Genetics in Medicine (2025) ■, 101251

ELSEVIER

Genetics in Medicine
An Official Journal of the ACMG

www.journals.elsevier.com/genetics-in-medicine

ARTICLE

Clinical and genetic delineation of autosomal recessive and dominant ACTL6B-related developmental brain disorders

Elisa Cali[1], Tania Quirin[2], Clarissa Rocca[1], Stephanie Efthymiou[1], Antonella Riva[3], Dana Marafi[4,5], Maha S. Zaki[6], Mohnish Suri[7,8], Roberto Dominguez[9], Hasnaa M. Elbendary[6], Shahryar Alavi[1,10], Mohamed S. Abdel-Hamid[11], Heba Morsy[1,12], Frederic Tran Mau-Them[13,14], Mathilde Nizon[15,16], Pavel Tesner[17], Lukáš Ryba[17], Faisal Zafar[18], Nuzhat Rana[18], Nebal W. Saadi[19], Zahra Firoozfar[10], Pinar Gencpinar[20], Bulent Unay[21], Canan Ustun[21], Ange-Line Bruel[22,23], Christine Coubes[24], Jennifer Stefanich[25], Ozlem Sezer[26], Emanuele Agolini[27], Antonio Novelli[27], Gessica Vasco[28], Donatella Lettori[28], Mathieu Milh[29], Laurent Villard[30], Shimriet Zeidler[31], Henry Opperman[32], Vincent Strehlow[32], Mahmoud Y. Issa[6], Hebatallah El Khassab[33], Prem Chand[34], Shahnaz Ibrahim[34], Ali Rashidi-Nezhad[35,36], Mohammad Miryounesi[37], Pegah Larki[37], Jennifer Morrison[38], Ingrid Cristian[38], Isabelle Thiffault[39,40,41], Nicole L. Bertsch[42], Grace J. Noh[43], John Pappas[44,45], Ellen Moran[46], Nikolaos M. Marinakis[47], Joanne Traeger-Synodinos[47], Susan Hosseini[48], Mohammad Reza Abbaszadegan[49], Roseline Caumes[50], Lisenka E.L.M. Vissers[51,52], Maedeh Neshatdoust[53], Mostafa Montazer Zohour[54], Elmostafa El Fahime[55], Christina Canavati[56], Lara Kamal[56], Moien Kanaan[56], Omar Askander[57], Victoria Voinova[58,59], Olga Levchenko[60], Shahzhad Haider[61], Sara S. Halbach[62], Rayana Elias Maia[63], Salehi Mansoor[64,65], Vivek Jain[66], Sanjukta Tawde[67], Viveka Santhosh R. Challa[68], Vykuntaraju K. Gowda[68], Varunvenkat M. Srinivasan[68], Lucas Alves Victor[69], Benito Pinero-Banos[70], Jennifer Hague[71], Heba Ahmed ElAwady[72], Adelia Maria de Miranda Henriques-Souza[73], Huma Arshad Cheema[74], Muhammad Nadeem Anjum[74], Sara Idkaidak[75], Firas Alqarajeh[76], Osama Atawneh[76], Hagar Mor-Shaked[77], Tamar Harel[77], Giovanni Zifarelli[78], Peter Bauer[78], Fernando Kok[79], Joao Paulo Kitajima[79], Fabiola Monteiro[79], Juliana Josahkian[79], Gaetan Lesca[80,81], Nicolas Chatron[80,81], Dorothe Ville[82], David Murphy[83], Jeffrey L. Neul[84],

The Article Publishing Charge (APC) for this article was paid by Wellcome Trust (WT093205MA and WT104033AIA).

*Correspondence and requests for materials should be addressed to Reza Maroofian, Department of Neuromuscular Diseases, University College London, Queen Square, Institute of Neurology, WC1N 3BG London, UK. *Email address:* r.maroofian@ucl.ac.uk

Affiliations are at the end of the document.

doi: https://doi.org/10.1016/j.gim.2024.101251

Sureni V. Mullegama[85], Amber Begtrup[85], Isabella Herman[86], Tadahiro Mitani[4], Jennifer E. Posey[4], Chee Geap Tay[87], Iram Javed[88], Lucinda Carr[89], Farah Kanani[90], Fiona Beecroft[90], Lee Hane[91], Elsayed Abdelkreem[92], Milan Macek[17], Luciana Bispo[93], Marwa Abd Elmaksoud[94], Farzad Hashemi-Gorji[95], Davut Pehlivan[4,86], David J. Amor[96], Rami Abou Jamra[32], Wendy K. Chung[97], Eshan Ghayoor Karimiani[98,99,100], Philippe M. Campeau[101,102], Fowzan S. Alkuraya[103], Alistair T. Pagnamenta[104], Joseph G. Gleeson[105,106], James R. Lupski[4,107,108], Pasquale Striano[3,109], Andres Moreno-De-Luca[110], Denis L.J. Lafontaine[2], Henry Houlden[1], Reza Maroofian[1,*]

ARTICLE INFO

Article history:
Received 7 December 2023
Received in revised form
4 September 2024
Accepted 5 September 2024
Available online 17 September 2024

Keywords:
ACTL6B
Autism
BAFopathies
Epileptic-dyskinetic encephalopathy
Ribosomopathies

ABSTRACT

Purpose: This study aims to comprehensively delineate the phenotypic spectrum of *ACTL6B*-related disorders, previously associated with both autosomal recessive and autosomal dominant neurodevelopmental disorders. Molecularly, the role of the nucleolar protein ACTL6B in contributing to the disease has remained unclear.

Methods: We identified 105 affected individuals, including 39 previously reported cases, and systematically analyzed detailed clinical and genetic data for all individuals. Additionally, we conducted knockdown experiments in neuronal cells to investigate the role of *ACTL6B* in ribosome biogenesis.

Results: Biallelic variants in *ACTL6B* are associated with severe-to-profound global developmental delay/intellectual disability, infantile intractable seizures, absent speech, autistic features, dystonia, and increased lethality. De novo monoallelic variants result in moderate-to-severe global developmental delay/intellectual disability, absent speech, and autistic features, whereas seizures and dystonia were less frequently observed. Dysmorphic facial features and brain abnormalities, including hypoplastic corpus callosum, and parenchymal volume loss/atrophy, are common findings in both groups. We reveal that in the nucleolus, ACTL6B plays a crucial role in ribosome biogenesis, particularly in pre-rRNA processing.

Conclusion: This study provides a comprehensive characterization of the clinical spectrum of both autosomal recessive and dominant forms of *ACTL6B*-associated disorders. It offers a comparative analysis of their respective phenotypes provides a plausible molecular explanation and suggests their inclusion within the expanding category of "ribosomopathies."

Introduction

Actin-like 6B (*ACTL6B*, HGNC:160) encodes an actin-related protein that constitutes part of the neuronal BRG1/brm-associated factor (nBAF) complex.[1] The BAF complex, also known as SWI/SNF complex, comprises at least 15 subunits that induce conformational changes in nucleosomes, thereby regulating chromatin structure and DNA accessibility in an ATP-dependent manner.[2,3] The nBAF complex plays a key role in epigenetic regulatory mechanisms guiding neurodevelopmental processes, including neurogenesis (cell proliferation and differentiation), cell migration, maturation, and neuronal integration.[4]

ACTL6B (also known as BAF53B) is specifically found in nBAF complexes of post-mitotic neurons where it appears to play a critical role in activity-dependent dendritic outgrowth and neuronal maturation.[5] Deficits in distinct subunits of this complex have been linked to a range of syndromic or non-syndromic neurodevelopmental disorders (NDD). Pathogenic loss-of-function (LoF) or dominant negative (DN)/gain-of-function (GoF) variants in numerous subunits, such as *ARID1A* (HGNC:11110), *ARID1B* (HGNC:18040), *ARID2* (HGNC:18037), *SMARCC2* (HGNC:11105), *SMARCD1* (HGNC:11106) (LoF) and *SMARCA4* (HGNC:11100), *SMARCE1* (HGNC:11109), *SMARCB1* (HGNC:11103)(DN/GoF), cause Coffin-Siris syndrome, which is an autosomal dominant disorder characterized by intellectual disability/global developmental delay (ID/GDD), speech impairment, feeding difficulties, coarse facial features, hypertrichosis, hypoplastic fifth finger and toenails, and corpus callosum agenesis[6-8] (MIM 135900, 614607, 614608, 614609, 616938, 617808, 618027, 618362, and 618779). Furthermore, pathogenic heterozygous variants in *SMARCA2* (HGNC:11098) are associated with 2 different conditions: Nicolaides-Baraitser

syndrome, characterized by severe GDD/ID, early-onset seizures, short stature, dysmorphic facial features, and sparse hair (MIM 601358), and a congenital disorder that comprises distinct facial features, blepharophimosis, and GDD (blepharophimosis-intellectual impairment syndrome, MIM 619293).[9,10] Pathogenic variants in *BCL11A* are associated with an autosomal dominant intellectual developmental disorder with dysmorphic features and asymptomatic persistence of fetal hemoglobin, referred to as Dias Logan syndrome (MIM 617101),[11] whereas monoallelic variants in *BCL11B* are linked to either intellectual developmental disorder with dysmorphic facies, speech delay, and T cell abnormalities (MIM 618092) or severe combined immunodeficiency with intellectual disability, spasticity, and craniofacial abnormalities (MIM 617237).[12] Biallelic pathogenic variants in *ACTL6B* have been associated with a subtype of developmental and epileptic encephalopathy (DEE) characterized by severe GDD, epilepsy, cerebral atrophy, and abnormal central system myelination (DEE76, MIM 618468).[5,13-17] Additionally, 2 recurrent de novo missense *ACTL6B* variants have been linked with a dominant NDD characterized by intellectual disability, and severe speech and gait disturbances (intellectual developmental disorder with severe speech and ambulation defects, MIM 618470).[5] Since 2015, 39 patients from 31 unrelated families carrying either biallelic (29 patients) or de novo (10 patients) variants have been reported in 6 separate publications.[5,13-17] Nevertheless, a comprehensive study describing the full range of genotypic and phenotypic manifestations associated with *ACTL6B* variants is lacking.

