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The expanding clinical and genetic spectrum of *DYNC1H1*-related disorders

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Intracellular trafficking involves an intricate machinery of motor complexes, including the dynein complex, to shuttle cargo for autophagolysosomal degradation. Deficiency in dynein axonemal chains, as well as cytoplasmic light and intermediate chains, have been linked with ciliary dyskinesia and skeletal dysplasia. The cytoplasmic dynein 1 heavy chain protein (DYNC1H1) serves as a core complex for retrograde trafficking in neuronal axons. Dominant pathogenic variants in *DYNC1H1* have been previously implicated in peripheral neuromuscular disorders (NMD) and neurodevelopmental disorders (NDD). As heavy-chain dynein is ubiquitously expressed, the apparent selectivity of heavy chain dyneinopathy for motor neuronal phenotypes remains currently unaccounted for. Here, we aimed to evaluate the full *DYNC1H1*-related clinical, molecular and imaging spectrum, including multisystem features and novel phenotypes presenting throughout life.

We identified 47 cases from 43 families with pathogenic heterozygous variants in *DYNC1H1* (aged 0–59 years) and collected phenotypic data via a comprehensive standardized survey and clinical follow-up appointments. Most patients presented with divergent and previously unrecognized neurological and multisystem features, leading to significant delays in genetic testing and establishing the correct diagnosis. Neurological phenotypes include novel autonomic features, previously rarely described behavioral disorders, movement disorders and periventricular lesions. Sensory neuropathy was identified in nine patients (median age of onset 10.6 years), of which five were only diagnosed after the second decade of life, and three had a progressive age-dependent sensory neuropathy.

Novel multisystem features included primary immunodeficiency, bilateral sensorineural hearing loss, organ anomalies and skeletal manifestations, resembling the phenotypic spectrum of other dyneinopathies. We also identified an age-dependent biphasic disease course with developmental regression in the first decade and, following a period of stability, neurodegenerative progression after the second decade of life. Of note, we observed several cases in

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whom neurodegeneration appeared to be prompted by intercurrent systemic infections with double-stranded DNA viruses (Herpesviridae) or single-stranded RNA viruses (Ross River fever, SARS-CoV-2). Moreover, the disease course appeared to be exacerbated by viral infections regardless of age and/or severity of neurodevelopmental disorder manifestations, indicating a role of dynein in anti-viral immunity and neuronal health.

In summary, our findings expand the clinical, imaging and molecular spectrum of pathogenic *DYNC1H1* variants beyond motor neuropathy disorders and suggest a life-long continuum and age-related progression due to deficient intracellular trafficking. This study will facilitate early diagnosis and improve counselling and health surveillance of affected patients.

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Introduction

Intracellular trafficking is a key process for transporting cargo within eukaryotic cells. The dynein-dynactin complex acts in concert with microtubule and intracellular adapter proteins[1,2] to enable nuclear positioning, spindle pole organization, Golgi maintenance and cargo transport, including deficient organelles and proteins to autophagolysosomes for degradation.[1-5] While these microtubule-directed transport proteins are vital in axonal filaments (axonemes) within cilia in respiratory airways and brain ventricles, virtually all cells within the human body require dynein for retrograde trafficking toward the microtubule minus-end.

The wide introduction of massively parallel sequencing for the diagnosis of monogenic disorders has identified a wide spectrum of defects in axonemal dyneins associated with ciliary dyskinesia. Monogenic defects in cytoplasmic dyneins are mostly limited to skeletal dysplasia (*DYNC2H1*, *DYNC2LI1*) and only a few recognizable neurological disorders that revolve around neuropathy: *DYNC1H1*-related disorders (detailed later), *DYNC1I2*-related neurodevelopmental disorders, *BICD2*-related spinal muscular atrophy, and *DCTN1*-related distal motor neuronopathy.

Cytoplasmic dynein 1 is a multi-subunit microtubule-based motor complex, with a core of dimerized dynein, cytoplasmic 1, heavy chain 1 (DYNC1H1).[4] The core phenotypes associated with pathogenic heterozygous variants in its gene *DYNC1H1* (OMIM #600112) are linked to a clinical and molecular spectrum including both neuromuscular disorders (NMD) and/or neurodevelopmental disorders (NDD) with muscular hypotonia, neurodevelopmental delay, axonal neuropathy and variable brain malformations.[6] NMD manifestations have been limited to peripheral nervous system phenotypes including Charcot-Marie-Tooth disease, axonal, type 2O (CMT2O)[7-9] and spinal muscular atrophy, lower extremity-predominant, 1 (SMALED1).[5,9-11] NDD manifestations associated with *DYNC1H1* variants extend into the CNS and include malformations in cortical development[9,12] and intellectual disability.[12-15]

We have previously delineated the spectrum of *DYNC1H1*-related disorders in a recent study with a clear focus on neurological manifestations and observed clustering of NMD manifestations with variants in the dimerization domain of its encoded protein. While DYNC1H1 is ubiquitously expressed in all human tissues, its selectivity for NMD and NDD remains elusive to this date. In this multicentre study, we identified a cohort of 47 patients with *DYNC1H1*-related disorders to expand the phenotype with clinical, molecular and imaging data that delineate a comprehensive and recognizable disease course with novel multisystem features in an age-dependent life-long continuum.

Materials and methods

Patient recruitment, data collection and phenotyping

We have recruited 47 patients from 43 families with heterozygous *DYNC1H1* (NM_001376.5) variants through international collaborations, GeneMatcher,[16] VarSome[17] and patient networks, including the recently established DYNC1H1 Foundation (dync1h1.org). Paired genotypic and health data was obtained from the IDDRC Brain Gene Registry for five individuals.[18] We also included updated information from previously published Patients P25 and P26.[12] Informed consent was obtained from all parents and/or patients. The study was approved by the local institutional review board at the Medical Faculty, University of Cologne (20–1711).[19,20] Detailed clinical data included assessments[21,22] from treating physicians based on a standardized clinical proforma, and imaging data from international collaborations, GeneMatcher[16] and VarSome.[17] We obtained a detailed follow-up medical history on patients through online consultations. The overall phenotypic picture was assessed using a proforma, standardized based on Human Phenotype Ontology (HPO).[23-25] See the Supplementary material and Supplementary Table 1 for detailed patient phenotyping. We assessed the prevalence of specific *DYNC1H1*-related phenotypic features in this cohort and compared those with the

numbers reported in the literature that we have additionally reviewed in a recent GeneReviews article.[7-12,14,26-43]

Molecular genetic investigations

DYNC1H1 variants in patients were detected via single-gene testing, a gene panel or whole exome investigations based on massively parallel sequencing. We investigated for co-segregation with the disorder in parents and/or relatives where available. All variants were analysed using *in silico* pathogenicity prediction via multiple lines of bioinformatic tools (Supplementary material). We conducted protein modelling on six selected amino acid residues in wild-type and mutant forms with DynaMut2,[44] to assess pathogenicity in patient variants (Supplementary material).

Results

General features

This study, focussing on autosomal dominant *DYNC1H1* disorders, included 47 patients from 43 families (27 male, 20 female), recruited through a multicentre study involving 13 countries. Forty-five of the 47 patients are reported here for the first time, while novel follow-up data were provided from two previously published patients, Patients P25 and P26.[12] The median age at last assessment was 12 years (range: 2–59). Figure 1 illustrates the general demographic features of our cohort.

Molecular genetic investigations

Our cohort of 47 patients included 41 different heterozygous (including 14 novel) pathogenic *DYNC1H1* variants distributed throughout the entire protein (4646 amino acids). Table 1 demonstrates the variants with selected pathogenicity prediction tools, while Supplementary Table 2 shows several more lines of pathogenicity prediction tools. The majority of pathogenic variants were missense variants (41/47). We also identified three patients with truncating variants (one with p.Arg3870* and two with p.Arg2179*), and one patient each with a frameshift-truncating (p.Phe1169Serfs*8), an in-frame deletion (p.Glu1548_Leu1560del) and a splice site variant (c.11595+3A>G). While all of these patients with truncating variants

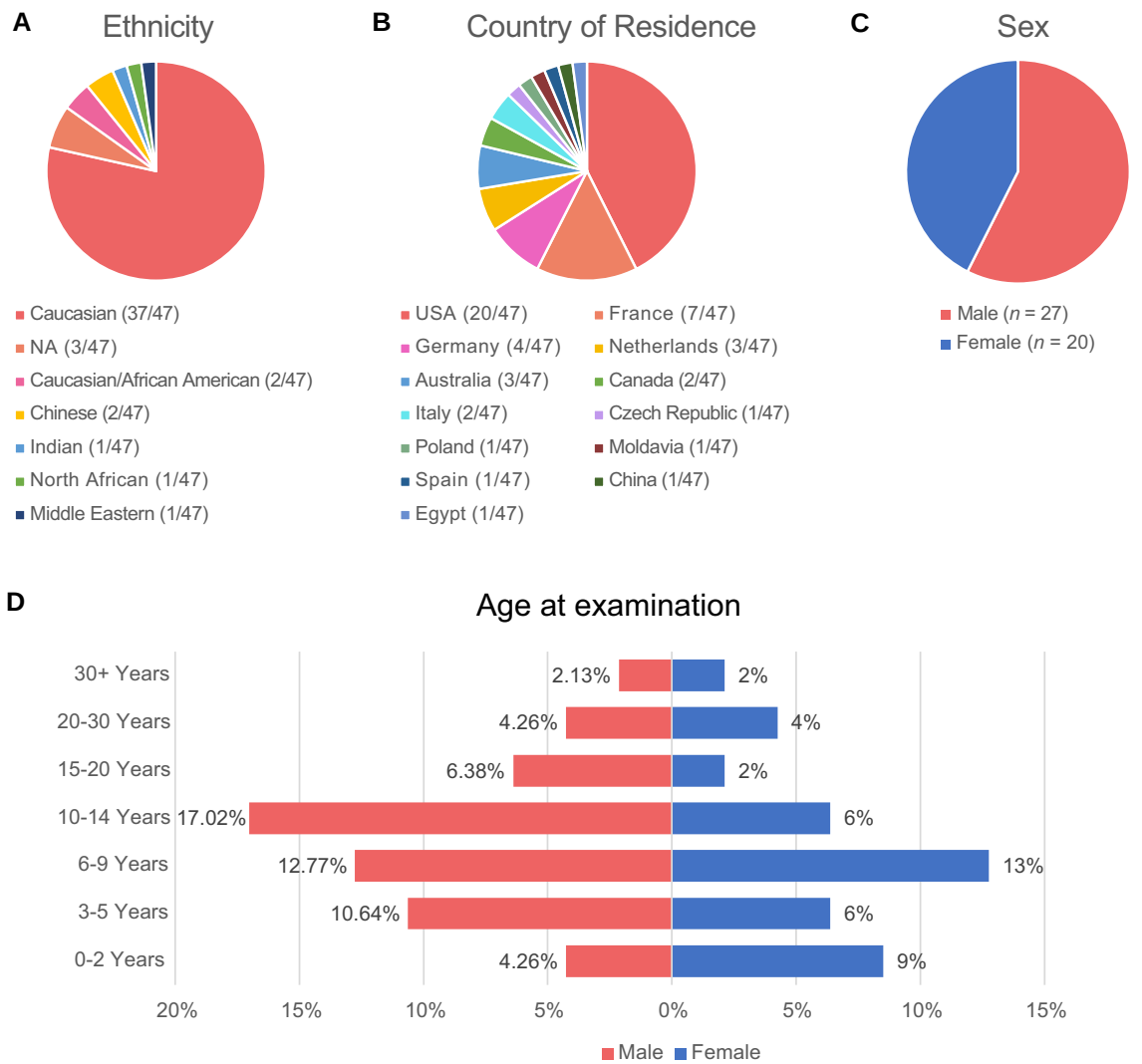

Figure 1 Demographic findings. The demographic data of our cohort in relation to (**A**) ethnicity, (**B**) country of residence, (**C**) sex and (**D**) age distribution at the last clinical examination and divided by sex.

