BRAIN
LETTER TO THE EDITOR

Expanding the mutational landscape and clinical phenotype of the YIF1B related brain disorder

Eva Medico Salsench,[1,†] Reza Maroofian,[2,†] Ruizhi Deng,[1] Kristina Lanko,[1] Anita Nikoncuk,[1] Belén Pérez,[3] Obdulia Sánchez-Lijarcio,[3] Salvador Ibáñez-Mico,[4] Antonina Wojcik,[5] Marcelo Vargas,[5] Nouriya Abbas Al-Sannaa,[6] Marian Y. Girgis,[7] Tainá Regina Damaceno Silveira,[8] Peter Bauer,[8] Audrey Schroeder,[9] Chin-To Fong,[10] Amber Begtrup,[11] Meisam Babaei,[12] Mehran Beiraghi Toosi,[13] Farah Ashrafzadeh,[13] Shima Imannezhad,[13] Mohammad Doosti,[14] Najmeh Ahangari,[14] Paria Najarzadeh Torbati,[14] Ehsan Ghayoor Karimiani,[14,15,16] David Murphy,[17] Elisa Cali,[2] Ibrahim H. Kaya,[18] Mohammad AlMuhaizea,[18,19] Dilek Colak,[20] Kelly J. Cardona-Londoño,[21] Stefan T. Arold,[21,22] Henry Houlden,[2] Aida Bertoli-Avella,[8] Namik Kaya[23] and Tahsin Stefan Barakat[1]

†These authors contributed equally to this work.

1 Department of Clinical Genetics, Erasmus MC University Medical Center, Rotterdam, The Netherlands
2 Department of Neuromuscular Disorders, UCL Queen Square Institute of Neurology, London WC1N 3BG, UK
3 Centro de Diagnóstico de Enfermedades Moleculares. Centro de Biología Molecular, Universidad Autonoma de Madrid, CIBER Enfermedades Raras, IdiPAZ, Madrid, Spain
4 Pediatric Neurology Unit, Arrixaca Universitary Hospital, Murcia, Spain
5 Gillette Children's Specialty Healthcare, St. Paul, MN 55101, USA
6 John Hopkins Aramco Health Care, Pediatric Services, Dhahran, Saudi Arabia
7 Pediatric Department, Children's Hospital, Cairo University, Cairo, Egypt
8 CENTOGENE, GmbH, 18055 Rostock, Germany
9 Division of Medical Genetics, University of Rochester Medical Center, Rochester, NY 14642, USA
10 Departments of Pediatrics and of Medicine, University of Rochester Medical Center, Rochester, NY 14642, USA
11 GeneDx, Gaithersburg, MD 20877, USA
12 Department of Pediatrics, North Khorasan University of Medical Sciences, Bojnurd, Iran
13 Department of Pediatrics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
14 Department of Medical Genetics, Next Generation Genetic Polyclinic, Mashhad, Iran
15 Molecular and Clinical Sciences Institute, St. George's, University of London, London SW17 0RE, UK
16 Innovative Medical Research Center, Mashhad Branch, Islamic Azad University, Mashhad, Iran
17 Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, University College London, UK
18 College of Medicine, AlFaisal University, Riyadh, Kingdom of Saudi Arabia
19 Department of Neurosciences, King Faisal Specialist Hospital and Research Centre (KFSHRC), Riyadh, Kingdom of Saudi Arabia
20 Department of Biostatistics, Epidemiology and Scientific Computing, KFSHRC, Riyadh, Kingdom of Saudi Arabia
21 King Abdullah University of Science and Technology (KAUST), Computational Bioscience Research Center (CBRC), Division of Biological and Environmental Sciences and Engineering (BESE), Thuwal 23955-6900, Saudi Arabia
22 Centre de Biologie Structurale, CNRS, INSERM, Université de Montpellier, 34090 Montpellier, France
23 Department of Translational Genomics, King Faisal Specialist Hospital and Research Centre, Riyadh 11211, Kingdom of Saudi Arabia