In a high-throughput study, ACTL6B was tentatively assigned to the nucleolus,[18] a multi-layered biomolecular condensate in which the initial steps of ribosome biogenesis occur. Ribosome biogenesis disorders, collectively referred to as "ribosomopathies," encompass an emerging class of syndromes, including neurological disorders, caused by pathogenic variants in ribosomal proteins or in ribosomal assembly factors.[19] Despite partial elucidation of the nBAF complex and ACTL6B protein's role in neuronal development, especially in association with DEE or NDD, no study has suggested ACTL6B's involvement in ribosome biogenesis so far. In this study, we define the spectra of *ACTL6B*-related disorders in 105 patients from 90 unrelated families, including 65 newly described patients harboring either biallelic ($n = 50$) or de novo ($n = 16$) variants in *ACTL6B*. In addition, we report a significant role of ACTL6B in ribosome biogenesis, providing a likely molecular explanation for the associated disorders. Thus, we propose that *ACTL6B*-related disorders are new forms of "ribosomopathies."

Materials and Methods

Patient ascertainment: Clinical and genetic evaluation

The affected individuals were identified through extensive data sharing with collaborators and database screening of several diagnostic and research genetic laboratories worldwide, as well as using GeneMatcher.[20] Informed consents were obtained from all study participants and/or their parents or legal guardians. Genome/exome sequencing was performed on genomic DNA extracted from blood in different diagnostic or research laboratories worldwide. When necessary, candidate variants were confirmed via Sanger sequencing in additional family members, especially in cases of presumed de novo variants or informative families with multiple affected or unaffected siblings. Comprehensive clinical data, including epileptic phenotype details (seizure duration/frequency) and family histories, were systematically collected from new and reported cases by recruiting clinicians and documented in structured data forms. Where available, electroencephalogram (EEG) recordings, clinical photos, videos, and brain magnetic resonance imaging (MRI) scans were reviewed by a multidisciplinary team comprising child neurologists, epileptologists, dysmorphologists, and neuroradiologists.

In silico analysis

Information on the ACTL6B protein was obtained from the AlphaFold Protein Structure database.[21] Protein tertiary structures were visualized using PyMOL (Schrödinger, LLC) and the variants were modeled in Coot.[22] All variants were standardized to NM_016188.5 /GRCh38/hg38.

Cellular studies

Cells were cultured under standard conditions. Knockdown experiments were carried out by reverse transfecting SH-Sy5y cells with dicer-substrate short interfering RNAs (DsiRNA) silencers (integrated DNA technologies), followed by total RNA extraction for subsequent denaturing RNA electrophoresis and northern blot analysis. Western blotting was performed on cell lysates collected 72 hours post-dicer-substrate short interfering RNAs treatment, with proteins separated by SDS-PAGE and transferred to a nitrocellulose membrane for detection using the BAF53B polyclonal antibody. Immunofluorescence assays were performed on SH-Sy5y and U2OS cells using antibodies targeting ACTL6B and nucleolar components. The acquired images were analyzed using FIJI (ImageJ).[23] Detailed explanation of the methods is available in the Supplemental Methods.

Results

Clinical characterization of disease spectrum

The comprehensive cohort, encompassing both autosomal recessive and dominant cases, comprises 105 patients from 90 unrelated families, with 62 females and 42 males (Supplementary Tables 1 and 2). Of these, 65 individuals are newly described, with ages at last evaluation ranging

from 3 months to 29 years (median 4, IQR 6.5). Table 1 provides an overview of the key clinical findings.

Autosomal recessive ACTL6B-related developmental and epileptic-dyskinetic encephalopathy

The autosomal recessive cohort comprises 46 females and 32 males from 65 families, with age at last evaluation ranging between 3 months and 17 years (median 4, IQR 7). Consanguinity was reported in 40 families. Compound heterozygous biallelic variants occurred in 6 of the 16 families without a historical report of consanguinity. Pregnancy and delivery were unremarkable for 26 of the 34 patients with available information. Prenatal manifestations, such as fetal abnormal or slow movements, oligohydramnios, and breech presentation, occurred in 2 fetuses requiring cesarean delivery. Preterm birth was reported in 8 infants, whereas 41 infants were born at term and 1 post-term (median 39 weeks, IQR 1.75). Birth parameters, including length and weight, when available, were within normal ranges for 85% and 89% of the newborns, respectively, whereas head circumference was reduced only in 1 case. Infantile hypotonia manifested in 34 infants (34/45, 76%). Failure to thrive (FTT) was documented in 95% of the children (53/56) and was likely due to feeding difficulties, often detected at birth or in infancy (42/46, 91%) and requiring the use of nasogastric tube or gastrostomy ($n = 17$).

Seizures manifested in 93% of the children (70/75) with the onset ranging from birth to 11 years (median age 2 months, IQR 6). A majority (81%) experienced their first seizure within the first year of life and 34% within the first month. In 4 individuals (6%), seizures occurred at birth and probably antenatally as well. Patients presented with both focal ($n = 18$) and generalized ($n = 40$) onset of seizures. Two exhibited focal-onset seizures with subsequent generalization, whereas 5 experienced only focal-onset seizures, and 26 exclusively had generalized seizures. Seizure types were variable and included myoclonic ($n = 30$, 8 focal, 17 generalized, 5 not specified), tonic ($n = 27$, 8 focal, 17 generalized, 2 not specified), tonic-clonic ($n = 11$, 2 focal with secondary generalization, 10 generalized), atonic ($n = 3$), and epileptic spasms ($n = 12$, 2 generalized, 10 not specified). Seizure type and onset were not specified for 16 and 13 individuals, respectively. When data on frequency were available, 92% of the patients presented seizures daily (up to 20-25/day), and clustering was reported in 64% of the cases (20/31). Seizure duration ranged from a few seconds to 5 minutes, and status epilepticus was reported in 10 children (12/32, 38%). When information on treatment was present, seizures were considered intractable in 88% of the patients. Treatment often included a combination of 2 to 5 antiseizure medications (ASMs) because seizures were rarely controlled by a single ASM (4/34, 12%). Although a distinct treatment pattern could not be determined, effective approaches often involved the combination of sodium valproate and other ASMs. Detailed information on the epileptic phenotype and individual treatment is available in Supplemental Table 3.

EEG abnormalities were recorded in 96% of the patients (53/55), including focal, multifocal, and diffuse epileptiform discharges, slowing of background activity, and subcortical changes. Notably, 1 patient exhibited EEG abnormalities despite the absence of clinical seizures. Although there were no distinctive or shared patterns among the abnormalities, certain age-related characteristics were found. A comprehensive summary of the results can be found in Supplemental Materials.

GDD and ID were reported in all the affected individuals (79/79, 100%). Motor delay was observed in 95% of individuals (62/65), with 74% failing to achieve unsupported sitting and 82% unable to accomplish walking (Figure 1D). A significant proportion (61/70, 87%) showed a complete lack of speech and language development, whereas the other 8 individuals (8/70, 11%) exhibited delayed speech, limited just to a few words. Ten patients had regression of motor and cognitive abilities (10/46, 22%). When formal cognitive assessments were performed, the degree of ID varied from moderate ($n = 3$), to severe ($n = 29$) and profound ($n = 22$). Behavioral abnormalities included attention deficit hyperactivity disorder, autism spectrum disorder (ASD), aggressivity, and sleep disturbance. Autistic-like features, such as sound hypersensitivity and stereotypic hand movements, were noted in 26 patients (26/42, 62%).

At the last evaluation, 78% of the patients (45/58) exhibited microcephaly (z-score range −7.5 to +1.5 SD). Notably, head circumference within normal ranges at birth suggested postnatal deceleration of head growth, indicative of progressive microcephaly. Tragically, 23% of patients succumbed to the disorder in infancy or early childhood (median age 2.83 years). The neurological examination showed limb hypertonia and spasticity in a substantial percentage of cases (53/70, 76%). Dystonia was observed in 55% of individuals (32/58), accompanied by increased deep tendon reflexes (35/52, 67%), muscle weakness (33/43, 77%), and axial hypotonia (34/70, 49%). The ophthalmological evaluation revealed a variable presence of refractive defects ($n = 9$), strabismus ($n = 3$), and nystagmus ($n = 4$) (16/31, 52%).

Neuroimaging studies were conducted on 63 individuals. Among these, 28 underwent independent review, whereas radiology reports were available for 35 (Supplementary Table 4). Of those with imaging available for independent review, most cases had low-resolution snapshots of brain MRIs and 3 had DICOM files. Fifty-two individuals (83%) exhibited abnormal findings, whereas 11 reportedly had a normal study (not independently reviewed). Of the reviewed cases ($n = 28$), the most common abnormalities included hypoplastic corpus callosum (96%), widened subarachnoid spaces (89%), global brain atrophy (86%), ventricular enlargement/ventriculomegaly (57%), white matter volume loss (57%), mega cisterna magna/enlarged cisterna magna (50%), hypomyelination (43%), and patchy white matter signal abnormalities/focal white matter lesions (21%).

