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Griscelli Syndrome Type 1: a novel *MYO5A* pathogenic variant and characterization of patient-derived fibroblasts

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Abstract

Griscelli syndrome (GS) is an autosomal recessive disorder classified into three subtypes according to the underlying gene mutation: GS-1 (*MYO5A*), GS-2 (*RAB27A*), and GS-3 (*MLPH* or *MYO5A* exon F). Silvery-gray hair and hypopigmentation of the skin are common features of all three subtypes and represent the only clinical manifestations of GS-3. In contrast, GS-2 is characterized by primary immunodeficiency and hemophagocytic syndrome, whereas GS-1 is associated with severe primary neurological impairment. Here, we report two siblings, a 23-year-old male and an 11-year-old female, from a non-consanguineous Brazilian family, both presenting with silvery-gray hair and severe neurological abnormalities. Clinical and histopathological findings support a diagnosis of GS-1/Elejalde syndrome in both patients. To our knowledge, the older sibling is the oldest reported patient with GS-1/Elejalde syndrome. Genetic analysis identified a homozygous *MYO5A* variant (c.2102G>A; Chr15:52,671,928 C>T) predicted to introduce a premature stop codon in place of tryptophan 701 (p.Trp701Ter; W701X), near the C-terminal end of the myosin-Va head domain. We subsequently established a fibroblast cell line from one patient and primary fibroblasts from a healthy donor as controls. Myosin-Va protein was undetectable in patient-derived fibroblasts, whereas expression of myosin-Vb and myosin-Vc was increased, suggesting potential compensatory mechanisms. These patient-derived fibroblasts provide a valuable model for investigating myosin-Va function and the potential compensatory roles of myosin-Vb and myosin-Vc, as well as for future studies involving induced pluripotent stem cells, *in vitro* differentiation, and genome editing to correct the pathogenic variant.

Key words: Griscelli Syndrome, Elejalde Syndrome; *MYO5A* variant, fibroblast model

Introduction

Griscelli Syndrome (GS) is a rare autosomal recessive disorder classified into three subtypes based on mutations in specific genes: GS-1 (*MYO5A*), GS-2 (*RAB27A*), and GS-3 (*MLPH* or *MYO5A* exon F). These genes encode proteins essential for the intracellular transport of melanosomes in melanocytes. Disruption of this transport process leads to the characteristic hypopigmentation seen in GS, typically presenting as silvery-gray hair and pale

skin. The *MYO5A* gene encodes myosin-Va, an actin-based molecular motor widely expressed in various tissues and responsible for the transport of organelles, vesicles, and other cellular components. It plays a particularly crucial role in neurons (Griscelli et al., 1978; Klein et al., 1994; Wu & Hammer, 2014). The clinical manifestations of GS vary depending on the subtype: GS-3 (MIM:609227) is limited to pigmentary abnormalities; GS-2 (MIM:607624) involves immune dysregulation, leading to primary immunodeficiency and hemophagocytic lymphohistiocytosis (HLH), a life-threatening hyperinflammatory syndrome; GS-1 (MIM:214450) is the most severe form caused by mutations in *MYO5A* and presents with profound neurological impairments such as developmental delay, progressive spasticity, seizures, and global hypotonia, highlighting the essential role of *MYO5A* in the nervous system. In melanocytes, myosin-Va interacts with Rab27a and melanophilin to form a heterotrimeric complex that anchors melanosomes to the cell cortex for transfer to keratinocytes. Loss-of-function mutations in *MYO5A* disrupt this mechanism, leading to perinuclear clustering of melanosomes and a resulting pigmentary dilution (da Silva Bizario et al., 2002; Hammer & Wagner, 2013; Pastural et al., 1997; Pastural et al., 2000). In this study, we describe the clinical, histopathological, and molecular features of two siblings (aged 27 and 12 years) from a non-consanguineous Brazilian family diagnosed with GS-1. We identified a novel pathogenic nonsense mutation in the coding region of *MYO5A* gene. To investigate its molecular consequences, we established primary fibroblast cultures from an affected sibling and compared them to control fibroblasts derived from an age-matched healthy donor. In the patient-derived cells, myosin-Va protein was undetectable, accompanied by a possible compensatory upregulation of myosin-Vb and myosin-Vc. These observations suggest the presence of adaptive mechanisms that may partially offset the functional loss of *MYO5A* in specific cell types. The patient-derived fibroblasts thus provide a valuable model for studying the molecular pathophysiology of Griscelli syndrome type 1 (GS-1) and for assessing potential therapeutic strategies.

Materials and Methods

Ethical Approval

This study was conducted in accordance with Brazilian national ethical standards which is aligned with international ethical standards. The research protocol, including the collection and use of human fibroblasts and clinical data, was reviewed and approved by the Ethics committee of Ribeirão Preto Medical School, University of São Paulo, approval number: 915/99. Written informed consent was obtained from all participants (or their legal guardians, when necessary) prior to inclusion in the study, authorizing the use of their biological samples, anonymized clinical information, and related images for research and publication purposes. All data were handled and presented to protect patient anonymity. Images were cropped to prevent recognition of any individual, and no identifying information is included in the text or figures.

Hair processing

Hair shafts collected from patients and healthy controls were mounted in Permount (Fisher Scientific) between a glass slide and coverslip (W. Knittel Glass, Germany) and analyzed without prior treatment. For each patient, two slides were prepared, each containing an average of four hair shafts. Optical microscopy images were acquired using a Nikon Eclipse E800 microscope equipped with a Nikon DXM1200 digital camera.

Skin biopsy and histological sections for optical microscopy

Skin biopsies from the patient and a healthy donor were obtained in a hospital setting at the Department of Dermatology, Hospital of Clinics, São Paulo, Brazil, to support the diagnosis. The tissue samples were divided into three portions for histological analysis by optical microscopy, transmission electron microscopy, and establishment of a primary fibroblast culture. For histological analysis, one portion was fixed overnight in 4% paraformaldehyde in 0.1 M phosphate buffer, washed three times with the same buffer, and dehydrated through a graded ethanol series (Merck). The tissue was then cleared in xylene, embedded in paraffin, sectioned using a microtome, and stained with hematoxylin and eosin (H&E). Histological sections were examined and documented using a Nikon Eclipse E800 microscope equipped with a Nikon DXM1200 digital camera.

Transmission electron microscopy (TEM)

The biopsied skin fragment was fixed in 2% paraformaldehyde (Electron Microscopy Sciences) and 2% glutaraldehyde (Ladd Research Industries) in 0.1 M cacodylate buffer (pH 7.4) containing 0.05% CaCl_2 for 24 hours

at 4 °C, followed by four washes with the same buffer. The sample was then post-fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 2 hours at 4 °C and washed twice with the same buffer. This was followed by two washes with 0.1 M sodium acetate buffer (pH 5.0) and block impregnation in 2% uranyl acetate (in acetate buffer) at 4 °C for 12 hours. The material was dehydrated in ethanol, infiltrated, and embedded in LX112 resin (Electron Microscopy Sciences) for 72 hours at 60 °C. Semi-thin sections (0.5 µm) were obtained and stained with toluidine blue for optical microscopy to delineate the area for analysis. Ultrathin sections were cut using a diamond knife, mounted on copper grids coated with polyol, and coated with a thin layer of carbon. The sections were then counterstained with 2% uranyl acetate for 20 minutes and 0.3% lead citrate for 3 minutes. Imaging was performed using a PHILIPS EM 208 transmission electron microscope.

Primary Fibroblast Culture

A small fragment of the skin biopsy from the patient and a healthy donor was washed four times with phosphate-buffered saline (PBS: 1.3 mM NaH₂PO₄, 1.4 mM KH₂PO₄, pH 7.3; 137 mM NaCl; 2.7 mM KCl), followed by two washes with Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12; Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% (v/v) fetal bovine serum (FBS), 100 mg/mL streptomycin, 100 U/mL penicillin, and 0.25 µg/mL amphotericin B (Invitrogen). Mechanical fragmentation of the tissue was then performed in a 100 mm cell culture-grade Petri dish (NUNC), using sterile surgical instruments (forceps, scalpel, and scissors) to obtain fragments of approximately 0.3 mm. These fragments were distributed into a 6-well plate (NUNC) containing 2 mL per well of the culture medium and incubated at 37 °C in a humidified 5% CO₂ atmosphere. The culture medium was replaced after four days of incubation and subsequently every 72 hours from day four until confluence was reached. Cells were harvested using 0.25% trypsin and 0.025% EDTA. Viability was assessed by Trypan Blue exclusion prior to storage and subsequent passaging for experiments.