Correspondence to: Namik Kaya
Department of Translational Genomics, King Faisal Specialist Hospital and Research Centre
Riyadh 11211, Kingdom of Saudi Arabia
E-mail: nkaya@kfshrc.edu.sa

Correspondence may also be addressed to: Tahsin Stefan Barakat
Department of Clinical Genetics, Erasmus MC University Medical Center
PO Box 2040, 3000 CA, Rotterdam, The Netherlands
E-mail: t.barakat@erasmusmc.nl

With great interest we read the article by Diaz and colleagues[1] providing further evidence of a neurodevelopmental disorder caused by bi-allelic variants disrupting the function of YIF1B, by reporting a second patient cohort and a mouse model. We had earlier reported six individuals from five unrelated families, harbouring bi-allelic protein truncating mutations in YIF1B, presenting with a progressive encephalopathy with various degrees of movement disorders, microcephaly and epilepsy.[2]

We here describe eight additional individuals from eight independent families harbouring protein altering YIF1B variants, including four individuals with homozygous or compound heterozygous missense variants (Fig. 1A–D and Supplementary Figs 1–6). We provide functional evidence that these missense variants impact on YIF1B function, and compare the clinical phenotype between these new and all previously reported cases to further delineate the mutational landscape and clinical phenotype associated with this new disease entity, which Online Mendelian Inheritance in Man (OMIM) recently named 'Kaya-Barakat-Masson syndrome' (KABAMAS, OMIM #619125).

Individual 1 is currently a 5-year-old male, the fifth child of consanguineous parents from Spain, born after an uneventful pregnancy and uncomplicated delivery. Developmental delay was noticed early on. He developed a severe encephalopathy, is nonverbal, has severe motor impairment with poor head control, axial hypotonia, peripheral hypertonia and upper extremity dystonia. He is unable to sit independently. He had a febrile seizure at 12 months and developed epileptic seizures at 21 months, initially treated with levetiracetam followed by valproate. EEG showed bilateral focal fronto-temporal activity and MRI showed cortical atrophy and thin corpus callosum. Hypotelorism and flat occiput were noticed. Whole exome sequencing (WES) identified a homozygous truncating variant in YIF1B (c.186dupT, p.Ala63fs), heterozygous in the unaffected parents. His 23-year-old sister was not investigated, but has a similar encephalopathy although without epilepsy.

Individual 2 is a female born to consanguineous Somali parents, who required hospitalization because of feeding problems at age of 2 months. She displayed severe global developmental delay, with no developmental milestones, and epileptic seizures. EEG showed frequent multifocal epileptiform discharges and at times evidence of burst suppression. Seizure control was obtained with phenobarbital and levetiracetam. MRI imaging at 10 months showed reduced cerebral white matter volume with atrophic prominence of ventricles and cerebellar hypoplasia. She became ventilation dependent and deceased at the age of 15 months. WES identified a homozygous truncating YIF1B variant (c.598G>T, p.Glu200*), heterozygous in the unaffected parents.

Interestingly, both p.Ala63fs and p.Glu200* are recurrent variants, identified in five and three independent families, respectively.[1,2] The p.Ala63fs variant was previously found in four Arab families, and given historic migrations of Arabs to Spain this might suggest a founder mutation. Similar, all families harbouring p.Glu200* are from Somali descent, likely indicating a founder mechanism.

Individual 3 is a female born to consanguineous parents from Saudi Arabia who showed lack of developmental milestones, congenital microcephaly, severe failure to thrive, spastic quadriparesis, axial hypotonia and hypoventilation with pons atrophy, cerebellar and corpus callosum hypoplasia and white matter alterations. WES identified a homozygous truncating YIF1B variant (c.336C>G, p.Tyr112*).

Individual 4 is a 1-year-old male born to consanguineous parents from Egypt, which showed severe developmental delay starting at the age of 2 months with spasticity and dystonia, with progressive psychomotor deterioration and feeding difficulties. Whole genome sequencing identified a homozygous chr19:38796532–38812925 (GRCh37/hg19) deletion that included the YIF1B promoter, YIF1B

exon 1–7, and exon 1 of KCNK6, a potassium channel without a known OMIM phenotype.