Facial photographs and/or videos were reviewed for 29 children from 23 families (Figure 2A). The predominant

Table 1 Overview of ACTL6B-related clinical features

		Recessive Cases			Dominant Cases		
Features	HPO Codes	Newly Reported	Previously Reported	Total	Newly Reported	Previously Reported	Total
Gender	-	29 F, 20M	17F, 12M	46F, 32M	8F, 8M	8F, 2M	16F, 10M
Median age at last follow-up (IQR)	-	4.875 (7.25)	2.25 (3.75)	4 (7)	6 (2.87)	5.7 (4)	6 (9)
Zygosity	-	42 H, 8 CH	23H, 6 CH	65 H, 14 CH	15DN	10DN	25DN
Growth							
Congenital microcephaly	HP:0011451	1/27 (4%)	-(0/7)	1/34 (3%)	2/9 (22%)	1/2 (50%)	3/11 (27%)
Progressive microcephaly	HP:0000253	24/34 (71%)	21/24 (88%)	**45/58 (78%)**	4/12 (33%)	5/10 (50%)	9/22 (41%)
Feeding difficulties	HP:0011968	41/45 (91%)	+(1/1)	**42/46 (91%)**	8/15 (53%)	n/a	**8/15 (53%)**
Failure to thrive	HP:0001508	40/42 (95%)	13/14 (93%)	**53/56 (95%)**	5/13 (38%)	n/a	5/13 (38%)
Short stature	HP:0004322	16/29 (55%)	7/12 (58%)	23/41 (56%)	- (0/14)	n/a	-(0/16)
Hypotonia in infancy	HP:0008947	33/44 (75%)	+(1/1)	**34/45 (76%)**	14/16 (88%)	n/a	**13/15 (87%)**
Motor development							
Motor delay	HP:0001270	45/48 (94%)	17/17 (100%)	**62/65 (95%)**	16/16 (100%)	8/9 (89%)	**24/25 (96%)**
Unsupported sitting not achieved	-	32/45 (71%)	5/5 (100%)	**37/50 (74%)**	3/10 (30%)	1/3 (33%)	4/13 (30%)
Unsupported walking not achieved	-	36/45 (80%)	5/5 (100%)	**41/50 (82%)**	7/12 (58%)	4/7 (57%)	11/19 (61%)
Intellectual development							
Absent speech	HP:0001344	39/48 (81%)	22/22 (100%)	**61/70 (87%)**	12/16 (75%)	9/10 (90%)	**21/26 (81%)**
Delayed speech	HP:0000750	8/48 (17%)	-	8/70 (11%)	4/16 (25%)	-	4/26 (15%)
GDD/ID	HP:0001263	50/50 (100%)	29/29 (100%)	**79/79 (100%)**	16/16 (100%)	10/10 (100%)	**26/26 (100%)**
	HP:0001249						
Mild-to-moderate	-	2	1	3	4	-	4
Severe	-	19	10	29	7	10	17
Profound	-	21	1	22	3	-	3
Autistic features	HP:0000729	17/32 (53%)	9/10 (90%)	**26/42 (62%)**	7/13 (54%)	7/8 (88%)	**14/21 (67%)**
Neurological examination							
Seizures	HP:0001250	44/49 (90%)	26/26 (100%)	**70/75 (93%)**	1/16 (6%)	1/10 (10%)	2/26(8%)
Increased tendon reflexes	HP:0001347	27/40 (68%)	8/12 (67%)	**35/52 (67%)**	4/9 (44%)	n/a	4/9 (44%)
Hypotonia	HP:0001252	24/45 (53%)	10/25 (40%)	34/70 (49%)	9/15 (60%)	5/6 (83%)	**14/21 (67%)**
Hypertonia/spasticity	HP:0001276	31/45 (69%)	22/25 (88%)	**53/70 (76%)**	5/15 (33%)	-(0/5)	5/20 (25%)
Muscle weakness	HP:0001324	31/41 (76%)	+(2/2)	**33/43 (77%)**	11/16 (69%)	n/a	11/16 (69%)
Dystonia	HP:0001332	19/42 (45%)	13/16 (81%)	32/58 (55%)	4/15 (27%)	n/a	4/15 (27%)
Limb contractures	HP:0003121	14/37 (38%)	-(0/3)	14/40 (35%)	1/14 (7%)	n/a	1/14 (7%)
Dyskinetic movements	HP:0100022	10/38 (26%)	2/3 (67%)	12/41 (29%)	8/15 (53%)	n/a	8/15 (53%)
Brain MRI abnormalities	HP:0410263	28/38 (73%)	24/25 (96%)	**52/63 (83%)**	10/13 (77%)	3/7 (43%)	**13/20 (65%)**
Miscellaneous							
Dysmorphic features	HP:0001999	30/39 (77%)	8/8 (100%)	**38/47 (81%)**	9/15 (60%)	6/9 (67%)	**15/24 (63%)**
Abnormal spine curvatures	HP:0010674	15/42 (36%)	+(3/3)	18/45 (40%)	6/16 (38%)	n/a	6/16 (38%)
Lethality	HP:0003819	12/32 (38%)	2/29 (6%)	14/61 (23%)	-	-	-

Bold: most frequent features.

CH, compound heterozygous; *DN*, de novo; *F*, females; *GDD*, global developmental delay; *H*, homozygous; HPO, human phenotype ontology; *ID*, intellectual disability; *IQR*, interquartile range; *M*, males; *n/a*, not available.

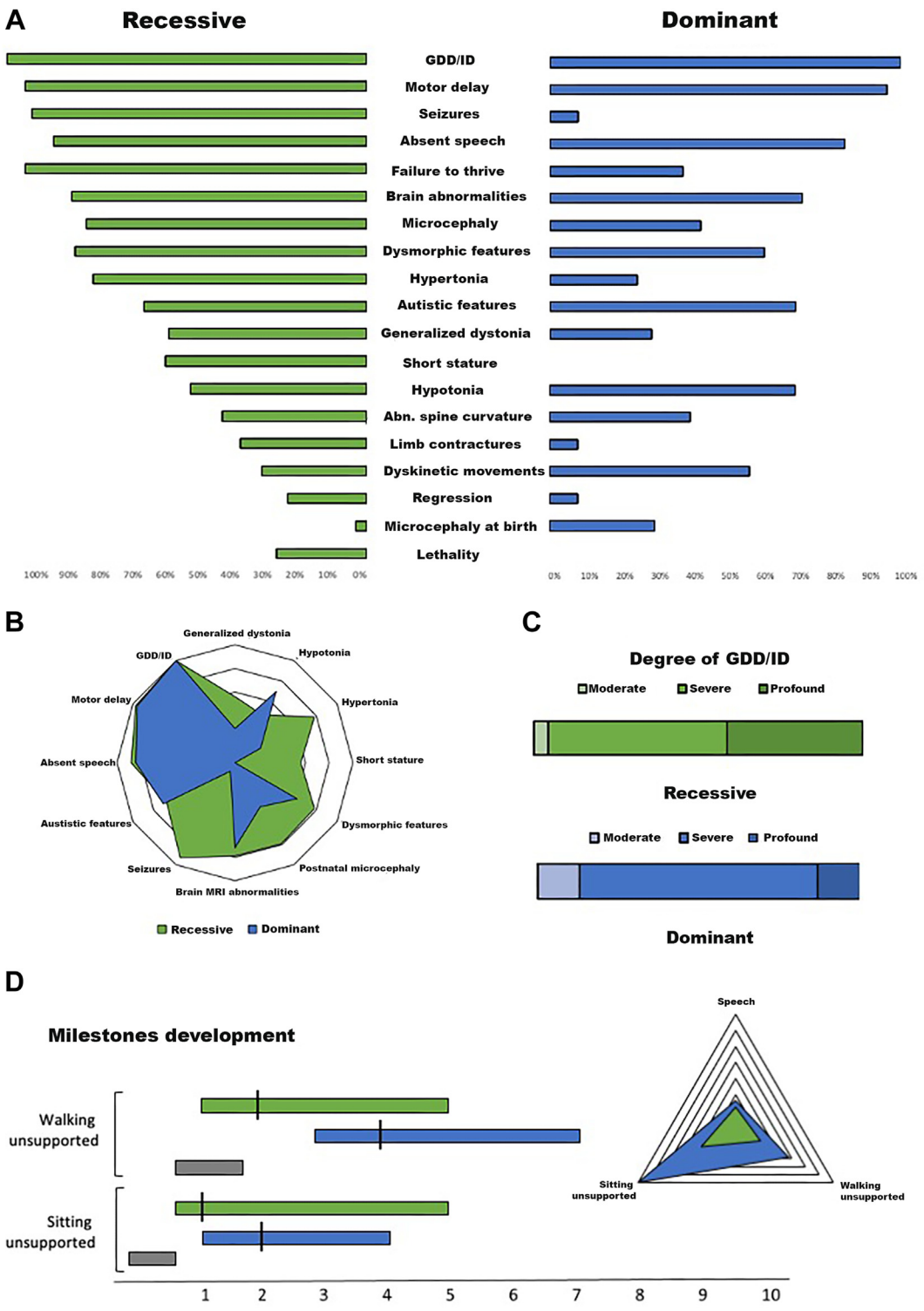

Figure 1 **Overview of dominant and recessive *ACTL6B*-related disorder.** A. Bar plot showing the frequency (%) of the main clinical features associated with *ACTL6B*-related disorder (on the left: recessive disorder; on the right: dominant disorder). B. Radar plot displaying the overlapping or distinguishable features of recessive and dominant disorder. C. Degree of global developmental delay (GDD)/intellectual disability (ID) in the disorders. D. Age span of achievement of developmental milestones in the recessive and dominant cohort compared with normal range. On the right, triangular radar plot showing the percentage of patients who achieved milestones at last evaluation.

Figure 2 **Dysmorphology, neuroimaging and epileptic findings in *ACTL6B*-related disorder.** A. Facial photographs of 18 newly reported patients with *ACTL6B* recessive disorder. B. Facial photographs of 4 newly reported patients with *ACTL6B* dominant disorder. C. Boxplot displaying head circumference measurements in SD to the norm. D. Bar plot showing the frequency (%) of the main dysmorphic features. E. Bar plot showing the frequency (%) of the main neuroimaging findings. F. Epileptic phenotype of patients with recessive disorder. On the left, bar plot documenting age at onset; in the middle, sunburst chart displaying seizure types, on the right, box plots documenting seizure duration (seconds) and frequency (seizures/day).

facial dysmorphic features observed in autosomal recessive *ACTL6B*-related NDD included narrow forehead/bifrontal/bitemporal narrowing (79%), full or broad nasal tip (76%), thick/full lower lip vermilion (65.5%), and broad, tall, or pointed chin (79%). Detailed facial dysmorphic features for all patients are shown in Supplemental Table 5 and tabulated using a unique Human Phenotype Ontology identification number to indicate the frequency of each feature. Supplemental Table 5 also includes patients for whom photographs were not available for assessment.