Table 1 Overview of results of computational prediction tools for *DYNC1H1* variants in the study's patient cohort

Variant (NM_001376.5)	Protein change (NP_001367.2)	Location (hg38)	Exon	CADD	ClinPred	M-CAP	gnomAD
c.17G>T	p.Gly6Val	14:101964708–101964708	1/78	23.6	0.43	0.89	N/A
c.623C>G	p.Pro208Arg	14:101979823–101979823	4/78	21	0.44	0.04	N/A
c.791G>A	p.Arg264Gln	14:101980380–101980380	5/78	32	0.93	1	N/A
c.791G>C	p.Arg264Pro	14:101980380–101980380	5/78	32	0.97	1	N/A
c.925C>T	p.Arg309Cys	14:101980514–101980514	5/78	32	0.98	0.98	N/A
c.1013A>G	p.Arg569Pro	14:101983070–101983070	6/78	23.8	0.72	0.87	N/A
c.1706G>C	p.Arg569Pro	14:101985931–101985931	8/78	29.6	0.88	0.98	N/A
c.1834G>A	p.Val612Met	14:101986059–101986059	8/78	27.9	0.76	0.99	N/A
c.3056A>G	p.Tyr1019Cys	14:101994224–101994224	12/78	29.9	0.85	0.96	N/A
c.3185A>T	p.Asp1062Val	14:101994701–101994701	13/78	28.9	0.83	0.98	N/A
c.3189G>A	p.Met1063Ile	14:101994705–101994705	13/78	23.1	0.38	0.62	N/A
c.3347T>C	p.Val1116Ala	14:101994999–101994999	14/78	29.2	0.80	0.93	N/A
c.3504delC	p.Phe1169Serfs*8	14:101995239–101995240	15/78	-	-	-	N/A
c.3543G>C	p.Lys1181Asn	14:101995279–101995279	15/78	23.4	0.65	0.54	N/A
c.3648_3652 delinsGGAGA	p.Trp1218Arg	14:101997118–101997122	16/78	-	-	-	N/A
c.3652T>C	p.Trp1218Arg	14:101997122–101997122	16/78	31	0.98	0.96	N/A
c.4376T>C	p.Lys1459Ser	14:102001335–102001335	20/78	32	0.98	0.92	N/A
c.4642_4680del	p.Glu1548_Leu1560del	14:102002635–102002674	22/78	-	-	-	N/A
c.4807A>G	p.Arg1603Gly	14:102002889–102002889	23/78	27.2	0.85	0.98	N/A
c.4868G>A	p.Arg1623Gln	14:102002950–102002950	23/78	32	0.99	0.99	N/A
c.5114T>C	p.Val1705Ala	14:102004826–102004826	25/78	28.3	0.70	0.88	N/A
c.5872G>A	p.Asp1958Asn	14:102008232–102008232	29/78	32	0.99	0.99	N/A
c.5884C>T	p.Arg1962Cys	14:102008244–102008244	29/78	29.5	1	0.99	N/A
c.6410T>C	p.Leu2137Pro	14:102010744–102010744	32/78	29.9	0.95	0.93	N/A
c.6535C>T	p.Arg2179*	14:102010869–102010869	32/78	37	-	-	N/A
c.6870C>A	p.Ser2290Arg	14:102012326–102012326	34/78	8.84	0.66	0.25	N/A
c.6880G>A	p.Glu2294Lys	14:102012336–102012336	34/78	26.1	0.96	0.97	N/A
c.6989G>A	p.Gly2330Glu	14:102012445–102012445	34/78	31	0.99	1	N/A
c.7793G>T	p.Gly2598Val	14:102016944–102016944	38/78	28.1	0.99	0.99	N/A
c.8080C>T	p.Arg2694Cys	14:102017407–102017407	40/78	32	0.98	0.72	N/A
c.8665T>A	p.Leu2889Ile	14:102026601–102026601	44/78	21.9	0.70	0.78	N/A
c.9041A>G	p.Asn3014Ser	14:102027537–102027537	46/78	23.2	0.73	0.45	N/A
c.9092C>T	p.Thr3031Met	14:102027662–102027662	47/78	25.3	0.87	0.89	N/A
c.10016G>A	p.Arg3339His	14:102032404–102032404	52/78	32	0.93	0.98	N/A
c.10352A>G	p.Tyr3451Cys	14:102033423–102033423	54/78	30	0.96	0.96	N/A
c.10573C>T	p.Arg3525Cys	14:102034135–102034135	55/78	32	0.98	0.98	N/A
c.11595+3A>G	p.?	14:102039549–102039549	intron 61	17.98	-	-	N/A
c.11608C>T	p.Arg3870*	14:102039650–102039650	62/78	46	-	-	N/A
c.11989C>T	p.Arg3997Trp	14:102041621–102041621	65/78	32	0.94	0.88	N/A
c.12214G>A	p.Gly4072Ser	14:102042124–102042124	66/78	62	0.87	0.86	N/A
c.13609G>A	p.Glu4537Lys	14:102049807–102049807	76/78	32	0.87	0.79	N/A

DYNC1H1 variants based on NM_001376.5/ENST00000360184.10 (GRCh38/hg38) and protein mutations based on NP_001367.2/ENSP00000348965.4 in our cohort of patients reveals multiple lines of damaging effects from pathogenicity prediction tools (PolyPhen, CADD, ClinPred, M-CAP). The scoring tools indicate pathogenicity mostly in a range from 0 = benign to 1 = pathogenic, except for CADD PHRED (0 = benign, 40 = pathogenic). All variants were absent from the healthy population database (gnomAD version 3.1.2). All prediction tools rounded up to the second decimal place. See Supplementary Table 2 for more lines of pathogenicity prediction tools. N/A = not available.

had a neurodevelopmental disorder and/or infantile muscular hypotonia, there was no clear genotype-phenotype correlation, or a more severe disease phenotype associated with truncating variants in our cohort and the previously published literature.

The locations of variants on the protein level are illustrated in Fig. 2. Of note, patients with variants in the dimerization domain mostly showed neuromuscular disorders with motor neurodevelopmental delays that are likely to be secondary to infantile muscular hypotonia. This is in line with the findings of our recent genotype-phenotype correlation study in *DYNC1H1*-related disorders.[12,15]

Protein modelling with DynaMut2 revealed pathogenic effects of patient variants on the protein level. In particular, Tyr1019Cys, Val1705Ala, Leu2137Pro and Glu2294Leu showed destabilizing effects with loss of hydrogen bonds to surrounding amino acid residues. Gly2598Val and Arg2694Cys were predicted to have a destabilizing effect with a gain in clash and/or hydrogen bonds to surrounding residues. All protein models are illustrated in Fig. 3 and detailed in Supplementary Table 3.

Clinical features

For a quantitative comparison of previously reported *DYNC1H1*-related phenotypes, we extracted data from previously reported cases based on our literature review.[43] Our analyses revealed a considerable divergence in neurological, skeletal and immunological phenotypes compared to previously published cohorts (Table 2). Figure 4 demonstrates clinical pictures (Fig. 4A) as well as the major brain imaging (Fig. 4B), skeletal (Fig. 4C) and histopathological muscle biopsy findings (Fig. 4D).

This detailed phenotypic characterization revealed a biphasic course of the disorder with an age-dependent progression on the

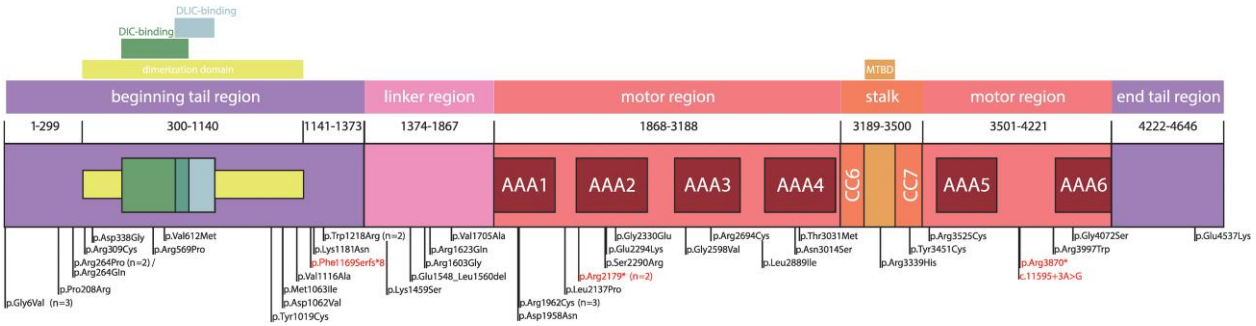

Figure 2 Genetic findings. DYNC1H1 protein structure, subdivided into different domains and with specified amino acid sequence length: beginning tail region (purple), including the dimerization domain (light green) and the overlapping dynein intermediate and light intermediate chain (DIC/DLIC) binding sites (dark green and blue), linker region (pink), motor region (light red) including AAA1-6 (dark red), stalk region (orange) consisting of CC6 and 7, MTBD region (yellow), as well as end tail region (purple). Below mutations are marked on protein level. The truncating variants p.Phe1169Serfs*8, p.Arg2179*, p.Arg3870*, as well as the splice site variant c.11595+3A>G are highlighted in red.