All but one previously reported family[1,2] presented with protein truncating variants. Interestingly, we identified four additional families with missense variants that resulted in a similar clinical phenotype.

Individual 5 is a 27-year-old female, born at full term to non-consanguineous parents of French and German descent. Because of developmental delay, she received medical attention at ~7 months of age. She was able to sit independently at age of ~2–3 years, but lost this capability. She never walked independently. Speech was limited to few words and lost upon regression. She currently communicates through noises and facial expression. She is currently managed for medically refractory generalized epilepsy, with EEG at age of 26 years noting tonic seizures, and multifocal and diffuse discharges. MRI at age of 25 years showed generalized cerebral and cerebellar volume loss with severe thinning of the corpus callosum. Dysmorphic features include a long face, widely-spaced teeth, a history of gingival hyperplasia, high arched palate, and bitemporal hirsutism. WES identified compound heterozygous missense variants in YIF1B (c.569C>A, p.Ala190Glu and c.621C>A, p.Ser207Arg), which were both absent in the unaffected brother.

Individual 6 is a currently 7.5-year-old male born to consanguineous parents from Iran that presented in early infancy with developmental delay and hypotonia. Epileptic spasms, axial dystonia and limb spasticity subsequently developed (Supplementary Video 1). His best motor achievements included independent sitting and pencil grasp, but no speech development or eye contact. WES identified a homozygous missense variant in YIF1B (c.691G>A, p.Val231Ile), heterozygous in the unaffected parents.

Individual 7 is an 11-month-old male born to consanguineous parents from Iran. He first came to medical attention at age of 4 years because of severe global developmental delay. He failed to develop any milestones and developed axial dystonia and limb spasticity but no epilepsy. Brain MRI showed a thin corpus callosum. WES identified a homozygous missense variant in YIF1B (c.377T>C, p.Leu126Pro), heterozygous in the unaffected parents and siblings.

Finally, Individual 8 is currently 4.5 years old, a female born to consanguineous parents from Iran that presented with global developmental delay, microcephaly and hypotonia, developing into limb spasticity, dystonia, dyskinesia and oculomotor apraxia, without epilepsy. Brain MRI showed global atrophy and a thin corpus callosum. She could sit independently but was unable to stand and spoke only a few words. Metabolic screening was unremarkable. WES identified a homozygous missense variant in YIF1B (c.803G>T, p.Arg268Leu).

Interestingly, all YIF1B missense variants encountered localized in or close to the transmembrane domains (previously shown to be required for YIF1B function[3]) and were absent from gnomAD, except for p.Val231Ile, which was found once in heterozygous state (minor allele frequency 0.00040). All changed highly conserved residues, had a CADD score >22, and *in silico* analysis predicted pathogenicity (Fig. 1B, Supplementary Figs 7–9, Supplementary Table 1 and Supplementary material).