Blood collection

Blood samples from patients and their parents were collected via peripheral venipuncture using Vacutainer® (Becton Dickinson) vacuum collection tubes containing either heparin or EDTA as an anticoagulant. The volume of blood collected ranged from 5 to 10 mL.

Separation of peripheral blood mononuclear cells (PBMCs)

Peripheral blood mononuclear cells (PBMCs) were isolated using a Histopaque®-1077 density gradient (Sigma), following the manufacturer's protocol. The collected blood was diluted 1:1 with sterile saline solution and carefully layered onto 4 mL of Histopaque-1077 in a 15 mL conical tube. For every 4 mL of Histopaque, 10 mL of the diluted blood mixture was added. The samples were centrifuged at $1300 \times g$ for 20 minutes at 4 °C. The resulting interphase layer containing the mononuclear cells was collected and transferred to a new conical tube containing 10 mL of sterile saline, followed by centrifugation at 2500 rpm for 10 minutes at 4 °C. This washing step was repeated once. After the second centrifugation, the supernatant was discarded, and the cell pellet was resuspended either in RPMI-1640 medium to establish cell cultures, in 1 mL of TRIzol™ reagent for total RNA extraction or processed according to the genomic DNA extraction protocol.

Genomic DNA Extraction

Genomic DNA was extracted from peripheral blood mononuclear cells and fibroblasts. The cells were cultured in 6-well plates until reaching at least 80% confluence. After cell sedimentation in a 1.5 mL tube, the medium was discarded and the pellet was washed four times with 1 mL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.4). After each wash, the tube was centrifuged at $14,000 \times g$ for 2 minutes at room temperature (RT). Next, the pellet was resuspended in 200 µL of autoclaved deionized water, and 5 µL 20 µg/mL Proteinase K, 20 µL of 10% SDS, and 20 µL of 10% sarcosine were added, followed by overnight incubation in a dry bath at 37°C.

After the incubation period, 250 µL of cold phenol-chloroform-isoamyl alcohol (25:24:1) was added, followed by vigorous vortexing. The sample was centrifuged at $14,000 \times g$ for 10 minutes at RT, and the aqueous phase containing the DNA was transferred to a new sterile tube. Then, 150 µL of cold analytical-grade isopropanol was added, and the sample was incubated overnight at -20°C to precipitate the DNA. After incubation, the sample was centrifuged at $14,000 \times g$ for 10 minutes at RT. The supernatant was discarded, and the pellet was carefully washed twice with 200 µL of cold 70% ethanol. For each wash, the tube was centrifuged at $14,000 \times g$ for 10 minutes at RT. After the final wash, the 70% ethanol was removed using a pipette, and the tube was left open on the bench for 15 minutes to allow the pellet to dry. Finally, the DNA pellet was resuspended in 30 µL of deionized water, with vortexing applied for more efficient dissolution. The sample was quantified using a spectrophotometer (260 nm/280 nm reading) and then stored at -20°C.

Southern Blotting

Genomic DNA extracted from PBMCs was digested with the restriction enzymes AflIII, AvaII, EcoRV, and HindIII, using 1.5 µg of DNA per reaction. Digestions were carried out overnight at 37 °C, following the manufacturer's instructions. Digested DNA (9 µL), combined with 1 µL of loading buffer (0.25% bromophenol blue, 30% glycerol), was subjected to electrophoresis in a 0.8% agarose gel at 50 V for approximately 8 hours. Following electrophoresis, the gel was stained with 1 µg/mL ethidium bromide for 10 minutes, rinsed in distilled water, and photographed under a UV transilluminator. Marker bands were visualized using HindIII-digested lambda phage DNA, and their positions were marked by punching holes in the gel. The gel was then treated as follows: washed once for 15 minutes in depurination solution (0.2 N HCl); washed twice for 15 minutes each in denaturation solution (1.5 M NaCl, 0.5 M NaOH); washed twice in neutralization solution (0.5 M Tris-HCl, pH 7.0; 1.5 M NaCl), first for 30 minutes, then for 15 minutes. Between each solution change, the gel was rinsed briefly with distilled water. Subsequently, a system for capillary transfer of DNA onto a Hybond-N nylon membrane (Amersham Pharmacia) was assembled, based on the method of Southern (1975). The setup included a flat surface with reservoirs at both ends containing SSPE buffer (1.5 M NaCl, 100 mM NaH₂PO₄, 10 mM EDTA, pH 7.4). A piece of 3MM filter paper, cut to a length sufficient to dip into both buffer reservoirs, was laid over the flat surface to initiate the transfer process. Following overnight capillary transfer, the membrane was washed in SSPE buffer and exposed to UV light (1200 J/s) using a CL-1000 Ultraviolet Crosslinker (Ultra-Violet Products). For Southern blot hybridization, a radiolabeled 434 bp human *MYO5A* cDNA fragment was used as a probe. This fragment corresponds to positions 5629 to 6063 of the *MYO5A* mRNA (RefSeq: NM_000259.3). The probe was generated by PCR amplification using cDNA synthesized from total RNA extracted from normal fibroblasts and the following primers (Forward – 5'-AGTCTCTGTGTCGTTTCATTCG-3'; Reverse – 5'-CCTGTATGTAACTCACGGTA-3'). The resulting PCR product was radiolabeled with [α -³²P]dCTP using a standard polymerase labeling reaction. Essentially, 50 ng of DNA, dATP [250 µM], dGTP [250 µM], dTTP [250 µM], [α -³²P] dCTP (25 µCi) (Amersham-Pharmacia), 2 µL of BSA, 1 U of the Klenow fragment of DNA polymerase, and autoclaved deionized water were mixed to a final volume of 50 µL. The reaction was incubated for 3 hours at 37°C. Then, 50 µL of dye (bromophenol blue and NT buffer) were added to the probe. The sample was then filtered through a Sephadex G-50 resin column

to remove unincorporated nucleotides and denatured at 95°C for 5 minutes. Following hybridization, the membrane was washed in SSC buffers of increasing stringency (i.e., decreasing salt concentrations). Autoradiographic detection was performed by exposing the membrane to X-ray film.

RNA extraction and obtention of *MYO5A* cDNA fragments

Peripheral blood mononuclear cells (PBMCs) were cultured for 72 hours in 5 mL of medium supplemented with phytohemagglutinin (PHA) in 25 cm² vented culture flasks. For total RNA extraction, cell suspensions were centrifuged, and the resulting pellets were homogenized in 1 mL of TRIzol™ reagent (Life Technologies, Gibco-BRL, Paisley, UK), following the manufacturer's instructions, except that RNA precipitation was carried out overnight at 20 °C. The dried RNA pellets were resuspended in diethyl pyrocarbonate (DEPC)-treated water and quantified by spectrophotometric analysis at 260/280 nm. For RT-PCR, total RNA was treated with DNase I (Promega) at 1 U per 2 µg of RNA in a 10 µL reaction volume and incubated for 30 minutes at 37 °C. Enzyme inactivation was performed by adding 1 µL of 20 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), followed by incubation at 65 °C for 15 minutes. cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems), by adding to the 2 µg of RNA, 1 µL of oligo(dT) primer [50 µM], 1 µL of dNTP mix [10 mM], and 0.5 µL of hexamers (random oligonucleotides) at 300 ng/µL, followed by incubation at 65°C for 5 minutes. After a brief centrifugation and 1 minute incubation on ice, the following were added: 4 µL of 5X First Strand Buffer, 1 µL of DTT [0.1 M], 1 µL of RNasin (RNase inhibitor), and 1 µL of SuperScript III (Invitrogen). The reaction mixture was incubated at 50°C for 1 hour, followed by inactivation at 70°C for 15 minutes. The resulting cDNA was used for amplification of target fragments of *MYO5A* gene. Nine primer pairs were designed to amplify regions spanning the full *MYO5A* (accession number NM_000259) coding sequence: F1: GCTGCGGGCGCTTCCTAG, R1: GCTGAGACTGTTTTTCCTGC; F2: GAACGAAATCAGTCCATCATC, R2: AGCTGTCTGCATCTCGGGAT; F3: CTGTGATTGAAGGAGTGGATGA, R3: CATAGTGAGGGGTAGTGGCAT; F4: GAACTCAATGCCACTACCC, R4: CAACTTTCGCTTCCTCTTCAC; F5: GAGCAGAACAAAGACTACAAAT, R5: AGCTCATCCTGCATCACCTG; F6: GAGCAGGAGAAGCAGGTGAT, R6: ACCCTTCATAAACCAGCCAC; F7:

AAAAATGAAAGATAAAGGTGAAAT, R7: GTTTTTGCTCATCCTCCTTCTT; F8: AAGGAGGATGAGCAAAACT, R8: TACTGGAGGTTCGCTTTCT; F9: AGGGCGTGTCTGGGGTGAAG, R9: GTATTCTAGGGAGATTCCAGTT. In addition, 17 additional primer pairs were designed to amplify the full exonic and flanking sequences spanning the coding region of *MYO5A* gene from genomic DNA. The primers anneal to 5' or 3' untranslated regions or intronic regions flanking the exons and were designed based on the genomic sequence (aligned to Refseq: NM_000259): F1: TGCGGGCGCTTCCTAGTCC, R1: TCCGCCGCCTTCACCATCT; F2: GATTGTAAAATAATTGATTGCTAG, R2: ACTCCCTTCACTTTATGCCAAT; F3: CCTCTTTCACAGTCCTTGCCA, R3: CAGCCTGCTTCCCCATGTAC; F4: TTTTCTTTCTTGTCCCCTACCC, R4: TCTAATAACTGCCATAAAATAACAA; F5: CCATAGAGACCATAGTTTCCC, R5: TTTTAGTGGCTGGAGACCCC; F6: CCCCTTTTCATATTATTTTGTGG, R6: CAACTTCATTCCAGGGATAAGC, F7: AGGTTGTTGTTGTTGAAAGCC, R7: GAACCCACTTATCTTCTGACC; F8: AAGGTTTGC GTTCAAGTTTGG, R8: TGAGCCAGAAAGGAAGGAGC; F9: TTACCTCACCTTTGACTTTGG, R9: GTTTGCTTGGCTGGTTTGGC; F10: AATGTTGTAGTATTTTGGTTGGA, R10: TGTGTACGGAGTAGATGGGG; F11: CATCTTTGTGATTCTGTTGGG, R11: GCTGAAATGGACATAAAGCCC; F12: CTTCACTGTTTTCGGTAGAGC, R12: GTCTTGAGCAGAACTCCCC; F13: CTTTTGAGTTGAATCTTAATGCG, R13: CAAGAGGCTGGCTTGAAGGC; F14: TTTGTGTTTTGCATTGTTGTTCC, R14: CAGGGGTGAAAGTCAGGAGG; F15: CAACTCGGGGTGTCGTCTCC, R15: GTTAAGAAGCATGTGGACCCG; F16: CATGCCTGCCAATTTTAGCC, R16: AAACCAAGACTCAGTGACCG; F17: AAAATTATAGGAACTTGGAGACC, R17: TTAACCCATGTGCCAGAAGG. This strategy ensured that the entire coding region of *MYO5A* gene was amplified and sequenced.

DNA sequencing

PCR fragments of the expected sizes were sequenced using the BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) in a 10 µL reaction. Each reaction contained 2 µL of BigDye reagent, 1 µL of either the forward or reverse primer (10 µM), and 50–200 ng of PCR product. Thermal cycling conditions were as follows: initial denaturation at 95 °C for 1 minute, followed by 24 cycles of denaturation at 95 °C for 10 seconds, primer-specific annealing for 5 seconds (temperature optimized for each primer), and extension at 60 °C for 4 minutes. After amplification, 30 µL of deionized water, 5 µL of 3 M potassium acetate (pH 5.2), and 100 µL of absolute ethanol were added to each tube. The contents were mixed by inversion and incubated at room temperature for 15 minutes. Samples were then centrifuged at 19,000 × g for 30 minutes at 4 °C. The supernatant

was carefully removed using absorbent paper. The pellet was washed by adding 200 μ L of 70% ethanol, incubated for 2 minutes at room temperature, and centrifuged again at $19,000 \times g$ for 6 minutes at 4 °C. After discarding the supernatant, a final wash with 70% ethanol was performed, and the tubes were air-dried at room temperature. The dried pellets were resuspended in sequencing buffer and analyzed on an ABI Prism® 3100-Avant Genetic Analyzer (Applied Biosystems).

Immunocytochemistry

Cells were cultured on coverslips placed in 24-well plates using the appropriate culture medium, as described above. After 18–24 hours, cells were washed and fixed with 2% paraformaldehyde (w/v) in PBS (pH 7.4) for 15 minutes. They were then permeabilized with 0.3% Triton X-100 (v/v) and blocked sequentially with 100 mM glycine and 3% BSA in PBS (w/v) at room temperature. Cells were incubated with the appropriate primary antibody, while coverslips processed without primary antibody served as negative controls. All samples were incubated overnight at 4 °C in a humidified chamber. After extensive washing with PBS, cells were incubated with 1 μ g/mL of secondary antibody: Alexa Fluor® 594 donkey anti-rabbit IgG (Abcam, ab150073) and/or Alexa Fluor® 488 donkey anti-mouse IgG (Promega, W4021; Madison, WI, USA). Coverslips were mounted using ProLong® Diamond Antifade Mountant with DAPI (Thermo Fisher Scientific, P36962; Waltham, MA, USA).

Western Blot

Cells were cultured in 6-well plates and, after 24 hours, washed and lysed in ice-cold lysis buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 20 mM NaF, 2 mM Na_3VO_4 , 5 mM MgCl_2 , 10 mM ATP (final pH 7.4), and a 1:100 dilution of protease inhibitor cocktail (Sigma-Aldrich). Lysates were passed through a 19-gauge needle 10 times and centrifuged at $11,000 \times g$ for 10 minutes at 4 °C. Supernatants were collected, and protein concentrations were determined using the Bradford assay (Bradford, 1976). Forty micrograms of total protein were loaded onto a 10% SDS–polyacrylamide gel and separated by SDS–PAGE. Proteins were then transferred to a nitrocellulose membrane (Bio-Rad, Richmond, CA, USA) and probed with the appropriate primary antibodies. Detection was carried out using HRP-conjugated secondary antibodies and enhanced chemiluminescence (ECL; GE Life Sciences, Buckinghamshire, England).

Antibodies

Rabbit polyclonal antibodies α -medial-tail; α -full-tail (α -8c) and α -head of myosin-Va, produced and purified in our laboratory. Mouse monoclonal antibody (anti-phosphoserine) against myosin-Va globular tail (phosphorylated serine 1650) (Pranchevicius et al., 2008). Anti- γ -Tubulin mouse monoclonal antibody (Merk T5326). Anti- α -Tubulin monoclonal antibody (Merk T6199). HRP-conjugated goat anti-mouse IgG polyclonal antibody (Horseradish Peroxidase, Promega W4021). Goat anti-rabbit IgG polyclonal antibody conjugated to HRP (HorseRadish Peroxidase, Promega V8051). Goat anti-Rabbit IgG Secondary Antibody, Alexa Fluor 488 (Thermo Fisher). Goat anti-Mouse IgG Secondary Antibody, Alexa Fluor 594 (Thermo Fisher).

RNA isolation and quantitative RT-qPCR

Total RNA was extracted from patient and healthy donor fibroblasts using TRIzol™ reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. RNA pellets were resuspended in DEPC-treated water, and RNA concentration and purity were assessed by spectrophotometry at 260/280 nm using a NanoVue spectrophotometer (GE Life Sciences, Buckinghamshire, England). For RT-qPCR, 2 μ g of total RNA was reverse transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Reverse transcription was performed at 25 °C for 5 minutes, followed by 30 minutes at 42 °C, and enzyme inactivation at 85 °C for 5 minutes. Quantitative PCR was performed using the GoTaq® qPCR Master Mix with SYBR Green (Promega) in 12 μ L reactions containing 6 μ L of master mix, 0.8 μ L of a primer mix (400 nM each of forward and reverse primers), and 2 μ L of cDNA (equivalent to 20 ng). Amplification and data analysis were carried out on an ABI PRISM® 7500 Sequence Detection System using the SDS v1.2 software (Applied Biosystems). Primers were designed to span exon–exon junctions or intron-flanking regions to prevent amplification of genomic DNA. The following primer pairs were used for the *MYO5A* gene: (Forward: CCTTTCAACCCATCTTCCCC, Reverse: TTGCCTGGACATCACTTTCA). The reference genes used for normalization were GAPDH, TBP, and β -actin. Their respective primers were: GAPDH (Forward: GTCTCCTCTGACTTCAACAGCG, Reverse: ACCACCCTGTTGCTGTAGCCAA); TBP (Forward: ACCCTTCACCAATGACTCCTATG, Reverse: ATGATGACTGCAGCAAATCGC); β -actin (Forward: GGCATCGTGATGGACTCCG, Reverse: GCTGGAAGGTGGACAGCGA). Statistical analysis of RT-qPCR data was performed using the comparative Ct ($2^{-\Delta\Delta C_t}$) method to determine relative gene expression levels. GAPDH, TBP, and β -actin were used

as internal reference genes for normalization. Each reaction was performed in triplicate, and results are expressed as mean $\pm$ standard deviation (SD). Statistical significance between groups was assessed using an unpaired two-tailed Student's *t*-test, considering $p < 0.05$ as significant. All analyses and graphical representations were generated using GraphPad Prism (version 6.03; GraphPad Software, San Diego, CA, USA).