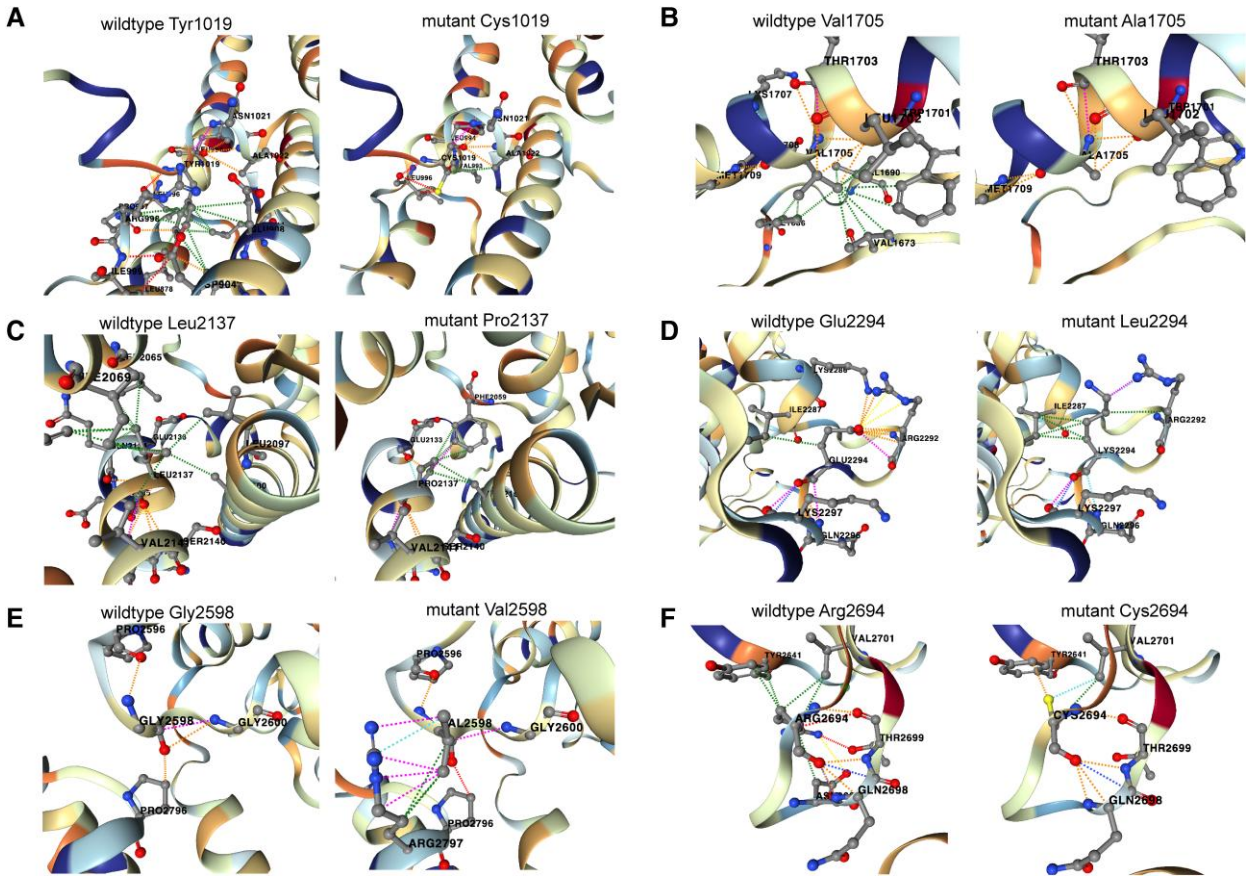

Figure 3 Protein modelling reveals pathogenic effects of patient mutations on protein level. (**A**) Tyr1019Cys with a predicted destabilizing effect (ΔΔGStability of −2.48 kcal/mol) modelled in PDB structure 7Z9J; mutant Cys1019 (*right*) with loss of hydrogen bonds to the illustrated surrounding residues in comparison to wild-type Tyr1019 (*left*). (**B**) Val1705Ala with a predicted destabilizing effect (ΔΔGStability of −2.26 kcal/mol) modelled in PDB structure 7Z8G; mutant Ala1705 with loss of hydrogen bonds to Phe1686 and Val1673 when compared to wild-type Val1705. (**C**) Leu2137Pro with a predicted destabilizing effect (ΔΔGStability of −1.27 kcal/mol) modelled in PDB structure 7Z8G; mutant Pro2137 with loss of hydrogen bonds to the illustrated surrounding residues when compared to wild-type Leu2137. (**D**) Glu2294Leu with a predicted destabilizing effect (ΔΔGStability of −0.71 kcal/mol) modelled in PDB structure 7Z8G; mutant Leu2294 with loss of hydrogen bonds to Ile2287 when compared to wild-type Glu2294. (**E**) Gly2598Val with a predicted destabilizing effect (ΔΔGStability of −1.33 kcal/mol) modelled in PDB structure 7Z8G; mutant Val2598 with a gain in clash to Arg2797 when compared to wild-type Gly2598. (**F**) Arg2694Cys with a predicted destabilizing effect (ΔΔGStability of −1.43 kcal/mol) modelled in PDB structure 7Z8G; mutant Cys2694 with gain of several hydrogen bonds to the illustrated surrounding residues when compared to wild-type Arg2694. Protein modelling with DynaMut2,[44] red = hydrogen bonds; orange = polar bonds; bright blue = van der Waals bonds; violet = clash.

Table 2 Overview of clinical features from our cohort with expanded phenotypes in comparison to previous published patients

	This cohort ($n = 47$)	Previously reported (GeneReviews 2024)
Peripheral nervous system findings		
Neuromuscular		
Hypotonia (HP:0001252)	65.9%	No data
Fatigue	18.18%	Not reported
Muscle weakness (HP:0001324)	UL (HP:0003484): 38.63%	UL (HP:0003484): >15%
	LL (HP:0007340): 63.63%	LL (HP:0007340): >80%
Muscle atrophy (HP:0003202)	UL (HP:0009129): 13.15%	UL (HP:0009129): No data
	LL (HP:0007210): 45.00%	LL (HP:0007210): No data
Hypo-/areflexia (HP:0001265)	UL (HP:0012391): 12.12%	UL (HP:0012391): <5%
	LL (HP:0002600): 48.48%	LL (HP:0002600): 50%
Sensory axonal neuropathy (HP:0003390)	18.18%	Singular cases
Exclusive motor developmental delay (HP:0001270)	8.69%	Singular cases
Abnormal gait (HP:0001288)	78.37%	>30%
Secondary skeletal abnormalities		
Abnormality of skull size (HP:0000240)	31.7%	>5%
(Limb) contractures (HP:0003121)	20.51%	>10%
Foot deformities (HP:0001760)	46.51%	>50%
Clinodactyly	18.18%	Not described
Spinal deformities (HP:0000925)	9.3%	20%
Congenital hip dysplasia (HP:0001385)	11.62%	No data
Femur fracture during delivery	4.76%	1 case
CNS findings		
Global developmental delay (HP:0001263)	61.36%	<5%
Motor development delay (HP:0001270)	75.00%	50%
Speech development impairment (HP:0000750)	71.73%	<10%
Intellectual disability (HP:0001249)	75.00%	40%
Behavioural disorders (HP:0000708)	73.8%	>15%
Seizures (HP:0001250)	50.00%	40%
Central movement disorders		10%
Paresis (HP:0010549)	9.3%	<5%
Tremor (HP:0001337)	13.95%	<5%
Ataxia (HP:0001251)	18.6%	<5%
Akinesia (HP:0002304)	0	<5%
Spastic grasp (HP:0034353)	6.81%	<5%
Vocal cord paresis (HP:0001604) & dysarthria (HP:0001260)	0	<5%
Parkinsonism (HP:0001300)	0	1 case
Brain malformations (HP:0012443)	61.53%	>60%
Abnormal cortical gyration (HP:0002536)	33.33%	<50%
Abnormal corpus callosum morphology (HP:0001273)	20.51%	>20%
Periventricular lesions	20.51%	Singular cases
Enlarged ventricles (HP:0002119)	23.07%	15%
Cerebellar hypoplasia (HP:0007360)	12.82%	<15%
Grey matter heterotopia (HP:0002282)	15.38%	>10%
Brainstem hypoplasia (HP:0007362)	10.25%	<10%
Autonomous nervous system findings		
Gastrointestinal manifestations overall	51.16%	<5%
Constipation	41.46%	<5%
Anomalies of the kidney and urinary tract	17.07%	<5%
Cardiovascular	15,00%	<5%
Respiratory and pulmonary involvement	21.95%	Singular cases
Severe dry eyes	6.38%	Not described
Failure to thrive	22.22%	Not described
Sleep issues apnoe	26.82%	Not described
Temperature and sudomotor dysregulation	14.28%	Not described
Other multi-organ manifestations		
Craniofacial dysmorphia (HP:0001999)	46.66%	<5%
Ophthalmologic findings	48.83%	>10%
Skin abnormalities	21.95%	
Primary immune deficiency	7.5%	Not described
Hearing loss	5.00%	Not described

Synopsis of clinical features and imaging findings in our cohort and previously reported cases with Human Phenotype Ontology terms.[43] LL = lower limb; UL = upper limb.

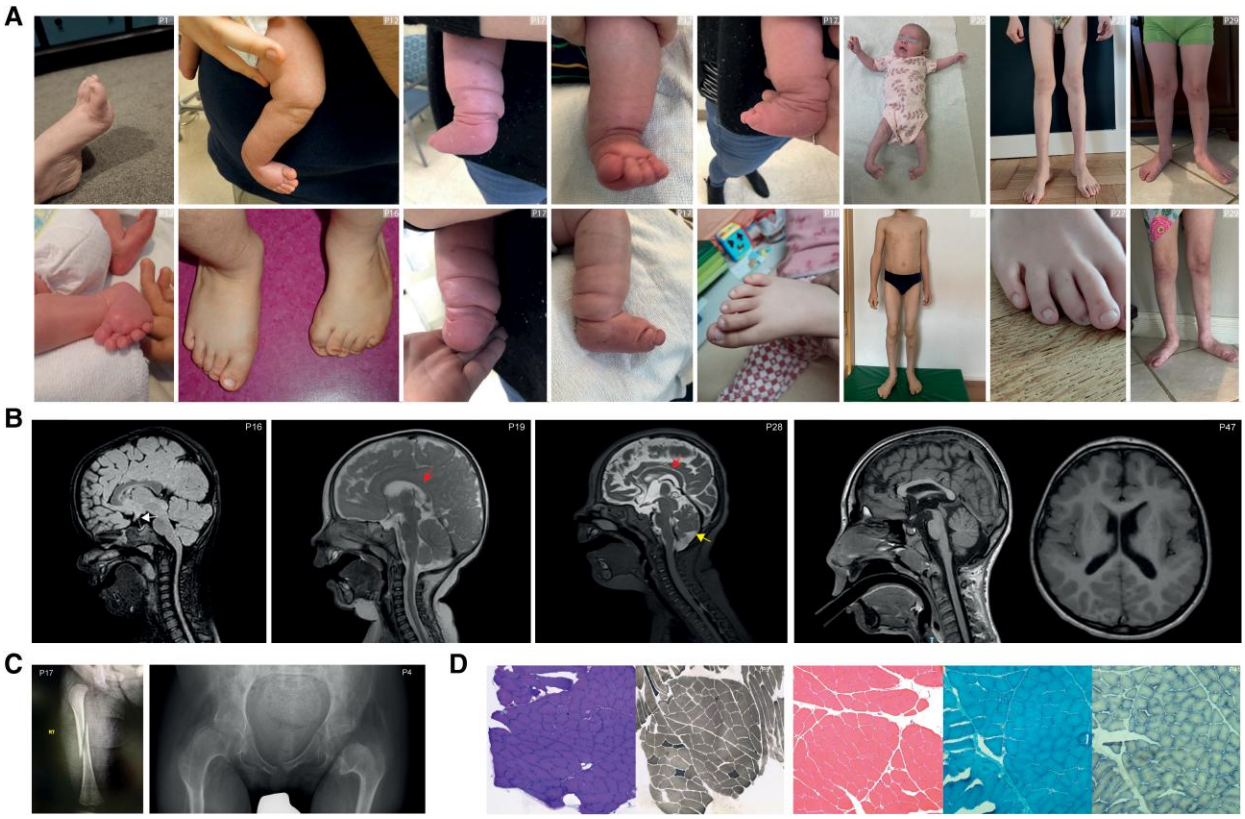

Figure 4 Clinical features of *DYNC1H1*-related disorders. (**A**) Photographs of patients in our cohort with pathogenic heterozygous *DYNC1H1* variants. Patient P1 age-related progressing pes cavus. Patient P12 bilateral talipes equinovarus. Patient P16 bilateral talipes equinovarus with predominant left-sided expression. Patient P17 left-sided talipes equinovarus and right vertical talus with hyperflexibility. Patient P18 clinodactyly. Patient P20 brachycephaly, plagiocephaly, face bones anomaly, bilateral developmental dysplasia of the hip and congenital bilateral talipes equinovarus. Patient P26 muscle weakness and atrophy of the lower limbs. Patient P27 muscle weakness and atrophy of the lower limbs. Slight pes caves and clinodactyly. Patient P29 pes valgus and planus. (**B**) Brain MRI studies indicated small pituitary gland in a T_1-weighted image of Patient P16 (white arrow), corpus callosum dysgenesis in T_2-weighted images of Patients P19 and P28 (red arrows), and a cerebellar hypoplasia in Patient P28 (yellow arrow). Brain MRI of Patient P47 showed a thinned mesencephalon as well as a simplified and thickened cortex of the temporal and insular regions, associated with deep sylvian valleys, suggestive of bi-opercular dysplasia. (**C**) X-ray of Patient P17 with a congenital femur fracture and bone density <10%. (**D**) Muscle biopsy histology in Patient P9 indicates type 1 fibre predominance in ATPase (pH 10.3) (*right*) and internalized nuclei haematoxylin and eosin (*left*), as well as few non-specific myopathic changes with only few internalized nuclei in haematoxylin and eosin, trichrome Gomorri and succinate dehydrogenase stainings in Patient P41.

background of a neurodevelopmental disorder, as outlined in the 'Biphasic neurodegenerative courses during development and early adulthood' section. Figure 5 illustrates the age-dependent continuum of this expanded phenotype in our cohort.