As primary cells of affected individuals were unavailable, we introduced the encountered missense variants by site-directed mutagenesis[4,5] in a YIF1B expression plasmid, and first tested protein expression of these mutants upon transient transfection in HEK cells. Missense variants assessed did not result in significantly reduced YIF1B proteins levels (Fig. 1E and F). To investigate subcellular localization of wild-type and mutant YIF1B, we performed co-staining for YIF1B and the endoplasmic reticulum (ER) marker calnexin (Fig. 1G). Whereas wild-type YIF1B showed a high co-localization with the ER, as previously found,[6] YIF1B variants showed
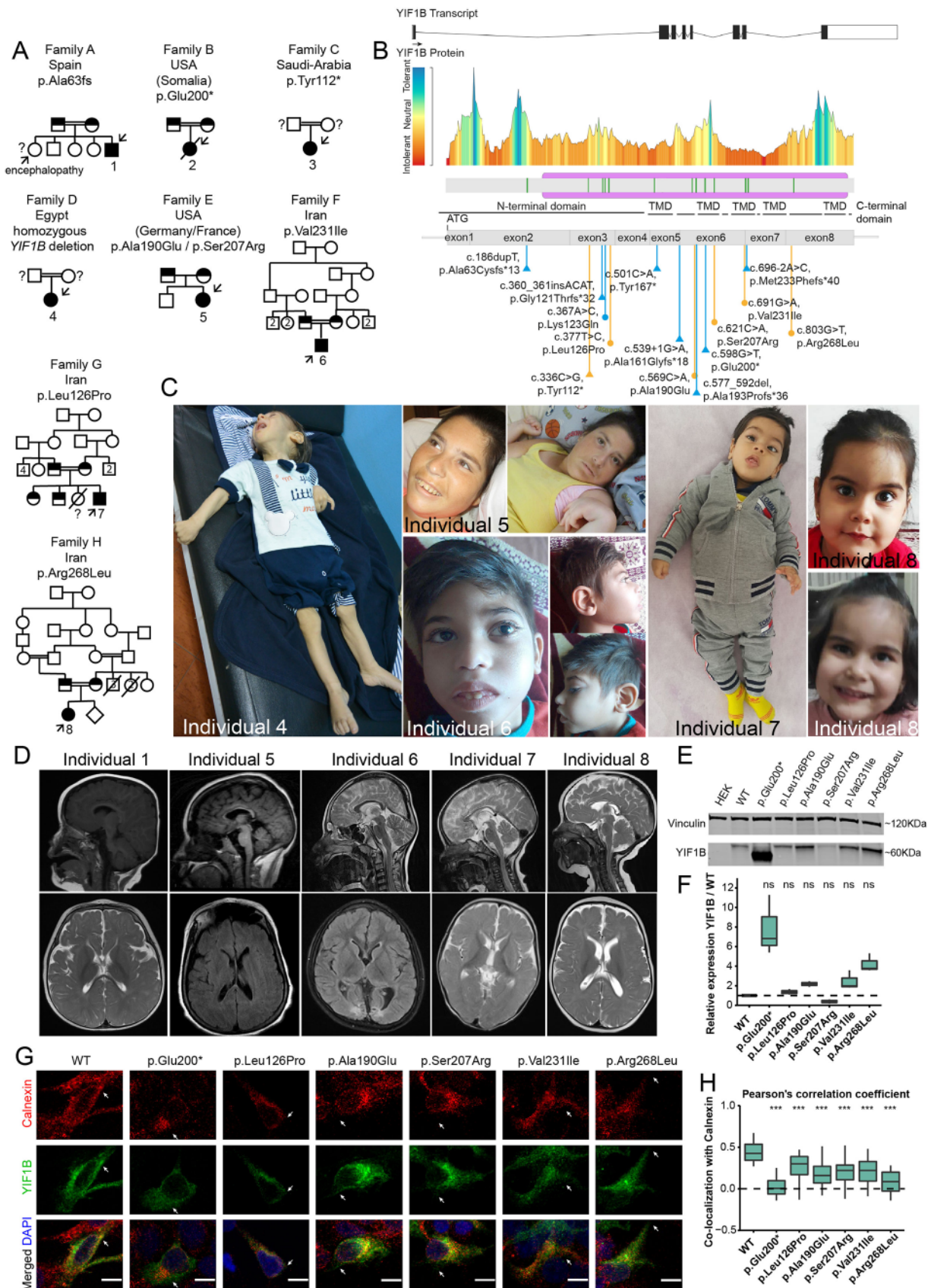
Figure 1 Pedigrees of families, clinical hallmarks and functional investigations of identified YIF1B variants. (A) Family pedigrees of ascertained families. Affected individuals with homozygous *YIF1B* variants and healthy parents with confirmed heterozygous *YIF1B* variants are indicated in filled and half filed symbols, respectively. Presumed carrier parents that were not available for segregation analysis are indicated with open circles or squares and a question mark. Affected individuals with confirmed genotype are indicated with an arrow and numbered. Not-tested affected siblings

