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Lab Resource: Multiple Cell Lines

Generation of two human induced pluripotent stem cell lines from peripheral blood mononuclear cells featuring normal or mutated STAB1 gene

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A B S T R A C T

Here, we report the establishment of a human induced pluripotent stem cell (hiPSC) line TUR-STAB1-B1 derived from peripheral blood mononuclear cells of a patient carrying heterozygous mutations 52,509,890 C>A 790L>I and 52,520,515 G>A 1872R>H on STAB1 gene in addition to a normal control cell line TUR-B1. The pluripotency, identity, quality, safety, and sequence of the mutation sites of the cell lines were confirmed. The generated patient-specific cell line will facilitate detailed studies of the STAB1 in various differentiated cell types.

Resource Table:

Unique stem cell lines identifier	UTUi002-A UTUi003-A
Alternative name(s) of stem cell lines	TUR-STAB1-B1 (UTUi002-A) TUR-B1 (UTUi003-A)
Institution	Institute of Biomedicine, University of Turku
Contact information of distributor	Elisa Närvä, elisa.narva@utu.fi
Type of cell lines	hiPSC
Origin	Human
Additional origin info required	Age: >40 Sex: Male Ethnicity: Caucasian
Cell Source	Peripheral blood mononuclear cells
Clonality	mixed
Method of reprogramming	Sendai-virus
Genetic Modification	Patient mutation, TUR-STAB1-B1: 3p21.1 STAB1
Type of Genetic Modification	TUR-STAB1-B1: 3p21.1 STAB1 52,509,890 C>A 790L>I and 52,520,515 G>A 1872R>H

(*continued on next column*)

(*continued*)

Unique stem cell lines identifier	UTUi002-A UTUi003-A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR
Associated disease	–
Gene/locus	TUR-STAB1-B1: 3p21.1 STAB1 52,509,890 C>A 790L>I and 52,520,515 G>A 1872R>H
Date archived/stock date	January 31, 2025
Cell line repository/bank	https://hpscreg.eu/cell-line/UTUi002-A https://hpscreg.eu/cell-line/UTUi003-A
Ethical approval	Ethics Committee for Human Sciences at the University of Turku (6/2023) Ethics Committee of the Hospital District of Helsinki and Uusimaa (HUS/2017/2020)

1. Resource utility

Pathogenic variants affecting the multifunctional scavenger receptor

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Clever-1 (*STAB1*) cause hyperferritinemia (Monfrini et al., 2023). The human induced pluripotent stem cell (hiPSC) line derived from a rare patient case carrying STAB1 mutations can provide an unlimited source of various cell types to study the effect of this mutation at the cellular level.

2. Resource details

Clever-1 (STAB1) is a receptor expressed on lymphatic endothelial cells, sinusoidal endothelial cells, immunosuppressive monocytes, and macrophages affecting scavenging, angiogenesis, and cell adhesion (Hollmén et al., 2020; Karikoski et al., 2009). A patient with recurrent severe infections (invasive staphylococcus) was found to carry heterozygous mutations 52,509,890 C>A 790L>I and 52,520,515 G>A 1872R>H on the STAB1 gene based on whole exome sequencing (WES). To produce hiPSCs, the patient's peripheral blood mononuclear cells (PBMC) were reprogrammed using a non-integrated Sendai virus reprogramming method. To establish a matching normal control cell line for further studies, PBMCs with the same ethical background were reprogrammed simultaneously.

Reprogrammed cell lines TUR-B1 and TUR-STAB1-B1 showed typical pluripotent stem cell morphology (Fig. 1A). Expression of undifferentiated hPSC markers NANOG, and SSEA-3, were validated by immunofluorescence staining (Fig. 1B). Additionally, expression of undifferentiated hPSCs marker SSEA-5 and differentiation marker SSEA-1 was validated with dual staining flow cytometry. Both cell lines showed over 98 % positivity for SSEA-5, whereas expression of the SSEA-1 remained under 1 % (Fig. 1C).

The pluripotency of the cells was confirmed as the ability of the cells to form three-dimensional embryonic bodies (EBs) (Fig. 1D). The expression of three germ layers (endo-, ecto-, and mesoderm) in these EBs was validated with RT-PCR (Fig. 1E) and with immunofluorescence staining (Fig. 1F).

