PCR products (10 μl) were visualized on a 1 % agarose gel. The correct size band indicates the presence of mycoplasma species in the cell culture.

4.8. Karyotype analysis

Karyotype analysis was performed using the Cytoscan HT-SMA 96 array for KaryoStat+ (Thermo Fisher Scientific). Chromosomal aberrations were identified by comparison to the reference set. In addition, GTG-banding analysis (Samkwang Medical Laboratories) was performed at a resolution of 500 bands per haploid set.

4.9. Short tandem repeat profiling

Short tandem repeat analysis was performed by KGIC Co. using the PowerPlex Fusion 6C system (Promega) and GeneMapper Software 5.

Table 2
Reagents details.

Antibodies and stains used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Stemness Markers	Rabbit anti-OCT4	1:300	Cell Signaling Technology Cat# 9656 AB_1658242
	Sox2	1:100	Thermo Fisher Scientific Cat# 53–9811-82 AB_2574479
	Tra 1–60	1:100	Thermo Fisher Scientific Cat# 13–8863-82 AB_891594
	SSEA-4	1:100	Thermo Fisher Scientific Cat# 46–8843-42 AB_2573850
Ectoderm Marker	Rabbit IgG anti-h/m/rNeuN	1:100	Thermo Fisher Scientific Cat # PA5-78639 AB_2736207
	Mouse anti-Neuron-specific beta-III Tubulin (Clone TuJ-1)	1:100	R&D SystemsCat # MAB1195 AB_357520
Mesoderm Marker	Rabbit IgG anti-h/mNkx2.5	1:100	Thermo Fisher Scientific Cat # PA5-49431 AB_2634885
	Goat IgG anti-h/mBrachyury	1:100	R&D SystemsCat # AF2085 AB_2200235
Endoderm Marker	Rabbit IgG anti-h/m/FOXA2	1:100	Thermo Fisher Scientific Cat # PA5-35097 AB_2552407
	Goat IgG anti-hSOX17	1:100	R&D SystemsCat # AF1924 AB_355060
Secondary antibodies	Alexa® Fluor 488 chicken anti-rabbit IgG	1:500	Thermo Fisher Scientific Cat# A-21441 AB_2535859
	Alexa® Flour 546 goat anti-mouse IgG	1:500	Thermo Fisher Scientific Cat# A-11030 AB_2534089

Site-specific nuclease		
Nuclease information	Nuclease type/version	pSpCas9-2A-Puro (PX459) V2.0 (Addgene)
Delivery method	Electroporation	Neon(Invitrogen-TFS), 2 pulses (850 V 30 ms)
Selection/enrichment strategy	Selection cassette	Puromycin

Primers and Oligonucleotides used in this study		
	Target	**Forward/Reverse primer (5′-3′)**
LMNA genotyping	Human *LMNA*	GGAGCTCAATGATCGCTTGG/ GCAAAGTTATCGGCCTCCAG
qPCR primers	GAPDH (House keeping gene)	GGAGCGAGATCCCTCCAAAAT/ GGCTGTTGTCATACTTCTCATGG
	LMNA	AGC AGC GTG AGT TTG AGA GC/ AGA CTG CCT GGC ATT GTC C
sgRNA_LMNA	Sequencing data from both alleles	CACCGAGTTTAAGGAGCTGAAAGCG/ AAACCGCTTTCAGCTCCTTAAACTC
CRISPR Plasmids	HU6/PX459	GAGGGCCTATTTCCCATGATT/ CAAGTGGGCAGTTTACCGT

gDNA from YCMi019-A-10 and CMC-hiPSC-011 at passage 8 were analyzed across 18 loci.