(continued)

significantly reduced co-localization (Fig. 1H). Previously, YIF1B was found to interact with RAB6A[7] and TAPL.[3] Upon co-transfection of RAB6A and YIF1B, we found significantly reduced co-localization between both proteins for all YIF1B variants, except p.Leu126Pro

and p.Ser207Arg (Supplementary Fig. 10A and D). In contrast, all tested YIF1B missense variants showed reduced co-localization with TAPL (Supplementary Fig. 10B and E). YIF1B overexpression diminishes co-localization of TAPL and lysosomal markers.[3]

Table 1 Overview of core clinical phenotypes of 24 individuals harbouring bi-allelic variants in YIF1B

Summary	Total (%)	Truncating (%)	Missense (%)	Fisher's exact test			
				OR	P	Low CI	High CI
Truncating mutation	18/24 (75)	–	–	–	–	–	–
Missense mutation	6/24 (25)	–	–	–	–	–	–
Female	13/24 (54.2)	10/18 (56.3)	3/6 (50)	1.238	1.000	0.128	11.999
Deceased	5/24 (20.8)	5/18 (27.8)	0/6 (0)	Infinity	0.280	0.305	Infinity
Growth parameters							
Microcephaly	15/23 (65.2)	11/17 (64.7)	4/6 (66.7)	0.920	1.000	0.065	8.949
Level of functioning							
Global developmental delay	23/23 (100)	17/17 (100)	6/6 (100)	0.000	1.000	0.000	Infinity
Head control	5/23 (30.4)	0/17 (0)	5/6 (83.3)	0.000	**0.000**	0.000	0.189
Sitting	4/23 (17.4)	0/17 (0)	4/6 (66.7)	0.000	**0.002**	0.000	0.351
Standing	2/23 (8.7)	0/17 (0)	2/6 (33.3)	0.000	0.059	0.000	1.733
Walking	2/22 (9.1)	0/16 (0)	2/6 (33.3)	0.000	0.065	0.000	1.844
Cognitive impairment	23/23 (100)	17/17 (100)	6/6 (100)	0.000	1.000	0.000	Infinity
Speech development	4/23 (17.4)	0/17 (0)	4/6 (66.7)	0.000	**0.002**	0.000	0.351
Progressive speech loss	3/4 (75)	n/a (n/a)	3/4 (75)				
Hypoventilation	5/18 (22.7)	5/16 (31.3)	0/2 (0)	Infinity	1.000	0.069	Infinity
Feeding problems/swallowing difficulties	19/23 (82.6)	16/17 (94.1)	3/6 (50)	13.390	0.040	0.796	883.373
Autistic behaviour	4/13 (30.8)	2/8 (25)	2/5 (40)	0.529	1.000	0.025	10.812
Neurology							
Seizures	14/22 (63.6)	10/16 (62.5)	4/6 (66.7)	0.840	1.000	0.059	8.247
Spasticity	21/22 (95.5)	15/16 (93.8)	6/6 (100)	0.000	1.000	0.000	103.833
Hypotonia	19/23 (82.6)	15/17 (88.2)	4/6 (66.7)	3.497	0.271	0.197	63.558
Dystonia	11/23 (47.8)	8/17 (47.1)	3/6 (50)	0.893	1.000	0.091	8.729
Dyskinesia or tremor	9/20 (45)	7/14 (50)	2/6 (33.3)	1.932	0.642	0.195	28.137
Eyes and ears							
Ear/hearing loss	0/16 (0)	0/12 (0)	0/4 (0)	0.000	1.000	0.000	Infinity
Strabismus	10/18 (55.6)	5/12 (41.7)	5/6 (83.3)	0.159	0.152	0.003	2.093
Optic atrophy	2/20 (10)	2/14 (14.3)	0/6 (0)	Infinity	1.000	0.078	Infinity
Retinal involvement	0/18 (0)	0/12 (0)	0/6 (0)	0.000	1.000	0.000	Infinity
Cataract	0/18 (0)	0/12 (0)	0/6 (0)	0.000	1.000	0.000	Infinity
Nystagmus	5/19 (26.3)	4/13 (30.8)	1/6 (16.7)	2.137	1.000	0.147	130.886
Brain imaging							
White matter/myelinization abnormalities	7/24 (29.3)	6/18 (33.3)	1/6 (16.7)	2.416	0.629	0.196	137.539
Cerebellar hypoplasia	8/23 (34.8)	7/17 (41.2)	1/6 (16.7)	3.333	0.369	0.275	188.570
Corpus callosum hypoplasia	12/22 (54.5)	9/16 (56.3)	3/6 (50)	1.271	1.000	0.128	12.729
Cerebral atrophy/parenchymal volume loss	10/24 (41.7)	8/18 (50)	2/6 (33.3)	1.570	1.000	0.170	21.636
Pons/brain stem atrophy	5/22 (18.2)	5/16 (31.3)	0/6 (0)	Infinity	0.266	0.352	Infinity