Both hiPSCs had normal male karyotypes (46, XY) (Fig. 1G). In addition, short tandem repeat (STR) analysis was utilized to confirm the identity of the hiPSC lines by verifying identical SNP profiles between these lines and the original PMBCs. In addition, STR analysis confirmed a male karyotype (XY) based on the primer pair AMEL. Sanger sequencing was used to verify the presence of heterozygous mutations 52,509,890 C>A 790L>I and 52,520,515 G>A 1872R>H on the STAB1 gene in the patient cell line TUR-STAB1-B1 and their absence in the control cell line TUR-B1 (Fig. 1H). In addition, both cell lines were tested to be mycoplasma negative (Fig. 1I) and virus-free (Fig. 1J).

Collectively, the generated hiPSC lines exhibited the characteristics of pluripotent stem cells (Fig. 1, Table 1).

3. Materials and Methods

3.1. Reprogramming

The PBMCs, isolated from the patient blood by Ficoll centrifugation, and healthy control (Charles River, PB009C-1) were cultured for 4 days in StemPro^{TM}-34 SFM medium (Gibco, 10639011) with 100 ng/ml SCF (Gibco, PHC2115), 100 ng/ml FLT-3 (Gibco, PHC9414), 20 ng/ml IL-3 (Gibco, PHC0034), and 20 ng/ml IL-6 (Gibco, PHC0065). According to the manufacturer's instructions, PBMCs were transduced using the CytoTune^{TM}-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific, A16517). 3 days after the transduction, the cells were plated to Matrigel-coated (12 μg/ml) (Corning, 354277) plates. 7 days after transduction medium was changed to Essential 8^{TM} medium (Gibco, A1517001), 25 U/ml penicillin–streptomycin, followed by daily medium change. 15 days after transduction, the cells split with 0.5 mM EDTA (Invitrogen, 15575–038) in PBS as clumps (ratio 1:3–1:4 every 2–3 days) without ROCK inhibitor and expanded at 37 °C, 5 % CO_2. The virus was inactivated using heat treatment at 39 °C for 5 days (TUR-B1, p25).

3.2. Immunofluorescence staining

HiPSCs (TUR-B1 p34, TUR-STAB1-B1 p38) fixed with 4 % paraformaldehyde for 15 min at 37 °C in Essential 8^{TM} medium and permeabilized with 0.05 % TRITON^{TM} X-100 (Sigma-Aldrich, 9036-19-5) in 1x PBS for 20 min at RT. Frozen sectioned EBs were fixed with 4 % paraformaldehyde for 30 min at RT in PBS. Samples were stained with primary antibodies in 30 % horse serum for 24 h at 4 °C followed by secondary antibodies and DAPI in 30 % horse serum for 1 h at RT (Table 2). The imaging was carried out using 3i CSU-W1 Spinning disk confocal microscopy.

3.3. Flow cytometry

HiPSCs (TUR-B1 p32, TUR-STAB1-B1 p39) were dissociated into single cells and fixed with 4 % paraformaldehyde. Cells were stained with conjugated antibodies and isotype controls for 30 min at 4 °C (Table 2). Samples were analyzed using BD LSRFortessa Blues flow cytometry and Flowing software.

3.4. In vitro differentiation by embryoid bodies

EBs were grown in Essential 8^{TM} medium from detached cells for 14 days using Low Attachment Surface plates (Corning, 3471) at 37 °C, 5 % CO_2. 20 % FBS was added for the final three days.

3.5. RT-PCR

RNA was isolated with RNeasy Mini Kit (Qiagen, 74104). RNA was treated with DNase I Amplification Grade Kit (Invitrogen, 18068015) and synthesized with a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystem, 4368814). RT-PCR was performed in triplicate reactions using TaqMan Fast Advanced Master Mix (2X) (Applied Biosystems, 4444557) and primers listed in Table 2. RT-PCR was performed on CFX96 Real-Time system thermocycler (Bio-Rad): 2 min at 50 °C, 20 sec at 90 °C, 40 cycles of 95 °C for 3 sec, 60 °C for 30 sec. Relative gene expression was determined using the $2^{-\Delta\Delta Ct}$ method relative to the housekeeping gene.