Peripheral nervous system features

Neuromuscular features were mainly present in the lower limbs comprising muscle weakness (63.6%), hypo- or hyper-reflexia or the persistence of primitive reflexes (48.4%), and muscle atrophy (45%). In total, 78.7% patients were able to walk (37/47), of which 78.3% (29/37) had an impaired gait. Apart from Patients P4 (11 years), P6 (29 years), P12 (7 years 8 months), P23 (10 years) and P46 (28 years), all patients with inability to walk were younger than 3 years. A total of eight patients were wheelchair-dependent. Patient P16 had a left positive Rossolimo reflex and Patient P33 had persistent primitive reflexes noted at 10 months. The upper limbs were affected to a lesser extent with weakness (38.6%), atrophy (13.1%) and hyporeflexia (12.1%). Another core clinical feature was muscular hypotonia (65.9%). Other manifestations included fatigue (18.1%), isolated motor developmental delay (8.6%), muscle fasciculations (Patients P1 and P6) and generalized muscle weakness (Patients P1, P2 and P17). Examination revealed a positive Gowers' sign after repeated testing in Patients P2 and P44. Sensory axonal neuropathy (18.1%), presenting with numbness, tingling, sensory impairment in all sensory modalities, and neuropathic pain, were found in the distal upper limbs and lower limbs. EMG and/or nerve conduction studies (NCS) were conducted in seven patients and indicated low amplitude tibial compound muscle action potentials with chronic denervation (Patients P1, P10, P11 and P26) with additional fasiculations (Patients P41 and P45), that may correspond to a primarily axonal neuropathy and/or anterior horn cell disorder. Skeletal abnormalities with contractures are illustrated in Fig. 4A and C and the Supplementary material.

Muscle biopsy in Patient P9 indicated type I fibre predominance with internalized nuclei on haematoxylin and eosin and myosin-ATPase stainings but no other abnormalities, including evidence of fibrosis, basophilic fibres, inflammation, necrosis or abnormal mitochondrial distribution and activity. Muscle biopsy in Patient P41 indicated only a few internalized nuclei and no other remarkable findings. Muscle biopsy findings are illustrated in Fig. 4D.

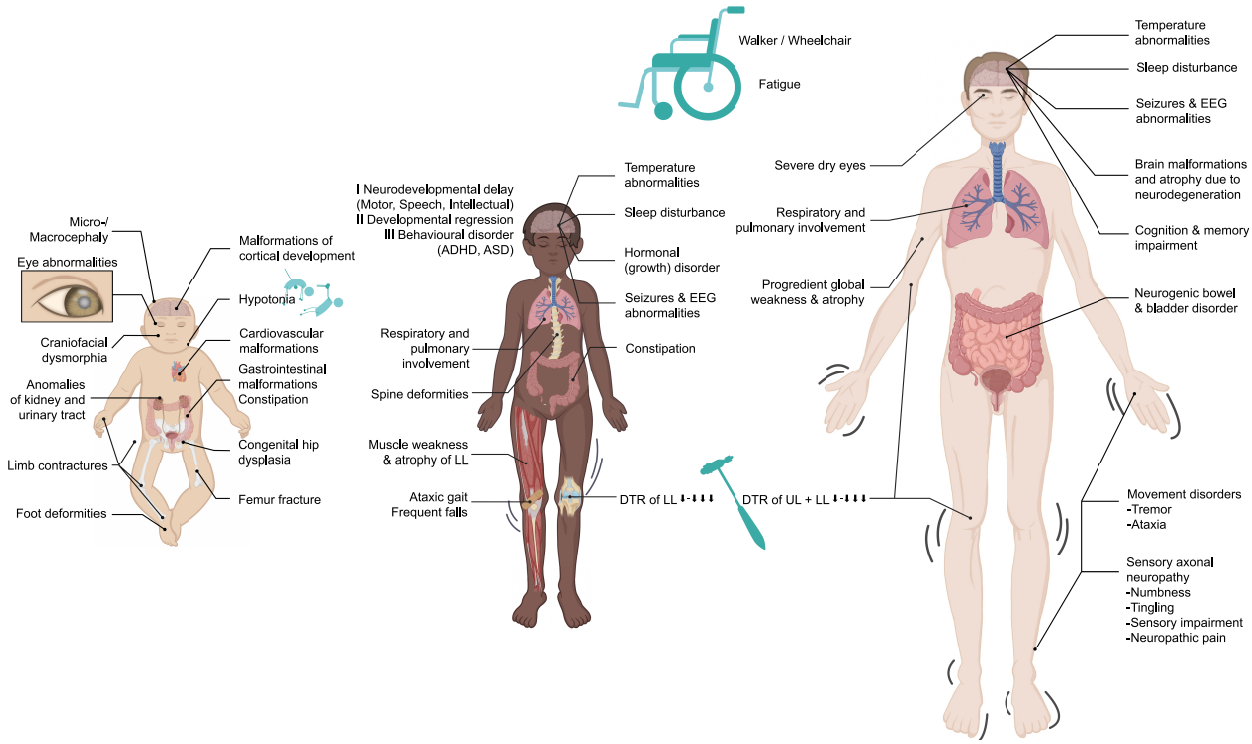

Figure 5 Evolution of *DYNC1H1*-related disorders over a life-long continuum. The age-related key findings of the patients in our cohort are shown as illustrative examples. We observed an occurrence of hypotonia, femur fracture, congenital foot deformities and contractures at infancy. Developmental delay and (speech) regression, muscle weakness and atrophy, abnormal gait, seizures and constipation occurred mainly at early childhood. We observed an age-related progression of hypo-/areflexia, muscle weakness and atrophy, cognition and memory disturbances, brain malformations and sensory axonal neuropathy at late adolescence and early adulthood. ADHD = attention deficit hyperactivity disorder; ASD = autism spectrum disorder; DTR = deep tendon reflexes; LL = lower limb; UL = upper limb. Created with BioRender.com.

Autonomous nervous system manifestations

Disorders of the autonomous nervous system ('dysautonomia') have rarely been reported in the literature on *DYNC1H1*-related disorders but were found in the majority of our patients. Gastrointestinal manifestations were present in 51.1% of patients. We reclassified constipation due to intestinal dysmotility as a dysautonomic phenotype, as recent advances in dysautonomia in neurological disorders suggest that any imbalance in autonomous enteric nerves and smooth muscle involvement may lead to a change in intestinal motility functions such as constipation,[45] a feature in 41.4% of our patients. Additional phenotypes included gastroesophageal reflux disease ($n = 5$, 10%), feeding difficulties (Patients P12, P31, P33 and P37), congenital gallstones (Patient P17), failure to thrive (Patient P20), diarrhoea (≥5×/day) (Patients P25 and P26), irritable bowel syndrome (frequent changes in constipation/diarrhoea), and neurogenic bowel (Patients P1 and P44). Urogenital anomalies were reported including neurogenic bladder (Patients P1, P12 and P30). Cardiovascular manifestations included palpitations, arrhythmia, postural tachycardia, syncope, hypotension and dizziness (Patients P1 and P2).

One of major early warning signs of dysautonomia include sleep disturbances as the autonomic nervous system regulates sleep architecture, visceral control on respiration and muscle tone during sleep with noctural hypoventilation, and hormonal arousal either during night- or daytime.[46] Sleep disturbances were described in 26.8% of patients. Patients P1 and P31 had age-related hypersomnia and fatigue was reported in 18.1%. Temperature and sudomotor dysregulation were described in 14.2% of patients, including repeated temperature spikes in Patient P17, suspected malignant hyperthermia in Patient P31, and seizures triggered by elevated environmental temperatures (≥24°C) in Patients P26 and P31. Paradoxical sweating was observed in five patients, while Patient P1 displayed nocturnal sweating and Patient P2 had inappropriate sweating only in early childhood.

Central nervous system manifestations

Global developmental delay was detected in 61.3% of patients as a core clinical feature. Developmental disorders in our patients included motor developmental delay (75%), intellectual disability (75%), behaviour (73.8%) and speech developmental delay (71.7%). Motor developmental delay was predominantly described with gross motor deficits. Fine motor developmental delay was noted in 29.5%. Patients P2, P7 and P22 had difficulties in coordination. Patients P4 (11 years), P6 (10 year) and P23 (29 year) never achieved independent walking. Furthermore, several cases of motor developmental arrest (Patient P27) and regression were observed ($n = 7$). Speech and language development and skills varied from normal to developmental delay ($n = 6$), non-verbal and/or language regression ($n = 12$).

The most common behavioural disorders were attention deficit (hyperactivity) disorder [AD(H)D, 45.2%] and autism spectrum disorder (ASD, 30.9%). There were additional reports of anxiety ($n = 8$), depression (Patients P1 and P2), (auto-)aggressive behaviour ($n = 8$), obsessive compulsive disorder (Patients P22 and P31), Tourette syndrome/tic disorders (Patients P31, P32 and P43), borderline personality disorder (Patient P11), attachment disorder

(Patient P41), disruptive behaviour (Patients P38 and P43), pica and excessive eating disorder with obesity (Patient 38), as well as motor and verbal stereotypic behaviours (Patients P22, P23 and P47), bruxism (Patient P44) and delayed social and emotional development (Patient P32). Patient P25 was treated with methylphenidate for an ADD, which led to neurodevelopmental improvement.

Abnormal head circumference was noted in 31.7%. Eleven patients had microcephaly, in some of them progressive. Patients P7 and P34 had macrocephaly.

We systemically reviewed brain MRI in 39 patients and found brain malformations/abnormalities in 61.5% of patients. The most common findings were abnormal cortical gyration/polymicrogyria (33.3%), enlarged ventricles (23%), periventricular lesions (20.5%), corpus callosum dysgenesis (20.5%, Fig. 4B), white matter changes (20.5%), grey matter heterotopia (15.3%), cerebellar hypoplasia (12.8%) and brainstem hypoplasia (10.2%). Additional abnormalities included vertically oriented hippocampi (Patient P7), hippocampal asymmetry (Patient P30), arachnoid cysts (Patients P11 and P26), widening of the Sylvian fissure (Patient P31), extensive perisylvian pachygyria (Patient P7), bi-opercular dysplasia and thinned mesencephalon (Patient P28, Fig. 4B), hypoplastic olfactory bulbs (Patients P16 and P23), small pituitary gland (Patient P16, Fig. 4B) and hydrocephalus (Patient P12). Transient leukoencephalopathy followed by cerebral atrophy was found in Patient P13 after febrile refractory status epilepticus.