Autosomal dominant ACTL6B-related neurodevelopmental disorder

The cohort of cases with autosomal dominant (AD) inheritance comprises 16 females and 10 males, with their age at last evaluation ranging from 1 to 29 years (median 6 years, IQR 9). Consanguinity was reported in only 2 families. Limited information was available on the prenatal/perinatal period. When gestational age at birth was provided, delivery occurred after a full-term pregnancy in 93% of the cases, with 1 baby having a preterm delivery. For those cases where birth parameters of length, weight, and occipitofrontal head circumference were provided, 3 newborns presented with normal ranges, whereas the other 3 were small for gestational age or had low birthweight, and 3 presented with microcephaly at birth (3/10, 30%). Most babies (87%) exhibited infantile hypotonia (13/15). Feeding difficulties and FTT were present in 53% and 38% of the cases, respectively. Epilepsy occurred only in 2 children (2/25, 8%). One child presented with infantile spasms at 3 months, which subsided at 18 months, unresponsive to vigabatrin, and subsequently developed focal tonic-clonic seizures with secondary generalization at 18 years. In the other affected individual, generalized seizures began at 1.6 years of age, with each episode lasting 30 seconds and occurring daily (up to 5/day). In both cases, seizures were responsive to ASMs. EEG recordings were not available in either of the cases. EEG abnormalities were reported in only 1 patient with no clinical seizures (1/8; 12.5%). Particularly, irregular, slow-wave/sharp-wave complexes and diffusely disorganized and slow background activity were documented. All affected children manifested GDD and ID (26/26). Motor delay was documented in 96% of the patients. Half of the cohort had not achieved independent sitting or walking at the last evaluation, with median ages of achievement of unsupported sitting and walking being 2 and 4 years, respectively (IQR 0.8 and 3.5). Absence of speech was reported in 81% of the children (21/26), and speech development was delayed in the remaining 5 children (19%; median age at first words is 1.5 y).When formal cognitive assessment was performed, the degree of ID ranged from moderate ($n = 4$), severe ($n = 17$) to profound ($n = 3$). Autistic features were present in 14 patients (67%). At the last evaluation, less than half of the patients (9/22, 41%) had microcephaly. Neurological examination showed axial hypotonia in 67% of the patients (14/21), and variable presence of hypertonia (5/20, 25%) and dystonia (4/15, 27%). Fourteen patients with AD disorder underwent neuroimaging studies, with 12 having only radiology reports available and 2 whose imaging were independently reviewed. Brain abnormalities were detected in 86% of the cases who had neuroimaging performed and common findings included: prominence of the subarachnoid spaces (29%), hypoplastic corpus callosum (21%), brain atrophy (21%), ventricular enlargement (21%), hypomyelination (21%), and mega cisterna magna (14%) (Supplemental Table 4). Facial photographs and/or videos from 10 children across 10 families were subjected to review (Figure 2B). Based on this assessment, the most prevalent facial dysmorphic features include narrow forehead/bifrontal/bitemporal narrowing (60%), full or broad nasal tip (60%), thick/full lower lip vermilion (50%), and broad, tall, or pointed chin (70%). Detailed facial dysmorphic features for all patients are presented in Supplemental Table 5, tabulated using unique human phenotype ontology IDs to demonstrate the frequency of each feature. Supplemental Table 5 also includes patients without available photographs for assessment.

Identification of biallelic and monoallelic *ACTL6B* variants delineate an allelic series

A total of 58 *ACTL6B* variants ($n = 54$ biallelic; $n = 4$ monoallelic) have been identified. Of those, 30 were novel and not previously reported in the literature. An overview of the molecular findings is available in Figure 3 and Supplemental Tables 6 and 7. Notably, all identified variants were consistently predicted as detrimental across a suite of in silico tools, affecting highly evolutionarily conserved residues. According to the American College of Medical Genetics classification, 35 were classified as pathogenic, 20 as likely pathogenic, and 3 as variants of uncertain significance. The biallelic variants identified in this study were found to be inherited from unaffected heterozygous parents. Specifically, among the patients, 60 inherited the variant in a homozygous state, whereas 14 patients inherited it in a compound heterozygous state. The molecular spectrum hereby described includes 24 nonsense variants (comprising 8 frameshift and 16 stop gain), 18 missense variants, 7 splicing variants, and 5 deletions.

Remarkably, all biallelic variants were either absent or found at very low allele frequencies in multiple variant frequency databases, ranging from 0.0 to 0.00002, (refer to Supplemental Table 6 for details). Notably, 6 variants were observed in more than 1 family. Particularly, variants p.(Phe147del) and p.(Ter427AspextTer33) were found in 8 and 4 independent families from different countries, respectively. This underscores the global relevance of these specific variants in the context of *ACTL6B*-disorders. These variants were divided into distinct groups based on their consequence, including missense, in-frame deletion, truncating, frameshift, and pre-mRNA splicing. Comprehensive comparisons were conducted across these genotypic groups and within individual variants in terms of clinical features. No evident genotype-to-phenotype correlation was identified in the recessive cohort (Figure 3). Four monoallelic

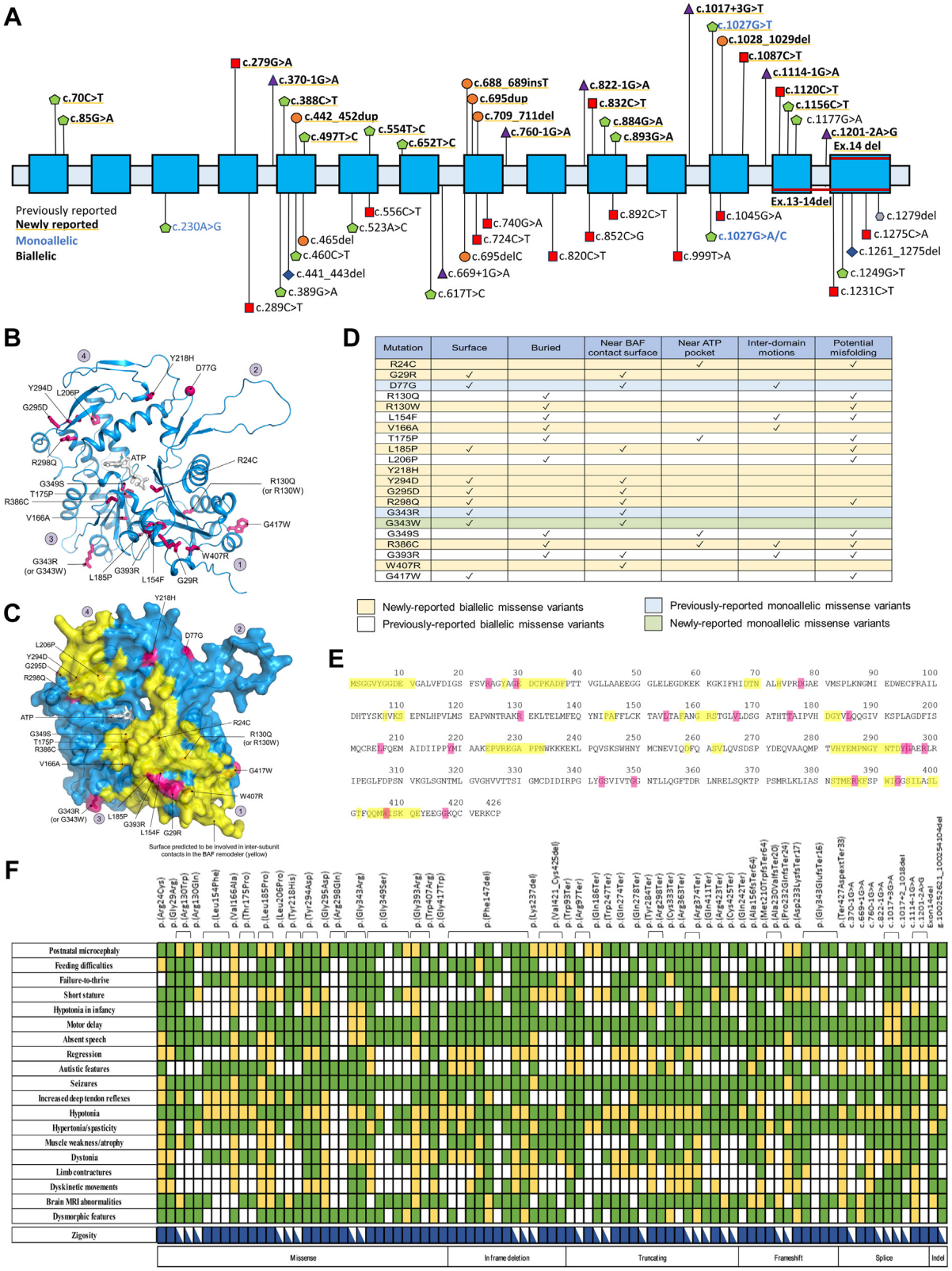

Figure 3 **Genetic findings and genotype-phenotype correlations.** A. Schematic drawing illustrating the gene structure of *ACTL6B* and the location of the biallelic and monoallelic variants identified. Green pentagon: missense variant. Red square: stop gain. Purple triangle: splice variant. Blue diamond: in-frame deletion. Gray octagon: frameshift. B. Ribbon representation of a 3-dimensional model of ACTL6B obtained with AlphaFold. The 4 missense variants identified in ACTL6B were modeled and are displayed in red. By analogy with actin, ATP is shown bound in the catalytic cleft of ACTL6B, and the 4 subdomains that characterize the reference actin fold are labeled (mauve-background circles). C. Partially transparent surface and ribbon representations of ACTL6B, showing the 4 missense variants in red.

variants were detected in the cohort, and all were de novo missense variants that were absent in several genetic variant frequency databases (listed in Supplemental Table 7). Notably, 3 of the variants occurred at the residue p.Gly343 in 24 independent families, indicating this location as a recurring hotspot for mutational events within a CpG dinucleotide.[24] Interestingly, 2 of the variants lead to the same amino acid change p.(Gly343Arg).