3.6. Karyotyping

HiPSCs (TUR-B1 p36, TUR-STAB1-B1 p28) were treated with 200 ng/ml colcemid (Roche Diagnostics, 10295892001) in Essential 8^{TM} medium for 2 h at 37 °C. Harvested single cells were treated with 0,75 M KCl 15 min at 37 °C and fixed (methanol, acetic acid 3:1). Giemsa-staining performed G-banding. 20 metaphases analyzed per cell line with ZEIZZ Imager Z2-microscope and MetaSystems CoolCube 1-MetaSystems Metafer 4 program.

3.8. Mycoplasma detection

Mycoplasma detection was performed using the LookOut® Mycoplasma PCR Detection Kit (Sigma-Aldrich, MP0035-1KT) (TUR-B1 p16, TUR-STAB1-B1 p19).

3.9. Sanger sequencing and STR-analysis

DNA was isolated using DNA NucleoSpin® Tissue Kit (Macherey-Nagel, 740952.50). For Sanger sequencing, the DreamTaq Green PCR master mix kit (Thermo Scientific, K1081) was used for the PCR reaction according to the manufacturer's instructions. The primers were designed to identify patient mutations in STAB1 (Table 2). The DNA was isolated from agarose gel using NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, 740609.250). Eurofins performed an STR analysis of 16 loci (Table 1) and Sanger sequencing.