CI = confidence interval; n/a = not applicable; n/d = not determined; OR = odds ratio. *P*-values in bold indicate significance.

Figure 1 Continued
presenting with similar phenotypes are indicated with a question mark. Consanguineous parents are indicated with a double connection line. Males are squares, females are circles; deceased individuals are marked with a diagonal line. (**B**) Drawing of the *YIF1B* transcript and YIF1B protein, including the tolerance landscape as determined by MetaDome analysis, which displays regional tolerance or intolerance to missense or synonymous variation. All currently known YIF1B variants from Almuhaizea *et al.*[2] and Diaz *et al.*[1] (blue) and those reported herein (orange) encountered in individuals with Kaya-Barakat-Masson syndrome are indicated. Missense and truncating variants are indicated with circles and triangles, respectively. (**C**) Images of Individuals 4–7 and 8 at age of 1 year, 27 years, 7.5 years, 11 months and 4.5 years, respectively. No gross dysmorphic features were observed. Note the neurological posture in Individuals 4, 5 and 7. (**D**) T_1 and T_2-weighted brain MRI images of Individuals 1 and 5–8 in sagittal and axial planes. Note the various degrees of cerebral atrophy, cerebellar hypoplasia, thin corpus callosum and white matter abnormalities. (**E**) Immunoblotting detecting proteins of wild-type and YIF1B variants upon transient transfection for 48 h in HEK293 cells. Endogenous vinculin served as a normalization control. Full length blots are provided in Supplementary Fig. 11. (**F**) Western blot quantification was performed using biological triplicates, normalized to vinculin for each sample and to the wild-type YIF1B control. Box plots represent interquartile range (IQR); line is median; and whiskers extend to $1.5 \times$ IQR, dots are outliers. Wilcoxon signed-rank test; ns = not significant. Full-length blots used for quantification are provided in Supplementary Fig. 11. (**G**) Representative images of immunofluorescence of HEK293 cells, transfected for 24 h with wild-type or mutant eGFP-YIF1B expression plasmids, and co-stained for the ER marker calnexin (red) and DAPI (blue). Scale bar = 10 μm. (**H**) Quantification of calnexin-YIF1B co-localization (*n* = 30 cells for each variant) using Pearson's correlation coefficient (range: –1 negative correlation, 1 max correlation). Box plots represent IQR; line is median; and whiskers extend to $1.5 \times$ IQR. ***$P < 0.001$, Kruskal-Wallis test with *post hoc* Dunn's test.

In agreement, whereas overexpression of wild-type YIF1B resulted in low co-localization correlation between TAPL and the lysosomal marker LAMP2, overexpression of mutant YIF1B failed to diminish this co-localization (Supplementary Fig. 10C and F). Also, co-localization correlation between YIF1B and LAMP2 was higher for mutant YIF1B. Together this indicates that the assessed missense YIF1B variants show mislocalization, reduced co-localization with known interactors and reduced functionality compared to wild-type YIF1B.