ACTL6B is a nucleolar protein required for efficient ribosome biogenesis

The subcellular distribution of endogenous ACTL6B was established by performing immunostaining in 2 distinct cell lines, namely, SH-Sy5y (neuroblastoma) and U2OS (osteosarcoma). In neuronal cells (SH-Sy5y), ACTL6B exhibited a distinctive localization pattern around the protein pescadillo ribosomal biogenesis factor 1 (PES1), forming a "ring". PES1 is known to label the granular subcompartment (GC) of the nucleolus, which is one of its most cortical parts. The ACTL6B ring colocalized perfectly with nucleophosmin (NPM1), another component of the GC (Figure 4A). Therefore, ACTL6B is positioned at the periphery of the GC of the nucleolus, as further supported by similar observations in U2OS cells in which ACTL6B also displayed a ring-like distribution (Figure 4B). To determine whether ACTL6B plays a role in ribosome biogenesis, particularly in pre-rRNA processing, SH-Sy5y cells were transfected with 2 independent silencers (#1 and #2) targeting the mRNAs encoding ACTL6B. After a 3-day incubation, total RNA was extracted and analyzed by northern blotting using a probe detecting major pre-rRNA processing intermediates (Figure 4C). Ethidium bromide staining of the agarose gel was also conducted to visualize the large mature rRNAs (18S and 28S) (Figure 4D). Efficient protein depletion was confirmed through Western blot analysis with a specific antibody (Figure 4E).

Although the levels of large mature rRNAs were not significantly affected by ACTL6B depletion, notable inhibitions in pre-rRNA processing were observed. Specifically, there was an accumulation of the 30S pre-rRNA (indicating inhibition of cleavage at site A0 in pathway 2, see Figure 4F) and an accumulation of the 21S pre-rRNA (indicating inhibition at site C). These observed phenotypes were consistent with both silencers. Additionally, other phenotypes were observed only with 1 of the 2 silencers (#2), including a reduction in the primary transcript, the 47S (possibly reflecting an increased turnover), and a mild reduction of 12S (suggesting inhibition at site 3′ and/or increased turnover). In conclusion, ACTL6B is a protein that localizes primarily at the periphery of the nucleolus where is it important for efficient pre-rRNA processing and thus for optimal ribosomal subunit biogenesis.

Discussion

This study provides a comprehensive overview of *ACTL6B*-related disorders, including those associated with rare NDD inherited as autosomal recessive (AR, biallelic) and dominant (AD, monoallelic) traits. The core phenotype observed in both AR and AD disorders manifests as moderate-to-profound GDD/ID, minimal or absent speech, and autistic features. Overall, our findings are consistent with previously reported cases and emphasize the cardinal features and clinical synopsis of the disorder. A critical distinguishing factor between AR and AD disorders lies in the presence or absence of an epileptic phenotype. Strikingly, seizures occurred in 93% of the recessive cases, 88% of which were classified as intractable seizures. In contrast, only 2 patients with monoallelic variants presented an epileptic disorder, effectively managed by ASMs. Such a marked contrast suggests a potential link to distinct pathomechanisms underlying these disorders. Unravelling the influence of these mechanisms on epilepsy's onset, progression, and response to treatment holds promise for tailoring more effective therapeutic interventions for affected individuals.

However, other clinical features exhibited a higher prevalence in the AR disorder when compared with the AD cases. Patients with AR disorder frequently presented FTT (95%) and short stature (56%), whereas FTT was less commonly observed in the AD cohort (38%), and instances of short stature were never reported.

Motor delay was documented in nearly all cases of both AR (95%) and AD (96%) disorders. Nevertheless, the severity of the delay differed between the 2 cohorts. The percentage of achievement of motor milestones (such as walking and sitting) was notably lower in the AR group (18%-27%) compared with the AD group (39%-70%). ID

ACTL6B is a subunit of the BAF chromatin remodeler related to the yeast SWI/SNF remodeler, whose 3-dimensional structure has been determined using cryo-electron microscopy. ACTL6B is most closely related to actin-related protein7 (ARP7) of yeast SWI/SNF. In the structures of yeast SWI/SNF, ARP7 makes contacts with other remodeler subunits. By analogy with ARP7, we show the surface of ACTL6B predicted to be involved in inter-subunit contacts within the BAF remodeler (yellow). D. Schematic table representation of the missense variants identified in ACTL6B and their localization/interactions. E. Sequence of ACTL6B highlighting residues undergoing missense variants (red) and residues likely to be involved in intersubunit contacts within the BAF remodeler (yellow). The diagrams in parts (D) and (E) were generated with the program PyMOL (Schrödinger, LLC). F. Phenotype-to-genotype correlations in the recessive cohort. Variants are grouped in the following categories: (1) missense, (2) in-frame deletion, (3) truncating, (4) frameshift, (5) pre-mRNA splice site, and (6) indel. Green: feature present; yellow: feature not present; blank: information not available. Blue rectangle: homozygous; blue triangle: compound heterozygous.

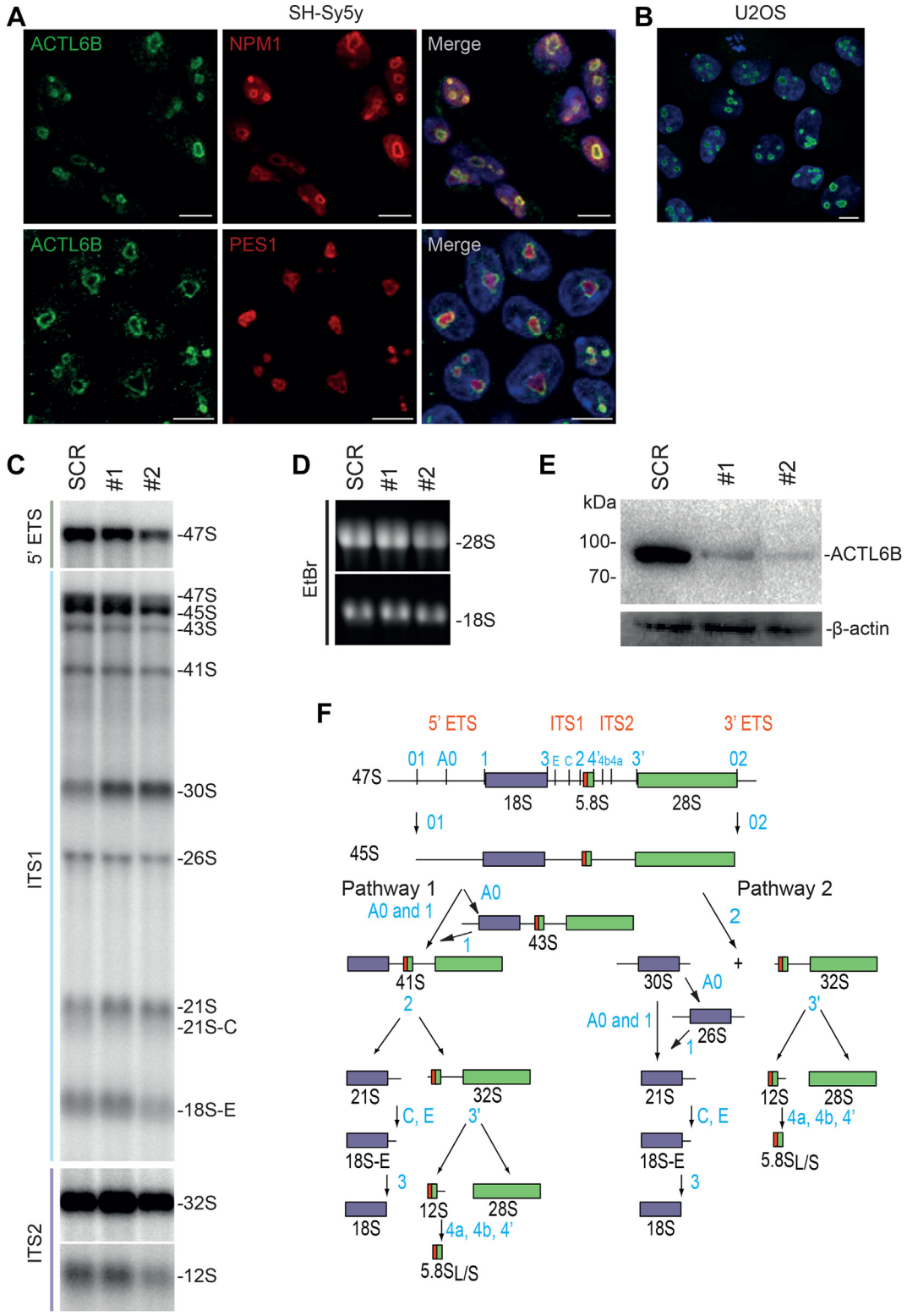

Figure 4 **ACTL6B is a nucleolar protein required for efficient pre-rRNA processing.** A. Subcellular distribution analysis of ACTL6B in SH-SY5Y cells. Cells were labeled with an antibody specific to ACTL6B (in green) and costained with an antibody specific to the nucleolar protein nucleophosmin (NPM1) or pescadillo (PES1) (in red). In the merge panels, the blue signal corresponds to the nucleoplasm (labeled with 4',6-diamidino-2-phenylindole). Scale bar, 1 µm. B. ACTL6B was immunostained (in green) with a specific antibody in U2OS cells. Blue, 4',6-diamidino-2-phenylindole. Scale bar, 1 µm. C. Pre-rRNA processing analysis upon ACTL6B depletion in SH-SY5Y cells. Cells were depleted with 25 nM siRNA for 3 days. Total RNA was extracted and separated on a denaturing gel, transferred to a nylon membrane, and processed for northern blotting with probes recognizing 5′ ETS, ITS1, or ITS2 sequences. Two silencers (#1 and #2) targeting a distinct region of *ACTL6B* transcripts were used independently. D. Mature rRNA analysis of samples described in panel (C). Ethidium

was universally observed in all patients (100%). Absent or limited speech was documented in the majority of cases with AR and AD disorders. There was a notable difference in the degree of ID, with a higher proportion of patients with biallelic variants exhibiting profound ID (41%) compared with the AD cohort (13%). However, both groups presented with a spectrum of severity ranging from moderate to profound.