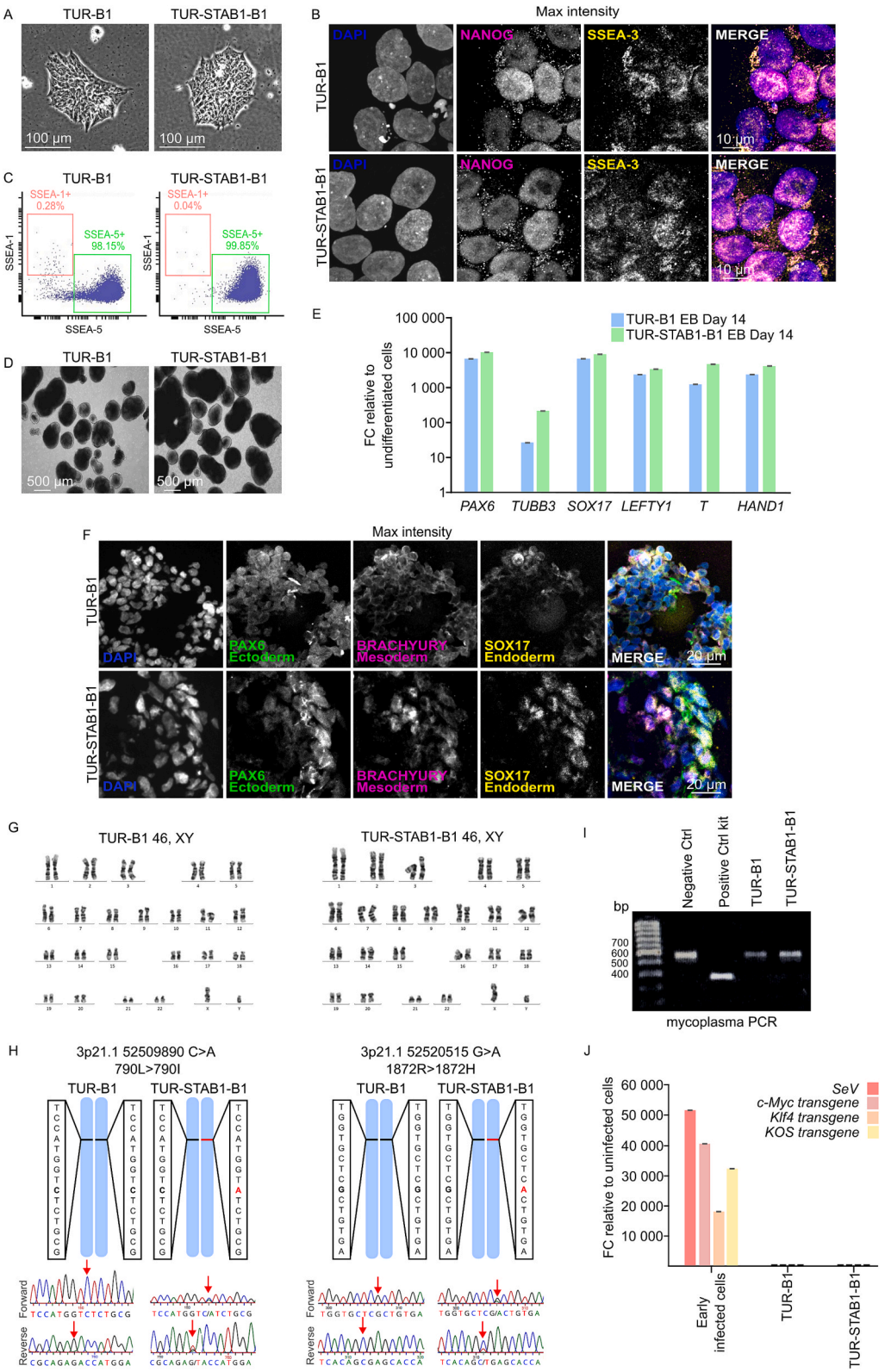

Fig. 1. Characterization of TUR-STAB1-B1 and TUR-B1 cell lines. (A) Morphology of established cell lines (scale bar 100 µm). (B) Immunofluorescence staining of undifferentiated hPSC markers NANOG and SSEA-3. DAPI used as nuclear control (scale bar 10 µm). (C) Expression of undifferentiated hPSC marker SSEA-5 and differentiation marker SSEA-1 detected by Flow cytometry. (D) Morphology of embryonic bodies generated from cell lines, 14 days (scale bar 500 µm). (E) Expression of three germ layers (endo-, ecto-, and mesoderm) measured by RT-PCR. (F) Expression of three germ layers (endo-, ecto-, and mesoderm) measured by immunofluorescence staining. DAPI used as nuclear control (scale bar 20 µm). (G) Karyotype of the cell lines based on G-banding. (H) Sanger sequencing of selected regions of STAB1 3p21.1. (I) Gel run of mycoplasma PCR test reactions. (J) RT-PCR of SeV, c-Myc, Klf-4, and KOS transgenes in early-infected cells and established cell lines.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Bright field microscopy	Compact colonies with a well-defined edge	Fig. 1 panel A
Phenotype	Qualitative analysis *(Immunocytochemistry)*	Positive expression of undifferentiated hPSC markers NANOG and SSEA-3	Fig. 1 panel B
	Quantitative analysis *(Flow cytometry)*	Expression of undifferentiated hPSC marker SSEA-5 > 98 %. Expression of differentiation marker SSEA-1 < 1 %.	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46, XY Resolution: 10 megabases	Fig. 1 panel G
Identity	Microsatellite PCR (mPCR) OR STR analysis	N/A 16 loci (AMEL, CSF1PO, D13S317, D16S539, D18S51, D19S433, D21S11, D2S1338, D3S1358, D5S818, D7S820, D8S1179, FGA, TH01, TPOX, and vWA) tested and all matched	N/A submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous	Fig. 1 panel H
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma Sendai-virus	Mycoplasma test by PCR was negative RT-PCR for Sendai virus RNA was negative	Fig. 1 panel I Fig. 1 panel J
Differentiation potential	Embryoid body formation and trilineage expression determined by immunofluorescence and RT-PCR	Embryonic bodies formed and expressed endoderm markers SOX17 and LEFTY1, mesoderm markers BRACHYURY and HAND1, and ectoderm markers PAX6 and TUBB3.	Fig. 1 panel D, E and F
List of recommended germ layer markers	**Expression of these markers has to be demonstrated at mRNA (RT PCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer**	Embryonic bodies formed and expressed endoderm markers SOX17 and LEFTY1, mesoderm markers BRACHYURY and HAND1, and ectoderm markers PAX6 and TUBB3.	Fig. 1 panel D, E and F
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Undifferentiated hPSC Markers	Goat anti-hNanog Alexa Fluor 647 anti-human/mouse SSEA-3 APC anti-human SSEA-5	1:50 1:100 1:100	R&D System Cat # AF1997 BioLgend Cat # 330307 BioLegend Cat # 355,209	RRID: AB_355097 RRID: AB_1227773 RRID: AB_2562012
Differentiation Markers	Alexa Fluor 488 anti-mouse/human CD15 (SSEA-1) PAX6 Antibody (PAX6/498) Human/Mouse Brachyury Antibody SOX17 Antibody	1:50 1:100 1:100 1:100	Biolegend Cat # 125609 NovusBio Cat # NBP2-34705 R&D System Cat # AF2085-SP NovusBio Cat # NBP1-80362	RRID: AB_1089191 RRID: AB_3291621 RRID: AB_2200235 RRID: AB_11054502
Secondary antibodies	Donkey anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 Donkey anti-Goat IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555 Donkey anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 Goat anti-Rat IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	1:400 1:400 1:400 1:400	Invitrogen Cat # A21202 Invitrogen Cat # A21432 Invitrogen Cat # A31573 Invitrogen Cat # A21247	RRID: AB_141607 RRID: AB_2535853 RRID: AB_2536183 RRID: AB_141778
Isotype Controls	Alexa Fluor 488 Mouse IgM, k Isotype Ctrl Antibody APC Mouse IgG1, k Isotype Ctrl (FC) Antibody	0,5 mg/ml 0,2 mg/ml	BioLegend Cat # 401617 BioLegend Cat # 400,121	RRID: AB_493552 RRID: AB_326443
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	