Seizures were present in 50% of patients and included generalized motor tonic-clonic ($n = 8$), infantile spasms ($n = 6$), focal motor (Patients P21, P27 and P28), generalized motor astatic/atonic (Patients P3, P4, P26 and P38), generalized non-motor absence ($n = 6$), generalized motor tonic (Patients P4, P13 and P40) and motor myoclonic onset seizures (Patient P40). Patient P45 had generalized motor reflex seizures, induced by pouring water during bathing between the ages 3–5 years, and responsive to clonazepam. Patients P12 and P19 developed seizures during a SARS-CoV-2 infection. Status epilepticus was reported in four patients (Patients P13, P17, P19 and P31). We observed seizures as key events in neurodegenerative courses of several patients (see later), ranging from persistent gait abnormalities after the first focal seizure (Patient P27) to severe visual impairment due to bilateral optic nerve atrophy with pale optic disc after onset of infantile spasms (right > left, Patient P28). EEG recordings included spike and wave discharges ($n = 6$), hypsarrythmia (Patients P20, P24, P28 and P46) and (multifocal) spikes (Patients P21, P24 and P25).

Movement disorders were observed in 34.8% of the 43 patients that were systemically examined by a neurologist. Ataxia was reported in eight patients (18.6%). Tremor was reported in five patients (10%). Patients P4, P13, P37 and P46 had obtained a diagnosis of spastic cerebral palsy and predominant lower limb involvement. Patient P24 showed myoclonic and generalized choreiform movements including the face. Patient P27 had upper limb myoclonus and Patient P46 had dystonia in all limbs.

Biphasic neurodegenerative courses during development and early adulthood

Several patients and/or their caregivers reported disease deterioration with concomitant infections. Patient P1 (43 years) reported progression of neurodegenerative features with generalized muscle weakness after viral infections with Epstein-Barr virus and Ross River fever, and during puberty, pregnancy and menopause. These neurodegenerative features included generalized muscle wasting, chronic pain syndrome, sensory peripheral neuropathy and numbness, urinary incontinence, irritable bowel syndrome, severe fatigue, hypersomnia, working memory impairment, and progressive pes cavus. Patient P2 (12 years) reported several periods with progression of neurodegenerative features beginning at 9 years and associated with regression of acquired skills, memory impairment, global muscle atrophy, low blood pressure and tachycardia. Each of these events were precipitated by viral or bacterial infection and the occurrence of seizures. In Patients P12 and P19, SARS-CoV-2 infection induced complex partial and absence seizures. Patient P11 (30 years) showed progressive motor deterioration with muscle weakness and atrophy of the upper and lower limbs, resulting in increased wheelchair dependence from the age of 30 years. Fatigue and tremor of the upper and lower limbs started at the age of 16 years and worsened after physical stress that also brought about keratoconjuntivitis sicca. The patient further reported frequent infections like pneumonia and an interstitial bacterial infection in the lower limbs. Patient P13 (5 years) had a febrile infection at the age of 3 years, which was followed by refractory status epilepticus. Analysis of CSF did not reveal a CNS infection. The patient subsequently developed acute leukencephalopathy with restricted diffusion on MRI that resolved after 2 weeks and was followed by brain atrophy with severe psychomotor regression. EEG recordings remained abnormal with continuous multifocal independent bilateral discharges. Antiepileptic drugs, immunosuppression and physiotherapy had no significant effect, but ketogenic diet reduced the seizure frequency.

Several patients and/or families reported changes in the disease course with onset of seizures. Patient P28 (2 years) had an age-appropriate development until onset of infantile spasms at 8 months of age with significant deterioration of psychomotor development. The patient responded to treatment with glucocorticoids and vigabatrin with complete seizure remission and has made continuous developmental progress. Patient P31 (59 years) was treated with antiseizure medication from early childhood. Discontinuation of medication led to status epilepticus with generalized motor tonic-clinic seizures at the age of 55 years. This event was followed by progressing weakness in all limbs, extreme fatigue and hypersomnia, tingling, pain and numbness progressing from distal to proximal limbs within 4 years, tremors, ataxia, drowsiness, major imbalance and falling, extreme keratoconjunctivitis sicca, dysphagia4, and prediabetes. Furthermore, severe cognitive decline occurred with memory loss and aphasia. Mild physical exertion, heat or sleep deprivation have since triggered additional seizures. Developmental progress stalled in Patient P44 (5 years) after the age of 3 years, especially with regards to fine motor skills. Patient P46 (28 years) with global developmental delay was ambulatory until 25 years, despite major imbalance and recurrent falls.

Other cases showed no correlations between disease course changes and any other apparent triggers. Patient P6 (29 years) showed age-related increasing weakness and numbness of the upper and lower limbs, starting distally and progressing proximally. At the last clinical review at the age of 28 years, there was hypaesthesia in the lower limbs, writing had become increasingly difficult over time, and the patient reported episodes of muscle hypertonia in the lower limbs at night. The increase in muscle weakness and sensory neuropathy occurred in the third decade of life. Patient P10 (14 years) described progressive reduction in muscle strength and pain primarily in distal lower limbs, progressive of sensory loss in the hands, as well as progressive pes cavus, leading to gait instability. Progression manifested as slow and steady deterioration of muscle strength and sensitivity. Patient P17 (2 years) had developmental regression with onset between 13 to 15 months of age.

At 12 months of age, the patient could turn, was beginning to crawl on his forearms and could sit independently for ~60 s. The regression included loss of forearm crawling, independent sitting, sleep disturbance with frequent nocturnal awakenings, a loss of consonant sounds, and a flattening of affect with no smiling and vocalizing. First absence and clonic seizures were noticed at the age of about 3 months, but no developmental regression or delay was noticed until 13 months of age. The seizures were responsive to levetiracetam. Patient P21 (9 years) is non-verbal. His receptive language skills are appropriate and he was diagnosed with an unspecified language regression. Patient P22 (14 years) had psychomotor regression at the age of 16 months. Before the regression he was using about 15 words, combining two words, and eventually regressed to usage of four to five words, stopped combining words, was unresponsive and was unable to transfer objects between hands. Patient P34 (4 years) showed a speech developmental disorder. He spoke his first words at the age of 13 months and in the following 3 months his vocabulary increased to >10 words. At the age of 16 months, he showed a regression in speech development to babbling. Patient P38 (20 years) could use 8–10 words at the age of 12 months. A speech regression followed at the age of 18 months, eventually leading to speech arrest. Both Patients P34 and P38 used augmentative and alternative communication from the age of 4 years.

Multisystem features

Primary immune deficiency was described in Patients P2, P33 and P34. Notably, Patient P2 had more than 20 infections per year, mostly respiratory tract infections, associated with IgA deficiency. Several bouts of bacterial pneumonia were accompanied by hospitalization and antibiotic treatments. The reduction of social contacts with homeschooling correlated with a decrease in upper respiratory tract infections and sinus infections down to 1–6 infections per year. Patients P33 and P34 were diagnosed with hypogammaglobulinemia and received intravenous immunoglobulin (IVIG) every 4 weeks. Patient P34 had recurrent infections, such as acute otitis media with tympanostomy tube placement, eczema, recurrent slow healing skin infections, and Eustachian tube dysfunction leading to recurrent acute otitis media. Patient P7 had recurrent acute otitis media of both ears and chronic otorrhea in the left ear even in-between infectious episodes with clinical suspicion of immunodeficiency. Detailed information on ophthalmologic features (48.8%), craniofacial dysmorphia (46.6%), endocrinological features (22.2%), pulmonary involvement (21.9%), skin abnormalities (21.9%), cardiac abnormalities (three patients) and auditory features (two patients) are available in the Supplementary material.

Discussion

Here we describe the currently largest cohort of 47 patients, with 41 variants in *DYNC1H1*, of which 14 variants have not previously been reported. As novel features we report involvement of the sensory and autonomic nervous system evolving throughout life, in line with recent molecular insights into the consequences of dynein motor complex dysfunction, with global effects on axonal transport mechanisms.[4] Defects in axonemal and cytoplasmic dynein subunits mostly cause a combination of immunological, skeletal and neurological disorders. Some of these disorders may fall under the umbrella of 'ciliopathies' with differential diagnoses including Kartagener syndrome or Meckel syndrome. However, until now, the cytoplasmic dynein heavy chain disorders have largely been reported as neurological disorders with differential diagnoses including diseases with brain malformations (lissencephalies), motor neuropathies in the central (5q-spinal muscular atrophy) and the peripheral nervous system (Charcot-Marie-Tooth disease). We discuss the intricate interactions between the dynein complex and viral infections with its implications for neuronal health, based on our observation how viral infections correlated with altered disease courses in some cases regardless of NDD severity or age at viral infection. Our findings extend the clinical, molecular and imaging spectrum of pathogenic *DYNC1H1* variants, and suggest an extended phenotypical spectrum presenting throughout life.

Autonomic and sensory nervous system involvement

Sensory neuropathy was found at a higher frequency in our cohort than previously shown (9/47, with a median age of onset at 10.6 years). Of note, five patients were reported with an onset after the second decade of life and three had progressive sensory neuropathy at a later age, including tingling and numbness with antecedent twitching, trembling and tremors.

More than half of the patients presented with multi-organ involvement related to dysautonomia. Autonomic nervous system (ANS)-related enteric neuropathies are observed in several neurodevelopmental and neurodegenerative disorders, often well before the onset of motor symptoms, for example in Parkinson's disease, due to loss of enteric neurons.[47] The manifestations of enteric neuropathies are diverse and include, for instance peristalsis dysfunction, constipation, dysphagia and gut dysmotility.[45,48-50] Recent studies in monogenic disorders with multisystem phenotypes, such as *EPG5*-related Vici syndrome or *UNC45A*-related osteo-oto-hepato-enteric syndrome, suggest a genetic contribution to enteric neuropathies.[51,52] In case reports of DYNC1H1 deficiency, <5% showed mild neuropathic gastrointestinal involvement, manifesting as dysphagia, pseudo-obstruction, bowel dysmotility, chronic constipation and faecal incontinence.[13,15,30,31] Although some of these features in theory may also be compatible with smooth muscle involvement, based on the observations in our series, we suggest that the occurrence of gastrointestinal symptoms may be caused by enteric neuropathy due to disturbed ANS development *in utero* or at early infancy.[48] As the ANS also regulates cardiopulmonary function, we specifically investigated our cohort for potentially relevant phenotypes. Primary monogenic disorders of the ANS can manifest as impaired arrhythmogenesis, orthostatic hypotension, or postural orthostatic tachycardia syndrome.[53,54] Recent findings also suggest an association of asthma and dysautonomia, due to aggravation by hypersensitive vagal reflex responses to stimuli such as cold air or exercise. An ANS-associated pathology was indicated by symptoms in our cohort as outlined earlier, and included other potentially relevant phenotypes, such as sleep disorders and fatigue, as well as disturbances of thermoregulation and lacrimation,[53,55,56] probably reflecting peripheral small nerve fibre dysfunction. Previous *DYNC1H1* cohorts reported only singular instances of ANS disorders, probably reflective of a stronger emphasis on other more obvious neurological features. Dedicated follow-up investigations of our cohort led us to examine this extension to disorders of the ANS.