During the neurological assessment, a substantial number of patients with biallelic variants exhibited increased tendon reflexes (69%) and hypertonia/spasticity (77%), whereas axial hypotonia was present in nearly half of the cases (48%). Conversely, individuals with de novo variations predominantly showed axial hypotonia (67%), with fewer cases presenting hypertonia or spasticity (25%) and hyperreflexia (44%). Dystonia was more frequent in AR disease (54% vs 27%), whereas dyskinetic movements were relatively more common in AD disorder (53% vs 30%). Autistic features were consistently present in both cohorts (62%-67%).

Neuroradiological abnormalities were observed in both groups, although the patients with AR disorder presented with a higher prevalence of abnormalities and additional findings consistent with hypoplastic corpus callosum, parenchymal volume loss/atrophy, and prominence of the subarachnoid spaces. It is crucial to note a limitation in this comparison due to the restricted availability of imaging for AD cases. Only 2 MRI snapshots were accessible for independent review, both of which unveiled the same abnormalities found in the AR cohort.

Upon examination of the available photographs, the most common facial features observed in both groups consist of a narrow forehead/bifrontal/bitemporal narrowing, full or broad nasal tip, thick/full lower lip vermilion, and broad, tall, or pointed chin. Our evaluation indicates the absence of a recognizable facial gestalt for either AR or AD *ACTL6B*-related NDD.

This study emphasizes the cardinal and distinct features of the 2 *ACTL6B*-related disorders (DEE76 and IDDSSAD), providing a differential diagnosis for BAFopathies, which includes various NDDs resulting from mutations in different subunits of the BAF complex (Supplemental Figure 1, Supplemental Table 8). Monoallelic variants in genes encoding BAF complex subunits are linked to different forms of syndromic intellectual development disorders, including Coffin-Siris syndrome and Nicolais-Baraitser syndrome. Although these disorders share commonalities with *ACTL6B*-related disorders, including GDD/ID, microcephaly, short stature, and autistic features, they are distinguishable by characteristic facial and hand/digital features. Unlike *ACTL6B*-related disorders, epilepsy is usually not a prominent feature in Coffin-Siris syndrome and Nicolais-Baraitser syndrome, and movement disorders or dystonia are not reported. Epilepsy is also extremely rare in the *ACTL6B* dominant cohort, suggesting its rarity in dominant BAFopathies.

Another autosomal recessive BAFopathy, involving biallelic variants in *SMARCD2*, has been reported. However, its distinct manifestations, centered around recurrent infections and hematologic abnormalities, set it apart from *ACTL6B*-related disorders. Although GDD/ID is observed, limited knowledge about its association with neurodevelopment complicates establishing a precise differential diagnosis. The nBAF complex plays a crucial role in various neurodevelopmental processes, influencing neurogenesis in terms of both cell proliferation and differentiation, cell migration, maturation, and integration of neurons. Recent studies have shed light on the involvement of the neuronal BAF complex in development of ASD development. Pathogenic variants in genes encoding BAF complex subunits, such as *ARID1B*, *SMARCA2*, *SMARCC1*(HGNC:11104), and *SMARCA4*, have been linked to an elevated risk of ASD. Autistic features were frequently observed in both AR and AD cases of *ACTL6B*-related disorders, providing additional evidence of the connection between nBAF complex dysfunction and ASD. However, the intricate relationship between the 2 remains not fully understood, warranting further research to elucidate the underlying mechanisms.

Ribosome biogenesis dysfunction has been linked to tissue-specific disorders affecting primarily the brain, the blood, and the bones.[26] Most of these diseases are deeply rooted in the embryonic development, and the brain disorders are no exception. It was notably shown using different animal models (frog, mouse, etc.) that optimal ribosome biogenesis is instrumental for proper maturation of neural crest cells, which are important for the development of the bone structure of the head and of the peripheral nervous system, among others.[27,28] The importance of ribosome biogenesis in neurological disorders was also illustrated by mutations in a gene encoding the methyltransferase METTL5-TRM112 acting on the small ribosomal subunit rRNA, the 18S. The absence of this methyltransferase leads to production of ribosomes lacking a single methyl group.[29] This could be considered a rather insignificant difference considering the ribosome is a multi-mega-kDa nanomachine, yet it is enough to cause GDD, microcephaly and associated manifestations,[30] including ID and unbalanced walking, which could be recapitulated in a mutant mouse and fly, respectively.[31,32]

bromide (EtBr) staining revealed the steady-state levels of mature 18S and 28S rRNAs. E. Western blot analysis confirming efficient protein depletion. The antibody used are described in the Materials and Methods. As control for loading, the blot was probed for β-actin. F. Pre-rRNA processing pathway depicting major intermediates and probes used. See https://doi.org/10.1016/j.biochi.2012.02.001[25] for details.

Our study expands the genotypic spectrum of both *ACTL6B*-associated disorders, introducing 26 biallelic and 1 monoallelic variant that were not previously reported. All biallelic variants are anticipated to result in LoF, according to Bell et al,[5] and are distributed across the protein in intradomain structured regions.[33] Conversely, monoallelic variants affecting the amino acid residue p.Gly343 are expected to exert DN effects, influencing the affinity to nucleosomal DNA by introducing a side chain upon mutation variation to arginine or tryptophan.[5,33] *ACTL6B* is among the first genes of the multisubunit protein BAF complex for which both recessive and dominant variants are pathogenic, with distinct consequences on protein function (LoF vs DN). Pathogenic monoallelic variants in other BAF complex genes are predicted to result in either LoF (*ARID1A*, *ARID1B*, *ARID2*, *SMARCC2*, and *SMARCD1*) or DN/GoF (*SMARCA4*, *SMARCE1*, and *SMARCB1*). Furthermore, disruptions in various structural hubs of the BAF complex (eg, histone binding, catalytic ATPase, and actin-related protein- module) might contribute to the variability in symptoms and severity observed in different BAFopathies.[33]

This study also investigates the role of ACTL6B in ribosome biogenesis and provides a likely molecular explanation for *ACTL6B*-related disorders. ACTL6B had been assigned tentatively to the nucleolus,[11] a multilayer biomolecular condensate where ribosome biogenesis is initiated. Our study has confirmed the localization of ACTL6B in the nucleolus and has revealed that the protein is enriched in a ring at the periphery of the organelle. In vitro, experiments on model cells demonstrated that mature rRNAs levels were not grossly affected upon ACTL6B depletion. However, the protein is required for efficient pre-rRNA processing at specific cleavage steps; therefore, its dysfunction does compromise important steps of ribosome biogenesis. Ribosome biogenesis disorders, collectively referred to as ribosomopathies, designate an emerging class of syndromes, including neurological disorders, caused by pathogenic variants in a ribosomal protein or a ribosomal assembly factor. The association between ribosome biogenesis dysfunction and NDDs has only started to unveil. Future research is warranted to establish fully connections between these disorders and ribosomes. With the evidences presented here, we suggest to include *ACTL6B*-associated disorders in the expanding spectrum of ribosomopathies (Supplemental Table 9).

Although our study provides novel insights into *ACTL6B*-related disorders, it is important to acknowledge the potential limitations arising from our relatively small and homogenous sample size. This constraint may not fully encompass the genetic and phenotypic diversity present within the broader population affected by these conditions. Nevertheless, our work offers a comprehensive overview of *ACTL6B*-related disorders, including the disease spectrum, genotype-to-phenotype relationships, and differential diagnosis based on mutational status. These insights contribute valuable information for improving clinical diagnosis and guiding therapeutic approaches. Additionally, our study proposes a probable molecular explanation for the pathology: inefficient ribosome biogenesis. This process has been previously linked to defects in the brain (neurodevelopmental and neurological issues) and bones (skeletal development, short stature, etc). Therefore, our findings improve the understanding of the *ACTL6B*-related syndrome and contribute to the expanding knowledge of diseases associated with ribosome biogenesis dysfunction.

Data Availability

The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files. For families R9, R16 and D5, data are available in the National Genomics Research Library v5.1, Genomics England (https://doi.org/10.6084/m9.figshare.4530893/7. 2020).

Acknowledgments

We are grateful for the important support from patients and families, our UK and international collaborators, brainbanks and biobanks, and grateful for essential funding from The Wellcome Trust, The MRC, The MSA Trust, The National Institute for Health Research, University College London Hospitals Biomedical Research Centre (NIHR-BRC), The Michael J Fox Foundation (MJFF), The Fidelity Trust, Rosetrees Trust, Dolby Family fund, Alzheimer's Research UK (ARUK), MSA Coalition, Parkinson's disease society, Parkinson's Foundation, The Guarantors of Brain, Cerebral Palsy Alliance, FARA, EAN, Victoria Brain Bank, the NIH NeuroBioBank, Queen Square BrainBank, The MRC Brainbank Network.

Funding

This study was supported by the Wellcome Trust (WT093205MA and WT104033AIA to H.H.), Medical Research Council (H.H.), European Community's Seventh Framework Programme (FP7/2007-2013, under grant agreement No. 2012-305121 to H.H.), the National Institute for Health Research, University College London Hospitals, Biomedical Research Centre, and Fidelity Foundation. This study was also supported in part by US National Institutes of Health R35NS105078 to J.R.L., and UM1 HG011758 to J.E.P. and J.R.L., and Muscular Dystrophy Association (MDA) (512848 to J.R.L.). D.M. was supported by a Medical Genetics Research Fellowship Program through the United States National Institute of Health (T32 GM007526-42). D.P. is supported by International Rett Syndrome Foundation, Grant/Award Number: #3701-1. J.E.P. was supported by NHGRI K08 HG008986. T.M. was supported by the Uehara Memorial Foundation. R.D. was supported by NIH grant R01 GM073791. Research in the Lab of D.L.J.L. was supported by the Belgian Fonds de la Recherche

Scientifique (F.R.S./FNRS), EOS [CD-INFLADIS], Région Wallonne (SPW EER) Win4SpinOff [RIBOGENESIS], the COST action TRANSLACORE (CA21154), and the European Joint Programme on Rare Diseases (EJP-RD) RiboEurope and DBAGeneCure. M.M. and P.T. were supported by NW24-06-00083.