Including the eight individuals described herein, 24 individuals from 19 families have currently been identified, harbouring bi-allelic truncating/whole gene deletion ($n = 18$), or missense variants ($n = 6$) in YIF1B (Table 1 and Supplementary Table 2). All individuals presented early in life with a progressive encephalopathy with global developmental delay and cognitive impairment, after uneventful perinatal development. Almost all had feeding problems, axial hypotonia and limb spasticity, with seizures (varying from myoclonic jerks, to generalized tonic clonic seizures and infantile spasms) in around two-thirds of the cases. Around half of the individuals showed signs of dystonia, dyskinesia or microcephaly. Whereas hypoventilation was relatively frequent in the cohort described by Diaz et al.,[1] in total this was only found in five individuals and seems to correlate to brainstem atrophy. Two-thirds of the individuals have brain imaging abnormalities, including white matter alterations, cerebral atrophy, corpus callosum hypoplasia and cerebellar hypoplasia. Interestingly, limited developmental milestones, such as head control, independent sitting, and limited speech were only observed in individuals harbouring missense variants, reaching statistical significant differences between the truncating and missense group after Bonferroni correction ($P = 0.0001783$, $P = 0.001694$, $P = 0.001694$, respectively). This might suggest that the encountered missense variants harbour some residual YIF1B activity in vivo, in agreement with our in vitro functional investigations. Other clinical features were not significantly different between the truncating and missense cohort (Table 1 and Supplementary Table 2).

Together, our previous work[2] and this study, and that of Diaz et al.[1] defines a previously unrecognized neurodevelopmental disorder, presenting with severe to profound neurodevelopmental delay, cognitive impairment, neurological sequelae, seizures and microcephaly. Long term follow-up of individuals with YIF1B variants will help to delineate this new disease entity further.

Data availability

All data are available from the corresponding author upon reasonable request, except for primary patient sequencing data, which cannot be made available due to consent regulations.

Acknowledgements

We would like to thank the individuals described herein and their families for participating in this study. We would like to thank all members of the Barakat lab for helpful discussion. We thank Rupert Abele (Frankfurt) for providing the TAPL plasmid, and Gerben Schaaf (Rotterdam) and Grazia M.S. Mancini (Rotterdam) for providing the LAMP2 and Calnexin antibody, respectively.

Funding

Competing interests

A.B. is an employee of GeneDx, Inc. T.R.D.S, P.B. and A.B.A. are employees of CENTOGENE GmbH. All other authors declare no conflict of interest.

Supplementary material

Supplementary material is available at Brain online.

References

1. Diaz J, Gérard X, Emerit M-B, et al. YIF1B mutations cause a postnatal neurodevelopmental syndrome associated with Golgi and primary cilium alterations. Brain. 2020;143(10):2911–2928.

2. AlMuhaizea M, AlMass R, AlHargan A, et al. Truncating mutations in YIF1B cause a progressive encephalopathy with various degrees of mixed movement disorder, microcephaly, and epilepsy. Acta Neuropathol. 2020;139(4):791–794.

3. Graab P, Bock C, Weiss K, et al. Lysosomal targeting of the ABC transporter TAPL is determined by membrane-localized charged residues. J Biol Chem. 2019;294(18):7308–7323.

4. Sanderson LE, Lanko K, Alsagob M, et al. Bi-allelic variants in HOPS complex subunit VPS41 cause cerebellar ataxia and abnormal membrane trafficking. Brain. 2021;144(3):769–780.

5. Weerts MJA, Lanko K, Guzmán-Vega FJ et al. Delineating the molecular and phenotypic spectrum of the SETD1B-related syndrome. Genet Med. Published online 3 August 2021. doi:10.1038/s41436-021-01246-2

6. Alterio J, Masson J, Diaz J, et al. Yif1B is involved in the anterograde traffic pathway and the Golgi architecture. Traffic. 2015;16(9):978–993.

7. Al Awabdh S, Miserey-Lenkei S, Bouceba T, et al. A new vesicular scaffolding complex mediates the G-protein-coupled 5-HT$_{1A}$ receptor targeting to neuronal dendrites. J Neurosci. 2012;32(41):14227–14241.