DYNC1H1 plays a major role as the only motor-supported retrograde cargo transport complex subunit within neuronal axons. This requires a coordinated action between Rab6a, BICD and the dynein–dynactin complex in Golgi–endoplasmic reticulum (ER) transport at ER exit sites (ERES) via a COPII-dependent mechanism.[57] The BICD2/Rab6 dynein adaptor was identified as vital for maintenance of

Golgi-associated ERES to enhance the efficiency of trafficking.[58] The RAB6-dynein-LIS1 complex for Golgi to apical surface transport in apical radial glia was identified as vital in the maintenance of neuroepithelial integrity during neuronal development, driving post-Golgi apical transport to prevent neuronal progenitor delamination.[59] Two retromer sorting nexins (SNX5 and SNX6) interact with the Rab6 effector and dynactin subunit p150^{glued}.[60] Zebrafish with dync1h1 mutations showed migrated Schwann cell progenitors with absent myelin membranes and reduced expressions of Pou3f1 and Egr2 due to failed interaction between Dync1h1 and myelin basic protein within both Schwann cells and axons.[61] Mice with pathogenic heterozygous *Dync1h1* variants displayed either an early-onset pure proprioceptive sensory neuropathy or a sensory neuropathy with muscle spindle deficiency and lack of an H-reflex despite normal hindlimb motor nerve function. Of note, proprioceptive sensory neurons were severely compromised in the lumbar dorsal root ganglia of newborn mice.[62] Mice with a deletion of Rab6a in intestinal epithelial cells (IECs, Rab6a$^{\Delta IEC}$ mice), showed IEC death, inflammation and intestinal bleeding with massive lipid accumulation shortly after birth, leading to early postnatal death.[63] In lung-injured mice through exposure to fine particulate matter, knockout of RAB6 inhibited pulmonary fibrosis, oxidative stress and cell death of type 2 alveolar epithelial cells (AEC2), stem cells in the adult lung that contribute to the lung repair process.[64] These studies indicate that Rab6 and dynein may be tasked with a more diverse range of tubule-dependent transport processes that extend beyond Golgi-to-ER tubular transport and may be relevant during neurodevelopment and in a multitude of other tissues.[65,66]

DYNC1H1 defects were primarily linked with motor neuropathy,[2-5] either in the peripheral and/or central nervous system.[12,13] However, corresponding with other hereditary neuropathies, here we identified a significant number of patients with additional sensory and autonomic nervous features. We therefore propose an expansion of the *DYNC1H1*-related neuropathies to include ANS disorders as part of a spectrum of global axonal trafficking disorders, including sensory and autonomic neuropathies developing *in utero* or during infancy. This is in line with recent molecular findings suggesting more global effects on axonal transport mechanisms associated with dysfunction of dynein motor complex and its interactors.[52] The coexistence of autonomic and sensory nervous system disorders will facilitate diagnoses of a *DYNC1H1*-related disorder in patients presenting with multisystem phenotypes.[57]

Correlations of exacerbated neurodegenerative courses with viral infections

The second major finding in our cohort was the observation of accelerated neurodegeneration that was temporally linked with episodes of systemic viral infections, in particular infection with double-stranded DNA viruses (Herpesviridae) or single-stranded RNA viruses (Ross River fever, SARS-CoV-2). While this correlation was found in several patients, it does not elucidate the exact pathomechanisms underlying this association. We suggest follow-up studies either with longitudinal observational studies in a cohort of patients with *DYNC1H1*-related disorders before and after viral infections, or with molecular studies from patient fibroblasts that are investigated before and after viral infection in cellular assays. Potential pathomechanisms may include neurodegeneration due to a general aggregation of all toxic substances during increased metabolic turnover that is unrelated to viral infection. An alternative pathomechanism may be an improper 'hijacking' of intracellular waste mechanisms by viruses and subsequent toxic aggregation with neurodegeneration.

Neurons are post-mitotic tissues that require several lines of toxic waste removal mechanisms for survival during time periods with increased metabolic turnover such as fasting, physical exertion or infection. Of note, we observed a correlation of exacerbating neurodegenerative disease courses with viral infections but not fasting or physical exertion, and suggest that this correlation may be specific to viral infections. There are several protective intracellular processes to protect neurons from infectious agents, one of which is termed 'xenophagy', a subtype of selective autophagy for the clearance of pathogens.[67-69] At health, xenophagy blocks viral replication by degrading viral particles and components, as well as endogenous factors essential for viral replication.[68,69] Dynein modulates the movement of autophagosomes along microtubules towards lysosomes for eventual degradation. Targeted inhibition of dynein was shown to impair autophagosome transport[4,70] and may also adversely affect xenophagy.

Recent molecular biological advances have demonstrated how several viruses interact with and ultimately hijack dynein for their own transport needs within the cell, including single-strand RNA viruses and double-strand DNA viruses. This mechanism may lead to defective shuttling of viruses from the cellular membrane to the nucleus and in turn may raise aggregation of viral particles, promoting chronic inflammation, inducing cellular oxidative stress, impairing mitophagy and mitochondrial morphology, and inducing protein aggregates associated with neurodegenerative diseases.[71]

Several ssRNA viruses were implicated in hijacking dynein for their own needs. The Zika virus (ZIKV) envelope protein was shown to interact with dimerization domain of cytoplasmic dynein heavy chain without dynactin or any cargo adaptor.[72] During ZIKV infection, autophagy is increased and ZIKV particles replicate in intracellular lipid droplets.[73] ZIKV infection is associated with postnatal microcephaly in newborns and Guillain-Barré syndrome in adults. Of note, several patients showed rapid-onset muscle weaknesses after systemic viral infections that were similar to Guillain-Barré syndrome. Another ssRNA virus is the porcine epidemic diarrhoea virus (PEDV), a member of the coronavirus family, that is shuttled to the nucleus by microtubule, dynein and kinesin-1, which can influence PEDV fusion and accumulation in the perinuclear region, but did not affect PEDV attachment or internalization.[74] Notably, perhaps the most recent member of the coronavirus family, SARS-CoV-2, was shown to lead to DYNC1H1 downregulation during infection.[75] Two of our patients had progression in the disease course of seizures upon SARS-CoV-2 infection, showing complex partial and absence seizures.

Notable dsDNA viruses include the herpes virus and adenovirus families. While mitochondria are essential to the energy metabolism in neurons, they also have an important role in orchestrating the induction of interferon response upon infection with the dsDNA herpes virus family member, Epstein-Barr virus (EBV). Dynein was found to be involved in the clustering of shortened mitochondria in a perinuclear 'mito-aggresome' during EBV infection and reactivation.[76] Of note, Patient P1 showed disabling and persistent generalized muscle weakness and leg muscle fasciculations after an infection with EBV, which may be on the background of a mitochondrial deficit with NCS indicating low amplitude tibial compound muscle action. Another member of the herpes virus family, herpes simplex virus 1 (HSV-1), was found to hijack dynein amongst other proteins for transportation of cell membrane to nucleus.[77] Inhibition of dynein was shown to reduce capsid transport of HSV1 from the cell periphery to the nucleus by 50%, although viral entry into the cells was not affected.[78] Adenovirus particles were shown to penetrate the endosomal membrane early during the course of an infection, and then move as naked cytosolic particles along microtubules to the microtubule-organizing centre[79] through

direct interaction of dynein intermediate and light intermediate chains with the adenovirus capsid subunit hexon, whereas knockdown of dynein heavy chain inhibited virus redistribution to the nucleus.[80]

While patients with pathogenic *DYNC1H1* variants are presumed with a loss-of-function in its encoded protein, we argue that any dsDNA and ssRNA viral hijacking of dysfunctional DYNC1H1 may redirect the residual protein to transporting viral particles for their own needs, further leading to accumulated autophagosomes, and ultimately exacerbating neurodegeneration through toxic aggregation in the cytoplasm.

Alternative pathomechanisms of an infection-bound exacerbation may include an increased general metabolic turnover during systemic inflammation that is unrelated to any defect in intracellular trafficking. A notable example of a herpes virus family member, HHV-6, with an association to epilepsy has been controversially discussed in the literature. While there is a clear link of HHV-6 as an agent in febrile seizures, its association to the neurodegenerative disease temporal lobe epilepsy has a variable range from 9.1% to 55.6%, and the presence of HHV-6 in neurons was found in patients without epilepsy.[81] This may suggest that viral infections in individuals without genetic predisposition to a neurodegenerative disease do not necessarily serve as causal triggers but may rather increase the risk in the general population towards unmasking patients with chronic neurological disorders. Several reports have indicated the presence of antibodies to herpes virus family members, most notably EBV, cytomegaly virus (CMV) and HHV-6, in correlation with multiple sclerosis.[82-84] While cellular models do indeed offer pathomechanistic insights into the role of viral infection as plausible causes for multiple sclerosis, the more correlative serological findings in patients are still under scientific debate.[85]

While we found no variants in disease genes associated with primary immunodeficiency, we observed an IgA deficiency in Patient P2 and hypogammaglobulinaemia in siblings Patients P33 and P34. We argue that this may be relevant to DYNC1H1-related intracellular trafficking in xenophagy. Several monogenic disorders of autophagy include decreased serum immunogloublins, e.g. IgA or IgG depletions in *EPG5*-related Vici syndrome.[86,87] *Rab6a* loss in mice led to hyperinflammation from lactation with decreased activation of STAT5.[88] Stat5 controls B-cell haematopoiesis and STAT5B deficiency has been previously linked to hypogammaglobulinaemia in a human monogenic disorder.[89] We propose that a DYNC1H1-Rab6 interaction defect may cause the IgA and IgG depletions in our Patients P2, P33 and P34. This may be an important finding for the monitoring of patients with recurrent infections, for whom we suggest an initial detailed immunological screening (immunoglobulins, complete blood count). If indeed the patients present with hypogammaglobulinaemia, we suggest treatment with IVIG, where appropriate. Recent guideline articles have not yet incorporated these findings and further studies in large cohorts of patients with *DYNC1H1*-related disorders are needed to screen for the incidence of hypogammaglobulinaemia before making general recommendations.

Legionella pneumophila was also shown to subvert ER-derived vesicles (ERDVs) by recruiting Rab33B and Rab6A.[90] While we did indeed observe a number of patients with neurodegenerative courses prompted by lower respiratory bacterial infections, there were no reports of confirmed *Legionella* infections in our cohort. Of note, recurrent infections of the upper respiratory tract (rhinitis, sinusitis, otitis media) as well the lower respiratory tract (bronchitis, pneumonia) are notable features in monogenic disorders of axonemal dynein, as well as cytoplasmic light or intermediate chains.[91,92] It is unclear if ciliary beating is altered in the affected patients. To our current knowledge, the implications of DYNC1H1 deficiency on ciliogenesis, or ciliary beating, remain elusive. Further insights may be obtained through ultrastructural and functional analysis, including the assessment of ciliary beat pattern and frequency. Our findings expand the current landscape of cytoplasmic dynein heavy chain disorders and place *DYNC1H1*-related disorders in the wider spectrum of other dyneinopathies. It remains elusive whether the contractures, congenital hip dysplasia and congenital bone fractures in our patients arise from early-onset NMD and NDD symptoms, as previously suggested, or whether there is an overlap in dynein trafficking disorders to the skeletal dysplasia associated with some cytoplasmic dynein chain disorders. Further research is needed to elucidate potential molecular biological links with other dynein trafficking disorders.