Author Contributions

Conceptualization: E.C., T.Q., C.R., S.E., D.L., H.H., R.M.; Data Curation: E.C., D.M., M.S., A.R., P.S., A.M.D.L., R.M.; Formal Analysis: E.C., T.Q., D.L., R.M.; Funding Acquisition: H.H., J.R.L., J.E.P., D.M., D.P., R.D., P.S., T.M.; Methodology: E.C., T.Q., C.R., M.S., R.D., A.M.D.L., S.E., D.L., R.M.; Supervision: D.L., H.H., R.M.; Writing-original draft: E.C., T.Q., M.S., R.D., A.M., D.L., R.M.; Writing-review and editing: all authors.

Ethics Declaration

Individuals (and/or their legal guardians) recruited in a research setting gave informed consent for their research participation. Those individual research studies received approval from the Review Boards and Bioethics Committees at University College London Hospital (project 06/N076) and the other institutions involved in this study. Permission for inclusion of their anonymized medical data in this cohort, including photographs, was obtained using standard forms at each local site by the responsible referring physicians.

Conflict of Interest

Sureni V. Mullegama and Amber Begtrup are employees of GeneDx. Lee Hane is an employee at 3billion. Christian Beetz is an employee at Centogene. All other authors declare no conflicts of interest.

Additional Information

The online version of this article (https://doi.org/10.1016/j.gim.2024.101251) contains supplemental material, which is available to authorized users.

Affiliations

[1]Department of Neuromuscular Diseases, University College London, Queen Square, Institute of Neurology, London, United Kingdom; [2]RNA Molecular Biology, Fonds de la Recherche Scientifique (F.R.S./FNRS), Université libre de Bruxelles (ULB), Biopark campus, Gosselies, Belgium; [3]Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa, Italy; [4]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; [5]Department of Pediatrics, Faculty of Medicine, Kuwait University, Safat, Kuwait; [6]Clinical Genetics Department, Human Genetics and Genome Research Institute, National Research Centre, Cairo, Egypt; [7]UK National Paediatric Ataxia Telangiectasia Clinic, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom; [8]Nottingham Clinical Genetics Service, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom; [9]Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [10]Palindrome, Isfahan, Iran; [11]Medical Molecular Genetics Department, Human Genetics and Genome Research Institute, National Research Centre Cairo, Egypt; [12]Human Genetics Department, Medical Research Institute, Alexandria University, Egypt; [13]Unité Fonctionnelle 6254 d'Innovation en Diagnostique Génomique des Maladies Rares, Pôle de Biologie, CHU Dijon Bourgogne, Dijon, France; [14]INSERM UMR1231 GAD, Dijon, France; [15]Service de génétique médicale, CHU de Nantes, Nantes, France; [16]Institut du thorax, INSERM, CNRS, UNIV Nantes, Nantes, France; [17]Department of Biology and Medical Genetics, 2nd Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic; [18]Department of Paediatric Neurology, Children's Hospital and Institute of Child Health, Multan, Pakistan; [19]College of Medicine/University of Baghdad, Unit of Pediatric Neurology, Children Welfare Teaching Hospital, Baghdad, Iraq; [20]İzmir Katip Çelebi University Tepecik Training and Research Department of Pediatric Neurology, Izmir, Turkey; [21]University of Health Sciences, Gülhane Faculty of Medicine, Department of Child Neurology, Ankara, Turkey; [22]Unité Fontctionnelle d'Innovation diagnostiques des maladies rares, FHU TRANSLAD, CHU Dijon Bourgogne, Dijon, France; [23]INSERM-Université de Bourgogne UMR1231 GAD "Génétique des Anomalies du Développement" Dijon, France; [24]Département de Génétique Médicale, Maladies rares et Médecine Personnalisée, et Centre de Référence Anomalies du Développement et Syndromes Malformatifs, CHRU de Montpellier, Montpellier, France; [25]Genetic Center, Akron Children's Hospital, Akron, OH; [26]Department of Medical Genetics, Samsun University, Faculty of Medicine, Samsun, Turkey; [27]Laboratory of Medical Genetics, Translational Cytogenomics Research Unit, Bambino Gesù Children Hospital, IRCCS, Rome, Italy; [28]Department of Neurosciences, Unit of Neurorehabilitation, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [29]Aix-Marseille Univ, APHM, department of Pediatrics Neurology. Timone children Hospital. Marseille, France; [30]Aix Marseille Univ, Inserm, MMG, Marseille, France Service de Génétique Médicale, AP-HM, Hôpital de La Timone, Marseille, France; [31]Department of Clinical Genetics, Erasmus MC,

Rotterdam, The Netherlands; [32]Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany; [33]Sulaiman Al Habib Hospital - Olaya Medical Complex-Riyadh, Saudi Arabia; [34]Department of Paediatric and Child Health, Aga Khan University Hospital, Karachi, Pakistan; [35]Maternal, Fetal and Neonatal Research Center, Family Health Research Institute, Tehran University of Medical Sciences, Tehran, Iran; [36]Genetics Ward, Yas Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran; [37]Department of Medical Genetics, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [38]Division of Genetics, Arnold Palmer Hospital for Children, Orlando Health, Orlando, FL; [39]Genomic Medicine Center, Children's Mercy Hospital, Kansas City, MO; [40]Kansas City School of Medicine, University of Missouri, Kansas City, MO; [41]Department of Pathology and Laboratory Medicine, Children's Mercy Hospital, Kansas City, MO; [42]The Community Health Clinic, Shipshewana, IN; [43]Department of Genetics, Southern California Permanente Medical Group, Fontana, CA; [44]Clinical Genetic Services, Department of Pediatrics, NYU Grossman School of Medicine, New York, NY; [45]Clinical Genetics, NYU Orthopedic Hospital, New York, NY; [46]Clinical Genetics, Center for Children, Hassenfeld Children's Hospital, New York University, New York, NY; [47]Laboratory of Medical Genetics, Medical School, National and Kapodistrian University of Athens, St. Sophia's Children's Hospital, Athens, Greece; [48]Pardis Pathobiology and Genetics Laboratory, Mashhad, Iran; [49]Department of Medical Genetics and Molecular Medicine, School of Medicine, Medical Genetics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran; [50]Service de Génétique Clinique, CHU Lille, Lille, France; [51]Department of Human Genetics, Radboudumc University Medical Center, Nijmegen, The Netherlands; [52]Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, The Netherlands; [53]Department of Cell and Molecular Biology and Microbiology, Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran; [54]Genetics of Non-Communicable Disease Research Center, Zahedan University of Medical Sciences, Zahedan, Iran; [55]Centre Mohamed VI for Research and Innovation (CM6RI) and University Mohamed VI for Health Science (UM6SS), Benguerir, Morocco; [56]Molecular Genetics Laboratory, Istishari Arab Hospital, Ramallah, Palestine; [57]Faculty of Medical Sciences, Mohammed 6 Polytechnic University of Benguerir, Ben Guerir, Morocco; [58]Veltischev Research and Clinical Institute for Pediatrics of the Pirogov, Russian National Research Medical University, Ministry of Health of Russian Federation, Moscow, Russia; [59]Mental Health Research Center, Moscow, Russia; [60]Research Centre for Medical Genetics, Moscow, Russia; [61]Paediatrics Wah Medical College NUMS, Wah Cantonment, Punjab, Pakistan; [62]University of Chicago Medicine, University of Chicago, Chicago, IL; [63]Department of Paediatrics and Genetics, Universidade Federal de Paraiba, Joao Pessoa, Paraiba, Brazil; [64]Cellular, Molecular and Genetics Research Center, Isfahan University of Medical Sciences, Isfahan, Iran; [65]Medical Genetics Research Center of Genome, Isfahan University of Medical Sciences, Isfahan, Iran; [66]Department of Pediatric Neurology, Neo Clinic Children's Hospital, Jaipur, India; [67]Department of Human Genetics, The University of Chicago, Illinois; [68]Department of Pediatric Neurology, Indira Gandhi Institute of Child Health, Bengaluru, India; [69]Department of Pediatric Neurology - Instituto de Medicina Integral Prof. Fernando Figueira (IMIP), Boa Vista, Recife, Brazil; [70]Oxford Genetics Laboratories, Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom; [71]Clinical Genetics service, Northampton General Hospital, Northampton, United Kingdom; [72]Department of Pediatrics, Fayoum University Hospitals, Fayoum, Egypt; [73]Instituto de Medicina Integral Prof. Fernando Figueira, Centro de Terapias Cetogênicas do IMIP, Recife PE, Brazil; [74]Department of Pediatric Gastroenterology Hepatology and Genetic diseases Children's Hospital and University of Child Health Sciences Lahore, Pakistan; [75]Al-Quds University, Jerusalem, Palestine; [76]PRCS hospital, Hebron, Palestine; [77]Department of Genetics, Hadassah Medical Center, Jerusalem, Israel Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israe; [78]CENTOGENE GmbH, Rostock, German; [79]Mendelics Genomic Analysis, São Paulo, Brazil; [80]Hospices Civils de Lyon, Service de Génétique, Bron, France; [81]Pathophysiology and Genetics of Neuron and Muscle (PGNM, UCBL - CNRS UMR5261 - INSERM U1315), Université Claude Bernard Lyon 1, Lyon, France; [82]Department of Neuropediatric, University Hospital of Lyon, Lyon, France; [83]Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, University College London, London, United Kingdom; [84]Department of Pediatrics, Vanderbilt Kennedy Center, Vanderbilt University Medical Center, Nashville, TN; [85]GeneDx, Gaithersburg, MD; [86]Section of Pediatric Neurology and Developmental Neuroscience, Department of Pediatrics, Baylor College of Medicine, Houston, TX; [87]Clinical Research Centre, Sunway Medical Centre, Malaysia; [88]Department of Paediatric Neurology, Children Hospital and Institute of Child Health, Faisalabad, Pakistan; [89]Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom; [90]West Midlands Clinical Genetics Service, Birmingham Women's Hospital, Birmingham, United Kingdom; [91]Division of Medical Genetics, 3billion, Inc, Seoul, South Korea; [92]Department of Pediatrics, Faculty of Medicine, Sohag University, Sohag, Egypt; [93]Laboratório Mendelics, Department of Genetic, São Paulo, Brazil; [94]Neurology Unit, Department of Pediatrics, Faculty of Medicine, Alexandria University, Egypt; [95]Genomic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [96]Department of Paediatrics, Murdoch Children's Research Institute and University of Melbourne, Royal Children's Hospital, Melbourne, Australia; [97]Department of Pediatrics, Boston Children's