Results

Case report: Clinical Findings

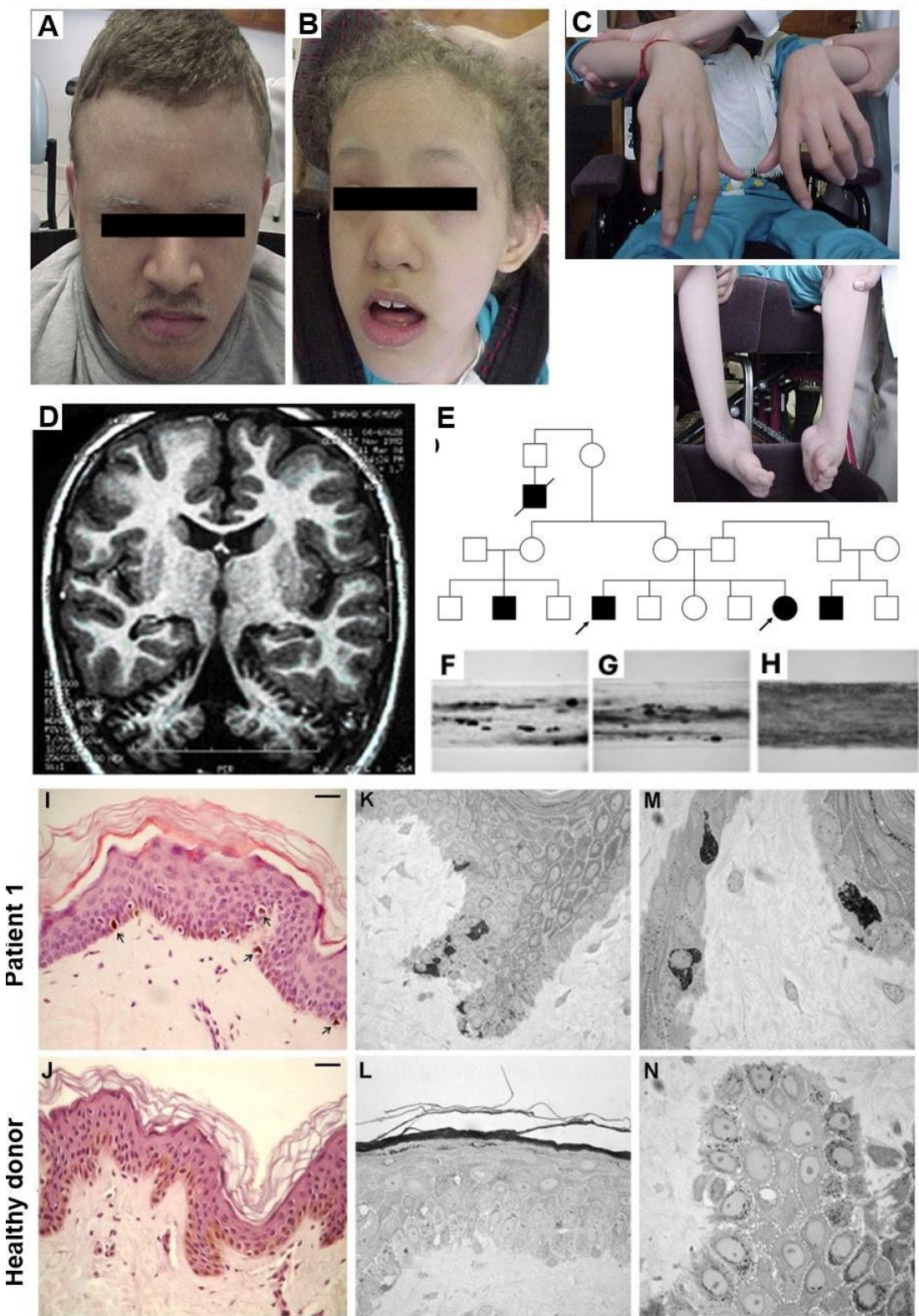
A male proband, hereafter referred to as *Patient 1* (**Fig. 1A**), and his sister, *Patient 2* (**Fig. 1B**), were evaluated at the Neuropediatric Outpatient Clinic of the Hospital of Clinics, University of São Paulo Medical School. They were born to non-consanguineous parents and were 23 and 11 years old, respectively, at the time of evaluation. Both siblings exhibited severe neuropsychomotor developmental delay, generalized hypotonia, and epilepsy since birth, with no clinical evidence of immunodeficiency. They had markedly limited awareness of their surroundings and communicated through non-verbal vocalizations, including grunting and groaning. On physical examination, both patients had silvery-gray eyebrows and eyelashes with normal distribution and texture. Patient 1 displayed a tanned skin tone with diffuse hypopigmented macules, particularly prominent in sun-exposed areas of the face. Patient 2 exhibited more extensive and irregular facial hypopigmentation and lighter hair with a silvery sheen, albeit less pronounced than her brother (**Fig. 1A, B**).

Neurologically, both patients presented with severe intellectual disability and fixed contractures involving the Achilles tendons, popliteal fossae, and wrists. Additional findings included scoliosis, pyramidal signs, and spastic paraparesis predominantly affecting the lower limbs, accompanied by axial hypotonia and appendicular hypertonia, as illustrated in the clinical photographs of Patient 2 (**Fig. 1 C**). Bilateral hypoplasia of the breast papillae was also noted. Cranial magnetic resonance imaging (MRI) of Patient 1 revealed marked cerebral and cerebellar cortical atrophy (**Fig. 1D**). Previous diagnostic evaluations included an electroencephalogram (EEG), which demonstrated spikes, paroxysms, and sharp-wave activity in the centroparietal and left frontal regions, as

well as occasional spikes in the right centroparietal region. Patient 1 was receiving continuous treatment with phenobarbital. Serum immunoglobulin levels (IgA, IgM, and IgG) were within normal limits. A skin biopsy was performed for histopathological and molecular analyses. The family history revealed additional affected individuals, including a maternal uncle and two cousins on both the maternal and paternal sides, as illustrated in the pedigree (**Fig. 1E**). Patient 2 died at 12 years of age. Contact with Patient 1 was subsequently lost, and his current clinical status is unknown. Written informed consent was obtained from the family for the collection of biopsies, storage, and analysis of clinical data, in accordance with institutional ethical guidelines (HCRP 915/99).

Fig. 1: Neurological and pigmentation features in two siblings consistent with Elejalde/Griscelli Syndrome type 1. (A, B) Frontal profile photographs of the affected siblings, Patient 1 (**A**) and Patient 2 (**B**), showing silvery-gray hair and eyebrows along with uneven skin pigmentation. (**C**) Photographs of the upper (hands/arms) and lower (legs/feet) limbs of Patient 2, illustrating abnormal posture. (**D**) Magnetic resonance imaging (MRI) of Patient 1's brain, revealing generalized cortical atrophy, most pronounced in the cerebellum. (**E**) Family pedigree indicating affected (black symbols) and unaffected (white symbols) individuals; males are represented by squares, females by circles. Arrows indicate the two affected siblings reported here. (**F–H**) Microscopic images of hair shafts from Patient 1 (**F**), Patient 2 (**G**), and a healthy donor (**H**). Note the clumps of pigment irregularly distributed along the hair shaft of patients (**F, G**), in contrast to the uniform pigmentation in the control (**H**). (**I–N**) Light micrographs of skin biopsy sections from Patient 1 (**I, K, M**) and a healthy donor (**J, L, N**), including Hematoxylin and Eosin-stained sections (**I, J**) and toluidine blue-stained 0.5 μm -thick sections (**K, M, L, N**). Note the accumulation of pigment in melanocytes located in the basal layer of Patient 1's epidermis (**I, K, M**), in contrast to the even pigmentation/melanosome distribution in the basal layer of control donor epidermis (**J, L, N**). Scale bar 25 μm .