Multisystem features in phenotype expansion

A primary assumption of any global effects on post-mitotic tissues might include a stall of autophagosomal trafficking due to DYNC1H1 defects. However, any trafficking disorder itself would not entirely explain an autophagy stall. Based on our observation in the phenotype expansion of patients with DYNC1H1 defects, we discuss that the multisystem features, such as immunodeficiency, cataracts, sensorineural deafness and skin abnormalities, do not only align with other dyneinopathies but more generally with other disorders of intracellular trafficking and autophagy.[93] *EPG5*-related Vici syndrome with an associated defect in autophagolysosomal tethering shows considerably phenotypic overlap with DYNC1H1 defects, such as NDD, cataracts, hearing loss, skin abnormalities, and immunological abnormalities.[94] PI4K2A deficiency with defective pools of Rab7-related endosomes presents as a paradigmatic disorder of intracellular trafficking presents with epileptic and dyskinetic encephalopathy and recurrent infections, similar to DYNC1H1 deficiency.[95] Recent studies indicated that any inhibition of HAP1-dynein-mediated autophagosomal transport disrupted autophagosomal maturation and inhibition of autophagosomal maturation perturbed the binding and function of dynein effectors.[96] JIP3 and JIP4 were identified as dynein adaptors on autophagosomes and lysosomes, regulated by ARF6 and RAB10.[97] Dynein supported retrograde flux of endosomes in dendrites, and inhibition of dynein delayed maturation of somatic endosomes with excessive accumulation of Rab7, which was vital for endosomal maturation and fusion with lysosomes after arrival in the soma.[98] These molecular biological reports indicate that there may be feedforward from defective trafficking to accumulated autophagosomes, as well as feedback from defective trafficking to failure of autophagosome maturation. Considering that autophagy is ubiquitous, we argue that the dynein-regulated tight link of both maturation and transport are vital in the normal functioning of autophagosomes to maintain health in post-mitotic tissues.[96]

Conclusion

In summary, our findings expand the spectrum of *DYNC1H1*-related disorders in a lifelong continuum with a newly recognized biphasic disease course. Consistent with molecular biology reports of global effects of a dysfunctional dynein motor complex, our findings suggest previously not recognized multi-organ involvement including the ANS but also non-neuronal tissues, as well as dynamic progression exacerbated by viral infections considerably overlapping with other dyneinopathies. These findings will aid early detection of multisystem phenotypes and genetic counselling of patients and families affected by *DYNC1H1*-related disorders.

Data availability

All anonymized patient data not presented in this study are available from the corresponding author upon reasonable request.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

References

1. Hoang HT, Schlager MA, Carter AP, Bullock SL. DYNC1H1 mutations associated with neurological diseases compromise processivity of dynein-dynactin-cargo adaptor complexes. *Proc Natl Acad Sci U S A*. 2017;114:E1597-E1606.
2. Reck-Peterson SL, Redwine WB, Vale RD, Carter AP. The cytoplasmic dynein transport machinery and its many cargoes. *Nat Rev Mol Cell Biol*. 2018;19:382-398.
3. Hirokawa N, Niwa S, Tanaka Y. Molecular motors in neurons: Transport mechanisms and roles in brain function, development, and disease. *Neuron*. 2010;68:610-638.
4. Schiavo G, Greensmith L, Hafezparast M, Fisher EMC. Cytoplasmic dynein heavy chain: The servant of many masters. *Trends Neurosci*. 2013;36:641-651.
5. Marzo MG, Griswold JM, Ruff KM, Buchmeier RE, Fees CP, Markus SM. Molecular basis for dyneinopathies reveals insight into dynein regulation and dysfunction. *Elife*. 2019;8:e47246.
6. Li JT, Dong SQ, Zhu DQ, et al. Expanding the phenotypic and genetic spectrum of neuromuscular diseases caused by DYNC1H1 mutations. *Front Neurol*. 2022;13:943324.
7. Strickland A V, Schabhüttl M, Offenbacher H, et al. Mutation screen reveals novel variants and expands the phenotypes associated with DYNC1H1. *J Neurol*. 2015;262:2124-2134.
8. Weedon MN, Hastings R, Caswell R, et al. Exome sequencing identifies a DYNC1H1 mutation in a large pedigree with dominant axonal Charcot-Marie-Tooth disease. *Am J Hum Genet*. 2011;89:308-312.
9. Poirier K, Lebrun N, Broix L, et al. Mutations in TUBG1, DYNC1H1, KIF5C and KIF2A cause malformations of cortical development and microcephaly. *Nat Genet*. 2013;45:639-647.
10. Harms MB, Allred P, Gardner R, et al. Dominant spinal muscular atrophy with lower extremity predominance: Linkage to 14q32. *Neurology*. 2010;75:539-546.
11. Harms MB, Ori-McKenney KM, Scoto M, et al. Mutations in the tail domain of DYNC1H1 cause dominant spinal muscular atrophy. *Neurology*. 2012;78:1714-1720.
12. Becker LL, Dafsari HS, Schallner J, et al. The clinical-phenotype continuum in DYNC1H1-related disorders—Genomic profiling and proposal for a novel classification. *J Hum Genet*. 2020;65:1003-1017.
13. Amabile S, Jeffries L, McGrath JM, et al. DYNC1H1-related disorders: A description of four new unrelated patients and a comprehensive review of previously reported variants. *Am J Med Genet A*. 2020;182:2049-2057.
14. Willemsen MH, Vissers LEL, Willemsen MAAP, et al. Mutations in DYNC1H1 cause severe intellectual disability with neuronal migration defects. *J Med Genet*. 2012;49:179-183.
15. Dafsari HS, Becker L, von der Hagen M, Cirak S. Genomic profiling in neuronal dyneinopathies and updated classifications. *Am J Med Genet A*. 2021;185:2607-2610.
16. 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;36:928-930.
17. Kopanos C, Tsiolkas V, Kouris A, et al. VarSome: The human genomic variant search engine. *Bioinformatics*. 2019;35:1978-1980.
18. Chopra M, Savatt JM, Bingaman TI, et al. Clinical variants paired with phenotype: A rich resource for brain gene curation. *Genet Med*. 2024;26:101035.
19. Körner RW, Bansemir OY, Franke R, Sturm J, Dafsari HS. Atopy and elevation of IgE, IgG3, and IgG4 may be risk factors for post COVID-19 condition in children and adolescents. *Children (Basel)*. 2023;10:1598.
20. Hentrich L, Parnes M, Lotze TE, et al. Novel genetic and phenotypic expansion in GOSR2-related progressive myoclonus epilepsy. *Genes (Basel)*. 2023;14:1860.
21. Dafsari HS, Kawalia A, Sprute R, et al. Novel mutations in SLC6A5 with benign course in hyperekplexia. *Cold Spring Harb Mol Case Stud*. 2019;5:a004465.

22. Allen NM, Dafsari HS, Wraige E, Jungbluth H. Neck-tongue syndrome: An underrecognized childhood onset cephalalgia. *J Child Neurol*. 2018;33:347-350.
23. Robinson PN, Köhler S, Bauer S, Seelow D, Horn D, Mundlos S. The human phenotype ontology: A tool for annotating and analyzing human hereditary disease. *Am J Hum Genet*. 2008;83: 610-615.
24. Köhler S, Gargano M, Matentzoglu N, et al. The human phenotype ontology in 2021. *Nucleic Acids Res*. 2021;49(D1): D1207-D1217.
25. Saffari A, Lau T, Tajsharghi H, et al. The clinical and genetic spectrum of autosomal-recessive *TOR1A* -related disorders. *Brain*. 2023;146:3273-3288.
26. Beecroft SJ, McLean CA, Delatycki MB, et al. Expanding the phenotypic spectrum associated with mutations of DYNC1H1. *Neuromuscul Disord*. 2017;27:607-615.
27. Niu Q, Wang X, Shi M, Jin Q. A novel DYNC1H1 mutation causing spinal muscular atrophy with lower extremity predominance. *Neurol Genet*. 2015;1:e20.
28. Ding D, Chen Z, Li K, et al. Identification of a de novo DYNC1H1 mutation via WES according to published guidelines. *Sci Rep*. 2016;6:20423.
29. Lin Z, Liu Z, Li X, et al. Whole-exome sequencing identifies a novel de novo mutation in DYNC1H1 in epileptic encephalopathies. *Sci Rep*. 2017;7:258.
30. Chan SHS, van Alfen N, Thuestad IJ, et al. A recurrent de novo DYNC1H1 tail domain mutation causes spinal muscular atrophy with lower extremity predominance, learning difficulties and mild brain abnormality. *Neuromuscul Disord*. 2018;28:750-756.
31. Gelineau-Morel R, Lukacs M, Weaver KN, Hufnagel RB, Gilbert DL, Stottmann RW. Congenital cataracts and gut dysmotility in a DYNC1H1 dyneinopathy patient. *Genes (Basel)*. 2016;7:85.
32. Chen Y, Xu Y, Li G, et al. Exome sequencing identifies de novo DYNC1H1 mutations associated with distal spinal muscular atrophy and malformations of cortical development. *J Child Neurol*. 2017;32:379-386.
33. Laquerriere A, Maillard C, Cavallin M, et al. Neuropathological hallmarks of brain malformations in extreme phenotypes related to DYNC1H1 mutations. *J Neuropathol Exp Neurol*. 2017;76: 195-205.
34. Hertecant J, Komara M, Nagi A, Suleiman J, Al-Gazali L, Ali BR. A novel de novo mutation in DYNC1H1 gene underlying malformation of cortical development and cataract. *Meta Gene*. 2016; 9:124-127.
35. Peeters K, Bervoets S, Chamova T, et al. Novel mutations in the DYNC1H1 tail domain refine the genetic and clinical spectrum of dyneinopathies. *Hum Mutat*. 2015;36:287-291.
36. Tsurusaki Y, Saitoh S, Tomizawa K, et al. A DYNC1H1 mutation causes a dominant spinal muscular atrophy with lower extremity predominance. *Neurogenetics*. 2012;13:327-332.
37. Das J, Lilleker JB, Jabbal K, Ealing J. A missense mutation in DYNC1H1 gene causing spinal muscular atrophy—Lower extremity, dominant. *Neurol Neurochir Pol*. 2018;52:293-297.
38. Punetha J, Monges S, Franchi ME, Hoffman EP, Cirak S, Tesi-Rocha C. Exome sequencing identifies DYNC1H1 variant associated with vertebral abnormality and spinal muscular atrophy with lower extremity predominance. *Pediatr Neurol*. 2015; 52:239-244.
39. Zillhardt JL, Poirier K, Broix L, et al. Mosaic parental germline mutations causing recurrent forms of malformations of cortical development. *Eur J Hum Genet*. 2016;24:611-614.
40. Fiorillo C, Moro F, Yi J, et al. Novel dynein DYNC1H1 neck and motor domain mutations link distal spinal muscular atrophy and abnormal cortical development. *Hum Mutat*. 2014;35:298-302.
41. Scoto M, Rossor AM, Harms MB, et al. Novel mutations expand the clinical spectrum of DYNC1H1-associated spinal muscular atrophy. *Neurology*. 2015;84:668-679.
42. Jamuar SS, Lam ATN, Kircher M, et al. Somatic mutations in cerebral cortical malformations. *N Engl J Med*. 2014;371:733-743.
43. Möller B, Coppolla A, Jungbluth H, Dafsari HS. DYNC1H1-Related Disorders. GeneReviews® [Internet]. Published online 14 March 2024. Accessed 28 March 2024. https://www.ncbi.nlm.nih.gov/books/NBK601997/
44. Rodrigues CHM, Pires DEV, Ascher DB. DynaMut2: Assessing changes in stability and flexibility upon single and multiple point missense mutations. *Protein Sci*. 2021;30:60-69.
45. Camilleri M. Gastrointestinal motility disorders in neurologic disease. *Journal of Clinical Investigation*. 2021;131:e143771.
46. Kim H, Jung HR, Kim JB, Kim DJ. Autonomic dysfunction in sleep disorders: From neurobiological basis to potential therapeutic approaches. *J Clin Neurol*. 2022;18:140-151.
47. Petry-Schmelzer JN, Krause M, Dembek TA, et al. Non-motor outcomes depend on location of neurostimulation in Parkinson's disease. *Brain*. 2019;142:3592-3604.
48. Goldstein AM, Thapar N, Karunaratne TB, De Giorgio R. Clinical aspects of neurointestinal disease: Pathophysiology, diagnosis, and treatment. *Dev Biol*. 2016;417:217-228.
49. De Giorgio R, Bianco F, Latorre R, Caio G, Clavenzani P, Bonora E. Enteric neuropathies: Yesterday, today and tomorrow. *Adv Exp Med Biol*. 2016;891:123-133.
50. Di Nardo G, Blandizzi C, Volta U, et al. Review article: Molecular, pathological and therapeutic features of human enteric neuropathies. *Aliment Pharmacol Ther*. 2008;28:25-42.
51. Cullup T, Kho AL, Dionisi-Vici C, et al. Recessive mutations in EPG5 cause vici syndrome, a multisystem disorder with defective autophagy. *Nat Genet*. 2013;45:83-87.
52. Esteve C, Francescatto L, Tan PL, et al. Loss-of-function mutations in UNC45A cause a syndrome associating cholestasis, diarrhea, impaired hearing, and bone fragility. *Am J Hum Genet*. 2018;102:364-374.
53. Wells R, Tonkin A. Clinical approach to autonomic dysfunction. *Intern Med J*. 2016;46:1134-1139.
54. Goldberger JJ, Arora R, Buckley U, Shivkumar K. Autonomic nervous system dysfunction: JACC focus seminar. *J Am Coll Cardiol*. 2019;73:1189-1206.
55. Dineen J, Freeman R. Autonomic neuropathy. *Semin Neurol*. 2015;35:458-468.
56. Sánchez-Manso JC, Gujarathi R, Varacallo M. Autonomic Dysfunction. StatPearls. Published online 24 October 2022. Accessed 28 March 2023. https://www.ncbi.nlm.nih.gov/books/NBK430888/
57. Matanis T, Akhmanova A, Wulf P, et al. Bicaudal-D regulates COPI-independent Golgi–ER transport by recruiting the dynein–dynactin motor complex. *Nat Cell Biol*. 2002;4:986-992.
58. Shomron O, Hirschberg K, Burakov A, et al. Positioning of endoplasmic reticulum exit sites around the Golgi depends on BicaudalD2 and Rab6 activity. *Traffic*. 2021;22:64-77.
59. Brault J, Bardin S, Lampic M, et al. RAB6 and dynein drive post-Golgi apical transport to prevent neuronal progenitor delamination. *EMBO Rep*. 2022;23:e54605.
60. Wassmer T, Attar N, Harterink M, et al. The retromer coat complex coordinates endosomal sorting and dynein-mediated transport, with carrier recognition by the trans-Golgi network. *Dev Cell*. 2009;17:110-122.
61. Langworthy MM, Appel B. Schwann cell myelination requires Dynein function. *Neural Dev*. 2012;7:1-15.
62. Chen XJ, Levedakou EN, Millen KJ, Wollmann RL, Soliven B, Popko B. Proprioceptive sensory neuropathy in mice with a