CRediT authorship contribution statement

So Hee Park: Writing – original draft, Visualization, Data curation. **David Suh:** Writing – original draft, Investigation. **Hyoeun Kim:** Validation. **Ru-Ri Lee:** Visualization. **Isabella Leite Coscarella:** Methodology. **Jaewon Oh:** Funding acquisition. **Sangwoo Kim:** Conceptualization. **Hyoung-Pyo Kim:** Funding acquisition, Conceptualization. **Chulan Kwon:** Writing – review & editing. **Chan Joo Lee:** Project administration. **Sahng Wook Park:** Supervision. **Seunghyun Lee:** Writing – review & editing, Data curation, 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.

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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.2025.103779.

References

Dechat, T., Adam, S.A., Taimen, P., Shimi, T., Goldman, R.D., 2010. Nuclear lamins. Cold Spring Harb. Perspect. Biol. 2 (11), a000547. https://doi.org/10.1101/cshperspect.a000547.

Fatkin, D., MacRae, C., Sasaki, T., Wolff, M.R., Porcu, M., Frenneaux, M., Atherton, J., Vidaillet Jr., H.J., Spudich, S., De Girolami, U., Seidman, J.G., Seidman, C., Muntoni, F., Muehle, G., Johnson, W., McDonough, B., 1999. Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. N. Engl. J. Med. 341 (23), 1715–1724. https://doi.org/10.1056/NEJM199912023412302.

Gonzalo, S., Kreienkamp, R., Askjaer, P., 2017. Hutchinson-Gilford Progeria Syndrome: a premature aging disease caused by LMNA gene mutations. Ageing Res. Rev. 33, 18–29. https://doi.org/10.1016/j.arr.2016.06.007.

Lee, J., Termglinchan, V., Diecke, S., Itzhaki, I., Lam, C.K., Garg, P., Lau, E., Greenhaw, M., Seeger, T., Wu, H., Zhang, J.Z., Chen, X., Gil, I.P., Ameen, M., Sallam, K., Rhee, J.W., Churko, J.M., Chaudhary, R., Chour, T., Wang, P.J., Snyder, M.P., Chang, H.Y., Karakikes, I., Wu, J.C., 2019. Activation of PDGF pathway links LMNA mutation to dilated cardiomyopathy. Nature 572 (7769), 335–340. https://doi.org/10.1038/s41586-019-1406-x.

Lin, F., Worman, H.J., 1993. Structural organization of the human gene encoding nuclear lamin A and nuclear lamin C. J. Biol. Chem. 268 (22), 16321–16326.

Shackleton, S., Lloyd, D.J., Jackson, S.N., Evans, R., Niermeijer, M.F., Singh, B.M., Schmidt, H., Brabant, G., Kumar, S., Durrington, P.N., Gregory, S., O'Rahilly, S., Trembath, R.C., 2000. LMNA, encoding lamin A/C, is mutated in partial lipodystrophy. Nat. Genet. 24 (2), 153–156. https://doi.org/10.1038/72807.

Stuurman, N., Heins, S., Aebi, U., 1998. Nuclear lamins: their structure, assembly, and interactions. J. Struct. Biol. 122 (1–2), 42–66. https://doi.org/10.1006/jsbi.1998.3987.

Sullivan, T., Escalante-Alcalde, D., Bhatt, H., Anver, M., Bhat, N., Nagashima, K., Stewart, C.L., Burke, B., 1999. Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. J. Cell Biol. 147 (5), 913–920. https://doi.org/10.1083/jcb.147.5.913.

Turgay, Y., Eibauer, M., Goldman, A.E., Shimi, T., Khayat, M., Ben-Harush, K., Dubrovsky-Gaupp, A., Sapra, K.T., Goldman, R.D., Medalia, O., 2017. The molecular architecture of lamins in somatic cells. Nature 543 (7644), 261–264. https://doi.org/10.1038/nature21382.

Wydner, K.L., McNeil, J.A., Lin, F., Worman, H.J., Lawrence, J.B., 1996. Chromosomal assignment of human nuclear envelope protein genes LMNA, LMNB1, and LBR by fluorescence in situ hybridization. Genomics 32 (3), 474–478. https://doi.org/10.1006/geno.1996.0146.