Fig.1

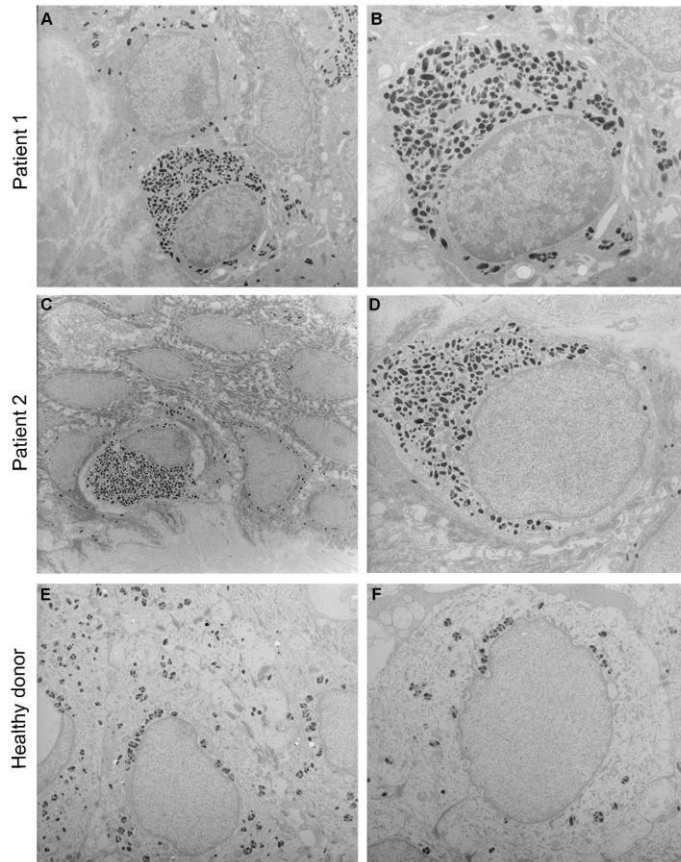


Microscopic Features of the Hair Shaft and Skin

Light microscopy of hair samples from both siblings revealed large pigment clumps irregularly distributed along the hair shafts (**Fig. 1F, G**). These findings contrasted with the uniform pigmentation observed in healthy controls (**Fig. 1H**) and with the smaller, more evenly distributed pigment clusters reported in hair shafts from patients with Chediak–Higashi syndrome (Klein et al., 1994; Meschede et al., 2008; Wolf et al., 2006). Histological analysis of skin sections from Patient 1 demonstrated pigment accumulation within melanocytes in the basal layer of the epidermis (**Fig. 1I**), in contrast to the more uniform pigmentation observed in skin sections from a healthy donor (**Fig. 1J**). Semi-thin sections further revealed clustering of melanosomes within melanocytes and irregular transfer of melanosomes to adjacent keratinocytes in samples from Patient 1 (**Fig. 1K, M**). In control samples, melanosomes were evenly distributed within basal keratinocytes (**Fig. 1L, N**). Transmission electron microscopy further demonstrated extensive accumulation of melanosomes in the perinuclear cytoplasm of melanocytes in samples from both Patient 1 and Patient 2 (**Fig. 2A–D**), whereas keratinocytes from a healthy donor exhibited a normal distribution of melanosomes (**Fig. 2E, F**). Together, these microscopic findings are consistent with the diagnosis of Griscelli syndrome type 1 (GS-1), also known as Elejalde syndrome, which is caused by pathogenic variants in *MYO5A*.

Fig. 2. Electron micrographs of skin biopsies from Patients 1 and 2 and a healthy donor. (A, B) Patient 1; **(C, D)** Patient 2; **(E, F)** healthy donor. Note the prominent accumulation of mature melanosomes in the cytoplasm of melanocytes in skin samples from both patients, in contrast to the healthy donor, in whom melanosomes are predominantly observed within keratinocytes.

Fig.2



Identification of a Novel Mutation in the *MYO5A* Gene

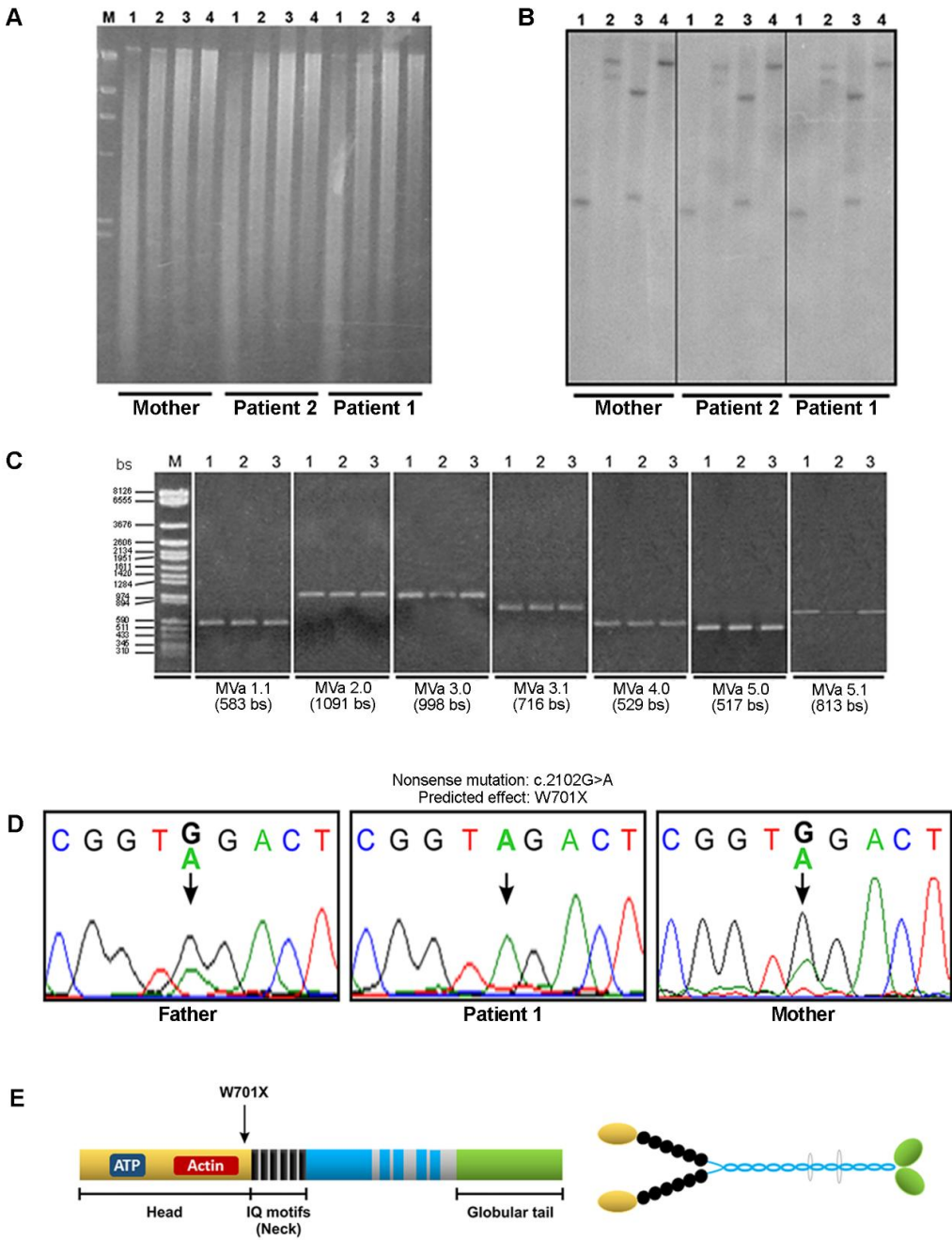
To investigate genetic alterations in *MYO5A*, Southern blot analysis was performed (**Fig. 3A, B**). Genomic DNA from the siblings and their mother was digested with restriction enzymes, separated by electrophoresis, and hybridized with a radiolabeled probe targeting the *MYO5A* gene. The banding patterns were identical in both siblings (Patients 1 and 2) and matched that of their mother, providing no evidence of large genomic deletions, insertions, or rearrangements detectable by this approach. The complete coding sequence and portions of the untranslated regions of *MYO5A* were subsequently obtained using cDNA and genomic DNA both from Patient 1 and from his parents. The gene was initially divided into nine overlapping fragments, seven of which were successfully amplified (**Fig. 3C**). The remaining regions, spanning nucleotides 155–768 at the 5' end and 4150–4709 at the 3' end, were amplified using additional primers designed for the genomic DNA. Full-length *MYO5A*

coding sequences were obtained from Patient 1 and from his parents. The *MYO5A* coding sequence from Patient 1 was deposited in GenBank under accession number **PZ761537**. Alignment of this complete consensus sequence with the *Homo sapiens MYO5A* reference sequence (RefSeq NM_000259.3) identified a single homozygous nucleotide substitution, **c.2102G>A** (Chr15:52,671,928 C>T), in Patient 1. This variant occurs at codon 701, changing TGG to TAG and resulting in a premature termination codon (**p.Trp701Ter; W701X**), predicted to truncate myosin-Va near the C-terminal end of its head domain (**Fig. 3D, E**). Both parents were confirmed to be heterozygous carriers (**Fig. 3D**). The reduced representation of the mutant allele in the parental cDNA sequencing electropherograms, as indicated by the relative heights of the A and G peaks, suggests reduced stability of the mutant transcript and is consistent with nonsense-mediated mRNA decay (**Fig. 3D**). To further investigate potential genetic causes of the phenotype, all exons and the canonical splice donor and acceptor sites of *RAB27A* were sequenced in Patient 1, and no pathogenic variants were identified. The pathogenicity of the *MYO5A* **c.2102G>A** variant is supported by its predicted protein-truncating effect and by previously reported *MYO5A* variants associated with similar clinical phenotypes (Meschede et al., 2008; Ménasché et al., 2003; Ménasché et al., 2000).