Hospital, Harvard Medical School, Boston, MA; [98]Department of Medical Genetics, Next Generation Genetic Polyclinic, Mashhad, Iran; [99]Molecular and Clinical Sciences Institute, St. George's, University of London, Cranmer Terrace, London, United Kingdom; [100]Innovative Medical Research Center, Mashhad Branch, Islamic Azad University, Mashhad, Iran; [101]CHU Sainte-Justine Research Center, Montreal, QC, Canada; [102]Department of Pediatrics, Faculty of Medicine, Université de Montréal, Montreal, QC, Canada; [103]Department of Translational Genomics, Center for Genomic Medicine, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia; [104]NIHR Biomedical Research Centre, Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; [105]Department of Neurosciences, University of California, San Diego, La Jolla, CA; [106]Rady Children's Institute for Genomic Medicine, San Diego, CA; [107]Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX; [108]Department of Pediatrics, Baylor College of Medicine, Houston, TX; [109]Pediatric Neurology and Muscular Diseases Unit, IRCCS Istituto Giannina Gaslini, Genova, Italy; [110]Department of Radiology, Neuroradiology Section, Kingston Health Sciences Centre, Queen's University Faculty of Health Sciences, Kingston, Ontario, Canada

References

1. Olave I, Wang W, Xue Y, Kuo A, Crabtree GR. Identification of a polymorphic, neuron-specific chromatin remodeling complex. *Genes Dev*. 2002;16(19):2509-2517. http://doi.org/10.1101/gad.992102
2. Wu JI, Lessard J, Olave IA, et al. Regulation of dendritic development by neuron-specific chromatin remodeling complexes. *Neuron*. 2007;56(1):94-108. http://doi.org/10.1016/j.neuron.2007.08.021
3. Lessard J, Wu JI, Ranish JA, et al. An essential switch in subunit composition of a chromatin remodeling complex during neural development. *Neuron*. 2007;55(2):201-215. http://doi.org/10.1016/j.neuron.2007.06.019
4. Sokpor G, Xie Y, Rosenbusch J, Tuoc T. Chromatin remodeling BAF (SWI/SNF) complexes in neural development and disorders. *Front Mol Neurosci*. 2017;10:243. http://doi.org/10.3389/fnmol.2017.00243
5. Bell S, Rousseau J, Peng H, et al. Mutations in ACTL6B cause neurodevelopmental deficits and epilepsy and lead to loss of dendrites in human neurons. *Am J Hum Genet*. 2019;104(5):815-834. http://doi.org/10.1016/j.ajhg.2019.03.022
6. Sekiguchi F, Tsurusaki Y, Okamoto N, et al. Genetic abnormalities in a large cohort of Coffin-Siris syndrome patients. *J Hum Genet*. 2019;64(12):1173-1186. http://doi.org/10.1038/s10038-019-0667-4
7. Santen GWE, Aten E, Vulto-van Silfhout AT, et al. Coffin-Siris syndrome and the BAF complex: genotype-phenotype study in 63 patients. *Hum Mutat*. 2013;34(11):1519-1528. http://doi.org/10.1002/humu.22394
8. Chen CA, Lattier J, Zhu W, et al. Retrospective analysis of a clinical exome sequencing cohort reveals the mutational spectrum and identifies candidate disease-associated loci for BAFopathies. *Genet Med*. 2022;24(2):364-373. http://doi.org/10.1016/j.gim.2021.09.017
9. Cappuccio G, Sayou C, Tanno PL, et al. De novo SMARCA2 variants clustered outside the helicase domain cause a new recognizable syndrome with intellectual disability and blepharophimosis distinct from Nicolaides-Baraitser syndrome. *Genet Med*. 2020;22(11):1838-1850. http://doi.org/10.1038/s41436-020-0898-y
10. Abdul-Rahman O. Nicolaides-Baraitser syndrome. In: Adam MP, Mirzaa GM, Pagon RA, et al., eds. *GeneReviews*. Seattle: University of Washington; 2015. Accessed October 23, 2024. https://www.ncbi.nlm.nih.gov/books/NBK321516/
11. Peron A, Bradbury K, Viskochil DH, Dias C. In: *BCL11A-related intellectual disability*. Seattle: GeneReviews. University of Washington; 2019. Accessed October 23, 2024. https://www.ncbi.nlm.nih.gov/books/NBK547048/
12. Lessel D, Gehbauer C, Bramswig NC, et al. BCL11B mutations in patients affected by a neurodevelopmental disorder with reduced type 2 innate lymphoid cells. *Brain*. 2018;141(8):2299-2311. http://doi.org/10.1093/brain/awy173
13. Fichera M, Failla P, Saccuzzo L, et al. Mutations in ACTL6B, coding for a subunit of the neuron-specific chromatin remodeling complex nBAF, cause early onset severe developmental and epileptic encephalopathy with brain hypomyelination and cerebellar atrophy. *Hum Genet*. 2019;138(2):187-198. http://doi.org/10.1007/s00439-019-01972-3
14. Maddirevula S, Alzahrani F, Al-Owain M, et al. Autozygome and high throughput confirmation of disease genes candidacy. *Genet Med*. 2019;21(3):736-742. http://doi.org/10.1038/s41436-018-0138-x
15. Yüksel Z, Yazol M, Gümüş E. Pathogenic homozygous variations in ACTL6B cause DECAM syndrome: developmental delay, epileptic encephalopathy, cerebral atrophy, and abnormal myelination. *Am J Med Genet A*. 2019;179(8):1603-1608. http://doi.org/10.1002/ajmg.a.61210
16. Wenderski W, Wang L, Krokhotin A, et al. Loss of the neural-specific BAF subunit ACTL6B relieves repression of early response genes and causes recessive autism. *Proc Natl Acad Sci U S A*. 2020;117(18):10055-10066. http://doi.org/10.1073/pnas.1908238117
17. Han X, Deng J, Chen C, et al. Developmental and epileptic encephalopathy 76: case report and review of literature. *Children (Basel)*. 2022;9(12):1967. http://doi.org/10.3390/children9121967
18. Stenström L, Mahdessian D, Gnann C, et al. Mapping the nucleolar proteome reveals a spatiotemporal organization related to intrinsic protein disorder. *Mol Syst Biol*. 2020;16(8):e9469. http://doi.org/10.15252/msb.20209469
19. Hetman M, Slomnicki LP. Ribosomal biogenesis as an emerging target of neurodevelopmental pathologies. *J Neurochem*. 2019;148(3):325-347. http://doi.org/10.1111/jnc.14576
20. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. *Hum Mutat*. 2015;36(10):928-930. http://doi.org/10.1002/humu.22844
21. Jumper J, Evans R, Pritzel A, et al. Highly accurate protein structure prediction with AlphaFold. *Nature*. 2021;596(7873):583-589. http://doi.org/10.1038/s41586-021-03819-2
22. Emsley P, Lohkamp B, Scott WG, Cowtan K. Features and development of coot. *Acta Crystallogr D Biol Crystallogr*. 2010;66(4):486-501. http://doi.org/10.1107/S0907444910007493
23. Abramoff M, Magalhaes P, Ram S, et al. Image processing with Image. *J Biophot Int*. 2004;11(7):36-42.
24. Hodgkinson A, Eyre-Walker A. Variation in the mutation rate across mammalian genomes. *Nat Rev Genet*. 2011;12(11):756-766. http://doi.org/10.1038/nrg3098
25. Mullineux ST, Lafontaine DLJ. Mapping the cleavage sites on mammalian pre-rRNAs: where do we stand? *Biochimie*. 2012;94(7):1521-1532. http://doi.org/10.1016/j.biochi.2012.02.001
26. Farley-Barnes KI, Ogawa LM, Baserga SJ. Ribosomopathies: old concepts, new controversies. *Trends Genet*. 2019;35(10):754-767. http://doi.org/10.1016/j.tig.2019.07.004
27. Delhermite J, Tafforeau L, Sharma S, et al. Systematic mapping of rRNA 2′-O methylation during frog development and involvement of the methyltransferase fibrillarin in eye and craniofacial development in Xenopus laevis. *PLoS Genet*. 2022;18(1):e1010012. http://doi.org/10.1371/journal.pgen.1010012
28. Dixon J, Jones NC, Sandell LL, et al. Tcof1/Treacle is required for neural crest cell formation and proliferation deficiencies that cause craniofacial abnormalities. *Proc Natl Acad Sci U S A*. 2006;103(36):13403-13408. http://doi.org/10.1073/pnas.0603730103

29. van Tran N, Ernst FGM, Hawley BR, et al. The human 18S rRNA m6A methyltransferase METTL5 is stabilized by TRMT112. *Nucleic Acids Res*. 2019;47(15):7719-7733. http://doi.org/10.1093/nar/gkz619
30. Richard EM, Polla DL, Assir MZ, et al. Bi-allelic variants in METTL5 cause autosomal-recessive intellectual disability and microcephaly. *Am J Hum Genet*. 2019;105(4):869-878. http://doi.org/10.1016/j.ajhg.2019.09.007
31. Leismann J, Spagnuolo M, Pradhan M, et al. The 18S ribosomal RNA m6 A methyltransferase Mettl5 is required for normal walking behavior in Drosophila. *EMBO Rep*. 2020;21(7):e49443. http://doi.org/10.15252/embr.201949443
32. Wang L, Liang Y, Lin R, et al. Mettl5 mediated 18S rRNA N6-methyladenosine (m6A) modification controls stem cell fate determination and neural function. *Genes Dis*. 2022;9(1):268-274. http://doi.org/10.1016/j.gendis.2020.07.004
33. Valencia AM, Sankar A, van der Sluijs PJ, et al. Landscape of mSWI/SNF chromatin remodeling complex perturbations in neurodevelopmental disorders. *Nat Genet*. 2023;55(8):1400-1412. http://doi.org/10.1038/s41588-023-01451-6