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CRediT authorship contribution statement

Sanne Sandelin: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Nikolaos**

Table 2 (*continued*)

	Antibodies used for immunocytochemistry/flow-cytometry		
	Antibody	**Dilution**	**Company RRID Cat #**
Sendai Virus genome (RT-PCR)	SeV	59 bp	Assay ID: Mr04269880_mr, ThermoFisher Scientific
Sendai Virus transgene (RT-PCR)	SeV-c-Myc	89 bp	Assay ID: Mr04269876_mr, ThermoFisher Scientific
Sendai Virus transgene (RT-PCR)	SeV-Klf4	67 bp	Assay ID: Mr04421256_mr, ThermoFisher Scientific
Sendai Virus transgene (RT-PCR)	SeV-KOS	80 bp	Assay ID: Mr04421257_mr, ThermoFisher Scientific
House-keeping Gene (RT-PCR)	GAPDH	157 bp	Assay ID: Hs02786624_g1, ThermoFisher Scientific
Differentiation marker (RT-PCR)	HAND1	65 bp	Assay ID: Hs02330376_s1, ThermoFisher Scientific
Differentiation marker (RT-PCR)	LEFTY1	136 bp	Assay ID: Hs00764128_s1, ThermoFisher Scientific
Differentiation marker (RT-PCR)	PAX6	86 bp	Assay ID: Hs01088114_m1, ThermoFisher Scientific
Differentiation marker (RT-PCR)	Sox17	149 bp	Assay ID: Hs00751751_s1, ThermoFisher Scientific
Differentiation marker (RT-PCR)	T	132 bp	Assay ID: Hs00610080_m1, ThermoFisher Scientific
Differentiation marker (RT-PCR)	TUBB3	134 bp	Assay ID: Hs00801390_s1, ThermoFisher Scientific
52,509,890 C>A 790L>I (PCR)		965 bp	ATCTGCCTGCCCATTGAGTC/ AAGGAGCGAATCCCAGATGC
52,520,515 G>A 1872R>H (PCR)		647 bp	CCAACCATGACTCCACTTGC/ GGGGACGTCCAGAACTTCG

Giannareas: Writing – review & editing, Supervision, Methodology, Investigation. **Mikko R.J Seppänen:** Writing – review & editing. **Juha Kere:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Maija Hollmén:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Elisa Närvä:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Maija Hollmén reports a relationship with Faron Pharmaceuticals Oy that includes: employment and equity or stocks.].

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References

Hollmén, M., Figueiredo, C.R., Jalkanen, S., 2020. New tools to prevent cancer growth and spread: a 'Clever' approach. Br. J. Cancer 123 (4), 501–509. https://doi.org/10.1038/s41416-020-0953-0.

Karikoski, M., Irjala, H., Maksimow, M., Miiluniemi, M., Granfors, K., Hernesniemi, S., Elima, K., Moldenhauer, G., Schledzewski, K., Kzhyshkowska, J., Goerdt, S., Salmi, M., Jalkanen, S., 2009. Clever-1/Stabilin-1 regulates lymphocyte migration within lymphatics and leukocyte entrance to sites of inflammation. Eur. J. Immunol. 39, 3477–3487. https://doi.org/10.1002/EJI.200939896.

Monfrini, E., Pelucchi, S., Hollmén, M., Viitala, M., Mariani, R., Bertola, F., Majore, S., Di Fonzo, A., Piperno, A., 2023. A form of inherited hyperferritinemia associated with bi-allelic pathogenic variants of STAB1. Am. J. Hum. Genet. 110, 1436–1443. https://doi.org/10.1016/J.AJHG.2023.07.004.