Fig. 3. Identification of a novel homozygous *MYO5A* variant associated with Griscelli syndrome type 1. (**A, B**) Southern blot analysis of the *MYO5A* gene in the mother (M) and both patients. Genomic DNA was digested with four different restriction enzymes: (1) *Ava*II, (2) *Hind*III, (3) *Afl*III, and (4) *Eco*RV. (**B**) Digestion products were separated by electrophoresis on a 0.8% agarose gel, transferred to a nylon membrane by capillary blotting, and hybridized with a radiolabeled probe corresponding to a 669-bp fragment of *MYO5A* cDNA (nucleotides 5201–5870 of the coding region). Identical banding patterns were observed in all three individuals, indicating no evidence of large deletions or insertions in the *MYO5A* gene. (**C**) PCR amplification of selected regions of *MYO5A* cDNA. Seven of the nine fragments required to span the entire *MYO5A* coding sequence were amplified using cDNA from the father (**1**), mother (**2**), and Patient 1 (**3**). Lane M: lambda-*Ava*II size markers. (**D**) DNA sequencing electropherograms showing a heterozygous G>A substitution at nucleotide 2102 of the *MYO5A* coding sequence in both parents and a homozygous A allele at the same position in Patient 1. The relative peak heights in the parental sequences are consistent with reduced abundance of the mutant transcript compared with the wild-type transcript. At least three independent PCR amplifications and sequencing reactions confirmed the variant. The c.2102G>A substitution introduces a premature termination codon at residue 701 (p.Trp701Ter; W701X), predicted to truncate the protein near the C-terminal end of the myosin-Va head domain. (**E**) Linear schematic

illustrating the structural domain organization of the myosin-Va heavy chain (Espreafico et al., 1992) and a model based on quick-freeze, deep-etch electron microscopy images (Cheney et al., 1993).

Fig.3

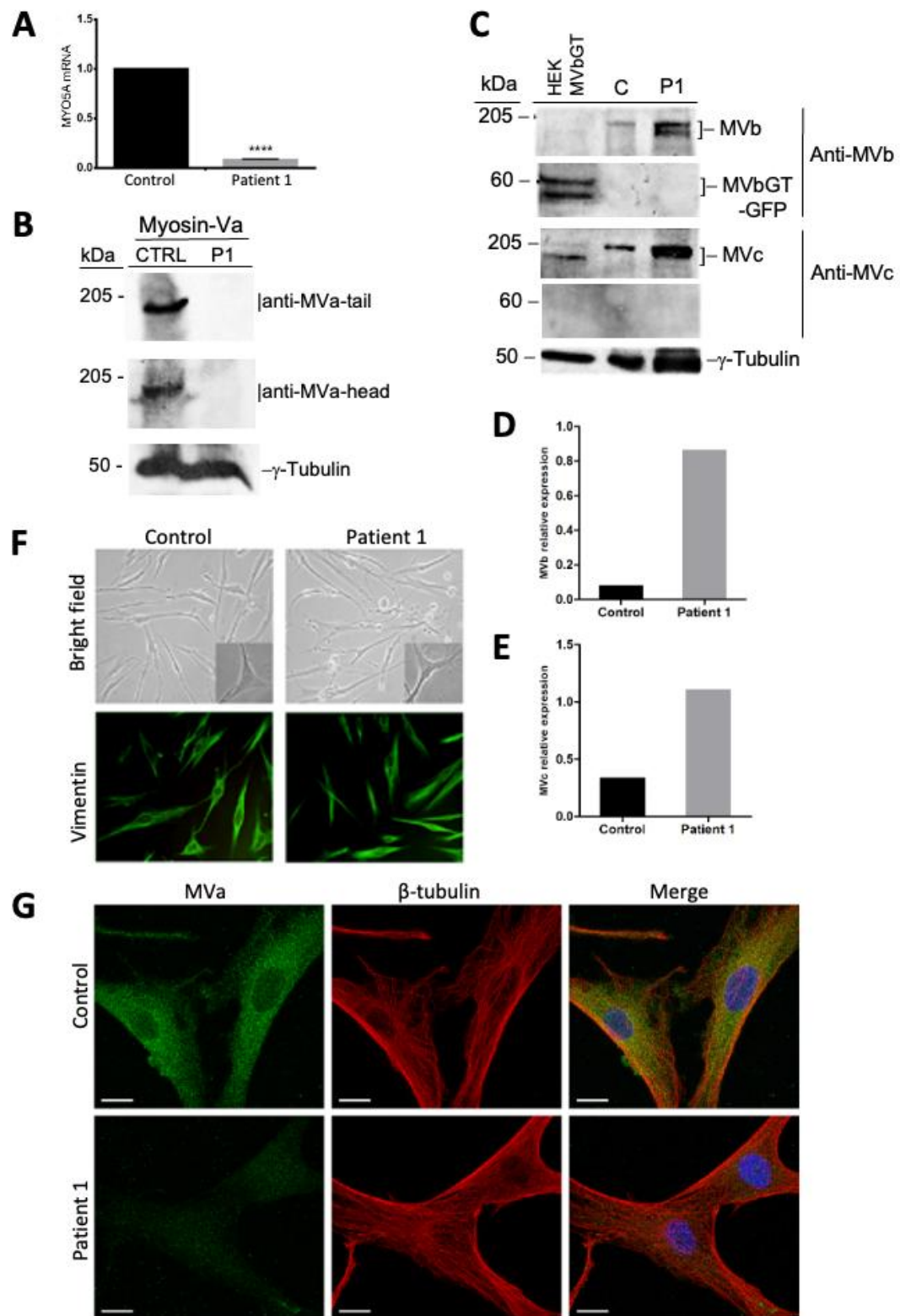


Isolation and Characterization of *MYO5A* Mutant Skin Fibroblasts

Primary fibroblast cultures were established from a skin biopsy of Patient 1 and compared with fibroblasts from a healthy donor (Control). Quantitative real-time RT-PCR (RT-qPCR) revealed significantly reduced *MYO5A* mRNA levels in fibroblasts from Patient 1 compared with the healthy donor control (**Fig. 4A**). Consistent with the reduced *MYO5A* transcript levels, Western blot analysis using antibodies targeting both the head and tail domains of myosin-Va failed to detect myosin-Va protein in patient-derived fibroblasts (**Fig. 4B**). In agreement with these findings, increased expression of the related class V myosins, myosin-Vb and myosin-Vc, was observed in Patient 1 fibroblasts compared with control fibroblasts (**Fig. 4C–E**), suggesting a potential compensatory response involving other class V myosins. Immunostaining for vimentin confirmed the fibroblastic identity of the skin-derived cells (**Fig. 4F**), whereas immunofluorescence staining for myosin-Va further confirmed the absence of detectable myosin-Va protein in patient-derived fibroblasts (**Fig. 4G**).

Fig. 4. Characterization of primary cultured fibroblasts derived from skin biopsies of Patient 1 and a healthy donor (Control). (A) RT-qPCR analysis of *MYO5A* expression in fibroblasts from the healthy donor (Control) and Patient 1. Relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method, with *GAPDH*, *TBP*, and *β -actin* as reference genes for normalization. **p* < 0.001; technical triplicates. (B) Western blot analysis of myosin-Va expression in Control (CTRL) and Patient 1 fibroblasts using two antibodies recognizing the myosin-Va head (anti-MVa head) and tail (anti-MVa tail) domains, with anti- γ -tubulin as a loading control. (C) Western blot analysis of myosin-Vb and myosin-Vc expression in Control (CTRL) and Patient 1 fibroblasts using antibodies to the tail domains of myosin-Vb and myosin-Vc, respectively, with anti- γ -tubulin as a loading control. (D, E) Quantification of myosin-Vb (D) and myosin-Vc (E) expression based on the myosin-Vb/ γ -tubulin and myosin-Vc/ γ -tubulin ratios, respectively, based on the Western blots shown in (C). (F) Phase-contrast bright-field microscopy and vimentin immunofluorescence staining (green) of cultured fibroblasts derived from skin biopsies of Patient 1 and the healthy donor. Vimentin staining is consistent with the fibroblastic phenotype of the cultured cells. Scale bar, 5 μ m. (G) Immunofluorescence images of Control and Patient 1 fibroblasts stained for myosin-Va (anti-MVa head, green) and β -tubulin (red).