mutation in the cytoplasmic dynein heavy chain 1 gene. *J Neurosci*. 2007;27:14515-14524.
63. Iwaki A, Moriwaki K, Sobajima T, et al. Loss of Rab6a in the small intestine causes lipid accumulation and epithelial cell death from lactation. *FASEB J*. 2020;34:9450-9465.
64. Yang L, Liu G, Li X, et al. Small GTPase RAB6 deficiency promotes alveolar progenitor cell renewal and attenuates PM2.5-induced lung injury and fibrosis. *Cell Death Dis*. 2020;11:827.
65. Dornan L G, Simpson J C. Rab6-mediated retrograde trafficking from the Golgi: The trouble with tubules. *Small GTPases*. 2023;14:26.
66. Buser DP, Spang A. Protein sorting from endosomes to the TGN. *Front Cell Dev Biol*. 2023;11:1140605.
67. Kuballa P, Nolte WM, Castoreno AB, Xavier RJ. Autophagy and the immune system. *Annu Rev Immunol*. 2012;30:611-646.
68. Sharma V, Verma S, Seranova E, Sarkar S, Kumar D. Selective autophagy and Xenophagy in infection and disease. *Front Cell Dev Biol*. 2018;6(NOV):147.
69. Mao K, Klionsky DJ. Xenophagy: A battlefield between host and microbe, and a possible avenue for cancer treatment. *Autophagy*. 2017;13:223.
70. Ye J, Zheng M. Autophagosome trafficking. *Adv Exp Med Biol*. 2021;1208:67-77.
71. Wongchitrat P, Chanmee T, Govitrapong P. Molecular mechanisms associated with neurodegeneration of neurotropic viral infection. *Mol Neurobiol*. 2023;61:2881-2903.
72. Zavala-Vargas DI, Visoso-Carbajal G, Cedillo-Barrón L, et al. Interaction of the Zika virus with the cytoplasmic dynein-1. *Virol J*. 2023;20:43.
73. Stoyanova G, Jabeen S, Landazuri Vinueza J, Ghosh Roy S, Lockshin RA, Zakeri Z. Zika virus triggers autophagy to exploit host lipid metabolism and drive viral replication. *Cell Commun Signal*. 2023;21:114.
74. Hou W, Kang W, Li Y, Shan Y, Wang S, Liu F. Dynamic dissection of dynein and kinesin-1 cooperatively mediated intercellular transport of porcine epidemic diarrhea coronavirus along microtubule using single virus tracking. *Virulence*. 2021;12:615-629.
75. Rosichini M, Bordoni V, Silvestris DA, et al. SARS-CoV-2 infection of thymus induces loss of function that correlates with disease severity. *J Allergy Clin Immunol*. 2023;151:911-921.
76. Glon D, Vilmen G, Perdiz D, et al. Essential role of hyperacetylated microtubules in innate immunity escape orchestrated by the EBV-encoded BHRF1 protein. *PLoS Pathog*. 2022;18:e1010371.
77. Banerjee A, Kulkarni S, Mukherjee A. Herpes simplex virus: The hostile guest that takes over your home. *Front Microbiol*. 2020;11:733.
78. Döhner K, Wolfstein A, Prank U, et al. Function of dynein and dynactin in herpes simplex virus capsid transport. *Mol Biol Cell*. 2002;13:2795-2809.
79. Suomalainen M, Nakano MY, Keller S, Boucke K, Stidwill RP, Greber UF. Microtubule-dependent plus- and minus end–directed motilities are competing processes for nuclear targeting of adenovirus. *J Cell Biol*. 1999;144:657-672.
80. Bremner KH, Scherer J, Yi J, Vershinin M, Gross SP, Vallee RB. Article adenovirus transport via direct interaction of cytoplasmic dynein with the viral capsid hexon subunit. *Cell Host Microbe*. 2009;6:523-535.
81. Wouk J, Rechenchoski DZ, Rodrigues BCD, Ribelato EV, Faccin-Galhardi LC. Viral infections and their relationship to neurological disorders. *Arch Virol*. 2021;166:733-753.
82. Bjornevik K, Cortese M, Healy BC, et al. Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. *Science*. 2022;375:296-301.
83. Grut V, Biström M, Salzer J, et al. Human herpesvirus 6A and axonal injury before the clinical onset of multiple sclerosis. *Brain*. 2024;147:177-185.
84. Fadda G, Yea C, O'Mahony J, et al. Epstein–Barr virus strongly associates with pediatric multiple sclerosis, but not myelin oligodendrocyte glycoprotein-antibody-associated disease. *Ann Neurol*. 2024;95:700-705.
85. Vietzen H, Berger SM, Kühner LM, et al. Ineffective control of Epstein-Barr-virus-induced autoimmunity increases the risk for multiple sclerosis. *Cell*. 2023;186:5705-5718.e13.
86. Chorin O, Hirsch Y, Rock R, et al. Vici syndrome in Israel: Clinical and molecular insights. *Front Genet*. 2022;13:991721.
87. Dafsari HS, Ebrahimi-Fakhari D, Saffari A, Deneubourg C, Fanto M, Jungbluth H. EPG5-Related Disorder. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Amemiya A, eds. *GeneReviews®*. University of Washington; 2022.
88. Cayre S, Faraldo MM, Bardin S, Miserey-Lenkei S, Deugnier MA, Goud B. RAB6 GTPase regulates mammary secretory function by controlling the activation of STAT5. *Development*. 2020;147:dev190744.
89. Acres MJ, Gothe F, Grainger A, et al. Signal transducer and activator of transcription 5B deficiency due to a novel missense mutation in the coiled-coil domain. *J Allergy Clin Immunol*. 2019;143:413-416.e4.
90. Kawabata M, Matsuo H, Koito T, et al. Legionella hijacks the host Golgi-to-ER retrograde pathway for the association of Legionella-containing vacuole with the ER. *PLoS Pathog*. 2021;17:e1009437.
91. Knowles MR, Daniels LA, Davis SD, Zariwala MA, Leigh MW. Primary ciliary dyskinesia. Recent advances in diagnostics, genetics, and characterization of clinical disease. *Am J Respir Crit Care Med*. 2013;188:913-922.
92. O'Callaghan C, Rutman A, Williams GM, Hirst RA. Inner dynein arm defects causing primary ciliary dyskinesia: Repeat testing required. *Eur Respir J*. 2011;38:603-607.
93. Bayram N, Kaçar Bayram A, Daimagüler HS, et al. Genotype-phenotype correlations in ocular manifestations of Marinesco–Sjögren syndrome: Case report and literature review. *Eur J Ophthalmol*. 2022;32:NP92-NP97.
94. Byrne S, Jansen L, U-King-Im JM, et al. EPG5-related vici syndrome: A paradigm of neurodevelopmental disorders with defective autophagy. *Brain*. 2016;139:765-781.
95. Dafsari HS, Pemberton JG, Ferrer EA, et al. PI4K2A deficiency causes innate error in intracellular trafficking with developmental and epileptic-dyskinetic encephalopathy. *Ann Clin Transl Neurol*. 2022;9:1345-1358.
96. Cason SE, Carman PJ, Van Duyne C, Goldsmith J, Dominguez R, Holzbaur ELF. Sequential dynein effectors regulate axonal autophagosome motility in a maturation-dependent pathway. *J Cell Biol*. 2021;220:e202010179.
97. Cason SE, Holzbaur ELF. Axonal transport of autophagosomes is regulated by dynein activators JIP3/JIP4 and ARF/RAB GTPases. *J Cell Biol*. 2023;222:e202301084.
98. Yap CC, Digilio L, McMahon LP, Wang T, Winckler B. Dynein is required for Rab7-dependent endosome maturation, retrograde dendritic transport, and degradation. *J Neurosci*. 2022;42:4415-4434.