Fig.4



Discussion

Here, we report a novel pathogenic variant in the *MYO5A* gene identified in two siblings clinically diagnosed with Griscelli syndrome type 1 (GS-1). Remarkably, one of the siblings, Patient 1, survived to at least 27 years of age, making him the longest-living individual with genetically confirmed GS-1 reported to date. Previously, the longest life span documented for GS-1 was 12 years (Ivanovich et al., 2001). This extended lifespan suggests that possible compensatory mechanisms or unidentified genetic modifiers may influence disease progression and clinical outcomes in GS-1.

The nonsense mutation (c.2102G>A, p.W701X) found in *MYO5A* coding region is a novel mutation that introduces a premature stop codon in the head domain of the myosin-Va protein. The head domain is critical for ATP hydrolysis and actin-binding, both essential for motor function. The absence of functional myosin-Va disrupts intracellular transport pathways in melanocytes and neurons, leading to the characteristic neurological and dermatological features of Griscelli Syndrome type 1. Importantly, this variant is absent from public databases, including ClinVar, underscoring its novelty. To date, approximately 28 *MYO5A* variants have been associated with GS-1, including two variants (c.2332C>T, p.R778Ter; c2110C>T, pG704Ter) located in the vicinity of the variant described here (Abd Elmaksoud et al., 2020; Pastural et al., 1997). In total, over 387 *MYO5A* variants have been reported in ClinVar. Selected variants associated with Griscelli syndrome are summarized in **Table 1**. Additionally, a large intronic deletion affecting exon F of *MYO5A* has been described in cases classified as Griscelli syndrome type 3 (MIM:609227), where the phenotype is limited to pigmentary abnormalities without neurological involvement (**Table 1**). These findings highlight the phenotypic heterogeneity of *MYO5A*-related disorders and underscore the importance of domain-specific effects on disease presentation across the Griscelli syndrome spectrum.

Table 1. Summary of human variants reported in MYO5A with clinical significance annotations related to Griscelli Syndrome type 1.

SNP ID	Clinical significance and condition	Chr15pos	Variation	Type	PMID	References
rs764371254	Pathogenic: Griscelli syndrome, Type 1	52,376,435(-)	Ref/Alt: G/A NM_000259.3(MYO5A):c.2332C>T (p.Arg778Ter)	nonsense	9207796 12058346	Pastural et al., 1997 Anikster et al., 2002
rs2141121140	Pathogenic: Griscelli syndrome, Type 1	52,379,723(-)	Ref/Alt: G/A NM_000259.3(MYO5A):c.2110C>T (p.Gln704Ter)	nonsense	32275080	Elmaksound et al., 2020
rs2043479059	Pathogenic: Griscelli syndrome, Type 1	52,416,294(-)	Ref/Alt: G/A NM_000259.3(MYO5A):c.463C>T (p.Arg155Ter)	nonsense	32275080	Elmaksound et al., 2020
rs769021352	Pathogenic: Griscelli syndrome, Type 1	52,383,090(-)	Ref/Alt: C/A NM_000259.3(MYO5A):c.2012+1G>T	splice donor	25326635	Yang et al., 2014
14070*	Pathogenic: Griscelli syndrome, Type 1	52,307,283(-)	47-BP INS, NT4634	insertion	10704277	Pastural et al., 2000
rs2141084327	Likely pathogenic: Griscelli syndrome, Type 1	52,370,192(-)	Ref/Alt: G/A NM_000259.3(MYO5A):c.3043C>T (p.Arg1015Ter)	nonsense	-	CENTOGENE, 2021+
rs201961375	Uncertain significance: Griscelli syndrome, Type 1	52,330,351(-)	Ref/Alt: G/A NM_000259.3(MYO5A):c.4480+2C>T	splice donor	17576681	Buratti et al., 2007
rs1446850592	Uncertain significance: Griscelli syndrome, Type 1	52,410,433(-)	Ref/Alt: C/T NM_000259.3(MYO5A):c.656G>A (p.Arg219His)	missense	-	KMC-MAHE, 2022+
rs2041830255	Uncertain significance: Griscelli syndrome, Type 1	52,383,173(-)	Ref/Alt: G/A NM_000259.3(MYO5A):c.1930C>T (p.His644Tyr)	missense	-	Revvity Omics, 2024+
rs374266181	Uncertain significance: Griscelli syndrome, Type 1	52,375,354(-)	Ref/Alt: C/T NM_000259.3(MYO5A):c.2527G>A (p.Val843Ile)	missense	-	Revvity Omics, 2024+
2627586*	Uncertain significance: Griscelli syndrome, Type 1	52,383,145(-)	Ref/Alt: G/A NM_000259.3(MYO5A):c.1958C>T (p.Thr653Ile)	missense	-	IHG-UKL, 2023+
rs561485938	Uncertain significance: Griscelli syndrome, Type 1	52,375,443(-)	Ref/Alt: C/T NM_000259.3(MYO5A):c.2438G>A (p.Arg813His)	missense	-	IIGK-IIG, 2024+
rs756082685	Uncertain significance: Griscelli syndrome, Type 1	52,319,260(-)	Ref/Alt: G/C NM_000259.3(MYO5A):c.4959C>G (p.Ile1653Met)	missense	-	IIGK-IIG, 2024+
rs751411629	Uncertain significance: Griscelli syndrome, Type 1; Inborn genetic diseases	52,367,093(-)	Ref/Alt: A/C NM_000259.3(MYO5A):c.3098T>G (p.Leu1033Trp)	missense	-	BMGL-KAMC, 2018+ Ambry Genetics, 2024+
rs752461164	Uncertain significance: Griscelli syndrome, Type 1 Likely benign: MYO5A-related disorder	52,375,404(-)	Ref/Alt: C/T NM_000259.3(MYO5A):c.2477G>A (p.Arg826His)	missense	-	Baylor Genetics, 2021+ Prevention Genetics, 2024+
rs72734962	Likely benign: Griscelli syndrome, Type 1 Benign: Inborn genetic diseases	52,317,044(-)	Ref/Alt: C/T NM_000259.3(MYO5A):c.5334+4G>A	intron	-	CG-ARHLCHC, 2023+ Ambry Genetics, 2024+
rs748268948	Likely benign: Griscelli syndrome, Type 1	52,416,223(-)	Ref/Alt: T/G NM_000259.3(MYO5A):c.534A>C (p.Arg178=)	synonymous	-	CG-ARHLCHC, 2023+
rs56132571	Likely benign: Griscelli Syndrome, Type 1; Intellectual disability; Inborn genetic diseases	52,367,055(-)	Ref/Alt: C/T NM_000259.3(MYO5A):c.3136G>A (p.Val1046Met)	missense	-	AJCGC-AJCSH, 2021+ CBPG-CHRU, 2020+ Ambry Genetics, 2024+
rs1058219	Benign: Griscelli syndrome, Type 1	52,351,367(-)	Ref/Alt: G/A NM_000259.3(MYO5A):c.3736C>T (p.Arg1246Cys)	missense	9207796 10733681	Pastural et al., 1997 Lambert et al., 2000
rs4774621	Benign: Griscelli syndrome, Type 1	52,383,064(-)	Ref/Alt: T/C NM_000259.3(MYO5A):c.2012+27A>G	intron	-	Genome Nilou, 2021+
rs11386962	Benign: Griscelli syndrome, Type 1	52,348,793(-)	Ref/Alt: T/TA NM_000259.3(MYO5A):c.3858+12dup	intron	-	Genome Nilou, 2021+
rs72623980	Benign: Griscelli syndrome, Type 1	52,410,502(-)	Ref/Alt: T/C NM_000259.3(MYO5A):c.613-26A>G	intron	-	Genome Nilou, 2021+
rs2414145	Benign: Griscelli syndrome, Type 1	52,375,355(-)	Ref/Alt: G/A NM_000259.3(MYO5A):c.2526= (p.Ile842=)	no sequence alteration	-	Genome Nilou, 2021+
rs1724577	Benign: Griscelli syndrome, Type 1	52,397,434(-)	Ref/Alt: T/G NM_000259.3(MYO5A):c.1086= (p.Asp362=)	no sequence alteration	-	Genome Nilou, 2021+
rs1724623	Benign: Griscelli syndrome, Type 1	52,389,376(-)	Ref/Alt: A/G NM_000259.3(MYO5A):c.1543-13T>C	intron	-	Genome Nilou, 2021+
rs61363656	Benign: Griscelli syndrome, Type 1	52,416,305(-)	Ref/Alt: T/C NM_000259.3(MYO5A):c.456-4A>G	intron	-	Genome Nilou, 2021+
rs71425744	Benign: Griscelli syndrome, Type 1	52,389,447(-)	Ref/Alt: AT/A NM_000259.3(MYO5A):c.1543-84del	intron	-	Mendelics, 2019+
rs2290333	Benign: Griscelli syndrome, Type 1	52,319,274(-)	Ref/Alt: G/T NM_000259.3(MYO5A):c.4945C>A (p.Arg1674=)	synonymous	-	Fulgent Genetics, 2022+

Genomic location annotation version GRCh38; Variants information is currently extracted from OMIM, ClinVar VCF, dbSNP and UniProt's Human polymorphisms and disease mutations database. * ClinVar Accession; + ClinVar Submitter.

Molecular analyses of patient-derived fibroblasts confirmed the lack of myosin-Va protein expression, since neither full-length nor truncated forms were detected by Western blot or immunofluorescence. The lack of detectable protein is consistent with nonsense-mediated mRNA decay, a well-characterized mechanism that degrades transcripts containing premature stop codons (He & Jacobson, 2015). This conclusion is supported by demonstration of very low levels of mutant *MYO5A* mRNA in the patient's fibroblasts, as well as reduced representation of mutant cDNA in sequencing reactions from heterozygous parental samples. An increased expression of myosin-Vb and myosin-Vc in patient fibroblasts suggested a possible compensatory mechanism within class V myosins, which may partially mitigate the loss of myosin-Va function in certain cell types and could contribute to the extended lifespan observed in Patient 1. However, further studies will be required to explore the role of a possible compensation in different cell types, particularly neurons, in which myosin-Va is essential for synaptic vesicle dynamics and organelle trafficking.

The fibroblast cultures generated in this study represents an important resource for investigating the molecular pathophysiology of GS-1. Although the control fibroblasts were derived from a genetically unrelated donor, they are age and gender matched, providing a useful reference for comparative analyses. For future studies, advanced tools of gene editing could be employed to correct the mutation in the patient fibroblasts, generating isogenic lines for functional studies. Conversely, introducing the mutation into fibroblasts from healthy donors would enable evaluation of its pathogenicity in diverse genetic backgrounds. Reprogramming these fibroblasts using protocols to induce pluripotent stem cells (iPSCs) would allow differentiation into relevant cell types, such as neurons and melanocytes, to model GS-1 with greater fidelity (Liu et al., 2018). This approach could provide critical insights into tissue-specific manifestations of the disease and facilitate the development of targeted therapies.

Beyond the established roles for *MYO5A* in melanocytes and neurons, several studies have shown the association of myosin-Va with the centrosome (Espreafico et al., 1998) and the primary cilia leading to the notion that myosin-Va is required for primary cilia biogenesis and maintenance (Assis et al., 2017; Kohli et al., 2017; Wu et al., 2018). Assis et al. (2017) demonstrated that myosin-Va interacts with RPGRIP1L, a ciliary protein required

for primary cilia maintenance. Pathogenic genetic variants of RPGRIP1L are the basis for defects in primary cilia implicated in autosomal recessive disorders such as Joubert syndrome type 7 (JBTS7, MIM:610937) and Meckel syndrome type 5 (MKS5, MIM:603194). These syndromes share overlapping neurological features with GS-1, including cerebellar atrophy, developmental delay, and hypotonia. Additionally, recent findings suggest that myosin-Va dysfunction may contribute to neurodegenerative processes, as seen in Parkinson's disease caused by LRRK2 mutations (Dhekne et al., 2021). In this context, loss of myosin-Va function has been linked to impaired cilia biogenesis and turnover, potentially underlying some of the neurological impairments observed in GS-1.

Previous study has identified an interaction between myosin-Va and spermine synthase (SMS), an enzyme involved in polyamine metabolism (Dolce et al., 2019; Timson, 2019). According to Dolce et al. (2019) data myosin-Va and SMS interaction could regulate spermine synthase expression at transcriptional or post-transcriptional levels, as reduced spermine synthase mRNA and protein levels were observed in *MYO5A* knockdown MCF-7 cells as well as in the myosin-Va-deficient fibroblasts derived from the GS-1 patient described here. Pathogenic variants in the *SMS* gene, which encodes spermine synthase, cause Snyder-Robinson syndrome, a condition characterized by intellectual disability, muscle hypotonia, and oxidative stress (Becerra-Solano et al., 2009; de Alencastro et al., 2008). The phenotypic overlap between Snyder-Robinson syndrome and GS-1 raises the possibility that polyamine dysregulation may contribute to the pathophysiology of GS-1. Future studies should explore this hypothesis further, as it could identify new therapeutic approaches for managing the neurological symptoms of GS-1. *MYO5A* mutant fibroblasts (F.O) and control fibroblasts (R.O) described here have recently been immortalized with expression of hTERT and have been proved useful in experiments that confirmed the requirement of myosin-Va in the transport and release of infectious Oropouche viral particles from infected cells (Concha et al., 2024).

By describing this novel *MYO5A* variant, we aim to raise awareness about the occurrence of GS-1 and emphasize the importance of distinguishing among the GS subtypes. GS-2 (MIM:607624) is fatal if untreated but can be managed with hematopoietic stem cell heterologous transplantation or ex-vivo gene therapy followed by autologous transplantation in newborns before the onset of hemophagocytic lymphohistiocytosis (Bizario et al.,

2004; Masri et al., 2008; Minocha et al., 2017). In contrast, GS-1 currently lacks effective treatments beyond palliative care to alleviate neurological symptoms (Abd Elmaksoud et al., 2020; Khorram et al., 2023; Pastural et al., 1997; Pastural et al., 2000). However, advances in gene therapy and genome editing offer hope for future interventions that could be applied pre- or postnatally. This study not only expands the mutational spectrum of *MYO5A* but also establishes a valuable cellular model for investigating GS-1 pathogenesis and developing potential therapies.

Declarations

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Competing interests

The authors declare that there are no competing interests associated with the manuscript.

Ethical Approval

This study was conducted in accordance with Brazilian national ethical standards which are aligned with international ethical standards and with the 1964 Helsinki Declaration. The research protocol, including the collection and use of human fibroblasts and clinical data, was reviewed and approved by the Ethics committee of Ribeirão Preto Medical School, University of São Paulo, approval number: 915/99. Written informed consent was obtained from all participants (or their legal guardians, when necessary) prior to inclusion in the study, authorizing the use of their biological samples, anonymized clinical information, and related images for research and

publication purposes. All data were handled and presented to protect patient anonymity. Images were cropped to prevent recognition of any individual, and no identifying information is included in the text or figures.

Data availability statement

All data supporting the findings of this study are available within the paper and raw data are available from the corresponding author upon request. The full-length coding sequence of the *MYO5A* transcript carrying the c.2102G>A (p.Trp701Ter; W701X) pathogenic variant described in this study has been deposited in GenBank under accession number **PZ761537**.

Author Contributions

Ingrid P. Meschede: Conceptualization, investigation, methodology, formal analysis, visualization, writing - original draft, and writing - review and editing. Guilherme P. F. Nader: investigation, methodology. Johnny A. Rockenbach Zenzen: visualization, writing - review and editing. Jackeline S. Araújo: investigation, methodology, formal analysis, writing - review and editing. Barbara Yasmin Andrade Garcia: investigation, methodology. Rui M. P. da Silva-Jr: writing - review and editing. Vitor Merino Loes: writing - review and editing. Tatiane C. Izidoro-Toledo: investigation, methodology. Fabíola A. Castro: resources. André L. S. Pessoa: neurological evaluation of the patients. Fernando Kok: general evaluation of the patients. Erasmo Casella: general evaluation of the patients. João Carlos Bizário: Conceptualization. Anelisa Ramão: writing - review and editing. Enilza M. Espreafico: Conceptualization, funding acquisition, project administration, formal analysis, writing - original draft, and writing - review and editing.

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