



## Truncating mutations in YIF1B cause a progressive encephalopathy with various degrees of mixed movement disorder, microcephaly, and epilepsy.

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# Acta Neuropathologica

## Truncating mutations in YIF1B cause a progressive encephalopathy with various degrees of mixed movement disorder, microcephaly, and epilepsy

--Manuscript Draft--

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	Erasmus MC Vriendenfonds (Human Disease Model Award)	Dr Tahsin Stefan Barakat

## Response letter

Dear Prof. Johannes Attems,

Thank you very much for further considering our work on YIF1B for publication in *Acta Neuropathologica*. We appreciate the time and effort that you and the reviewers have dedicated for providing your valuable feedbacks on our manuscript. Please find attached our revised version, where we have incorporated the very helpful reviewers' comments and the editorial text changes as requested. Below is a point-to-point reaction. We hope to hear from you soon,

Best wishes,

Namik Kaya  
Stefan Barakat

### Point-to-point replies to Reviewers:

Reviewer #1: This is an important short correspondence describing for the first time how mutations in the YIF1B gene cause human disease. By detailing 5 patients in 4 families, the authors are able to describe the hallmark features of microcephaly and dyskinetic encephalopathy. This manuscript could be improved by just a few revisions.

*We thank the reviewer for the appreciation of our work.*

#### Major suggestions:

-The clinical data presented in the 4th paragraph is a little confusing, as it is unclear how many of the features are found in EVERY or MOST of the patients. The first 6 sentences seem to describe shared features. The paragraph then focuses on the individual from family 2, 2:II, and it is unclear whether the rest of the paragraph is then focused on a single person, or all patients.

*We thank for the comment. We have made some changes to clarify this section based on the comment. Due to the word limits of this short correspondence we were limited in space to give a full description of each case. However, a detailed clinical description can be found in the supplementary table and materials and methods. We hope that this sufficiently addresses the reviewers comment.*

-The data presented in supplementary figures 2, 3 and 4 appear to be presenting that available directly from public websites, and not generated or filtered by the authors in a particular manner. It might be more appropriate to merely cite the original sources.

*We have added citations to the source of these publically available data as requested.*

Minor suggestions:

- "whole exome Sequencing" in the 3rd paragraph should either be all lower case, or all three words capitalized.

*Updated as requested*

- There is no description of who the "healthy controls" in supplementary figure 1 are.

*These are healthy members of families 1 and 3. This is noted in the revised supplementary figure 1*

Reviewer #2: This is a well written and straight-forward manuscript. In this study the investigators used WES to identify the causal mutation that cause microcephaly with dyskinetic encephalopathy. They find a mutation in the YIF1B gene in a single case and then they leverage their own biobank to identify additional similar cases with novel mutations in YIF1B. All patients were unrelated by shared a common haplotype suggesting a common ancestor. Studies in mice suggests that this protein is involved in protein traffic.

It is clear that additional functional studies are needed to understand the role of this protein in health and diseases, although this is out of the scope of this study.

*We thank the reviewer for his/her positive feedback. We have added more information in the supplemental material as requested.*

On the other hand, although this is corresponded and therefore quite focused study, the authors need to explain in much more details in material and methods or in the supplementary material how they performed the filtering of the WES (even they used the same approach as before).

*We thank for the comment. We explained this in the supplementary material and methods as requested.*

It is also not clear reading the main text, material and methods or even the supplementary material where the mRNA is coming from, how it is processed QCed and analyzed, and should be explained better.

*The data is taken from the publicly available sources. We have added citations to the source of these databases in the supplementary figures' captions.*

Besides this minor critiques, this is a good manuscript

*We highly thank the reviewer for his/her positive feedback.*

1) Ensure the word count is under 1000 including references and figure legends, as stipulated for correspondence format in instructions for authors.

*As discussed by mail, we have aimed to reduce the number of words to ~1150 (incl main text, refs and the main figure legend), and have finally come to a total of 1183 words. Further cutting on references or text would significantly affect the content of the manuscript, so we hope that this slightly higher word limit is acceptable (also considering that our main figure display is quite small).*

2) Submit Figure 1 as a tiff file with a resolution of at least 300dpi and ensure lettering in figure panels is in lower case.

*Done as requested*

3) All supplementary material should be submitted as one document (preferably as a word or pdf file), and consistently refer to supplementary material throughout the manuscript text as e.g Supplementary Fig.3, online resource, as the material will only be available in the online version of the manuscript and not the printed version

*Done as requested*

Please carefully consider the following technical requirements:

(1) Figures should be of high resolution (at least 300 dpi).

*Done as requested*

(2) Figure parts should be identified with lower case roman letters on figures and throughout text including legends (a, b, c etc, not A, B, C etc).

*Done as requested*

(3) References should be arranged in alphabetical order and formatted exactly according to journal style.

*Done as requested*

(4) Letters (correspondence) should consist of less than 1,000 words, including references and legends. They do not have an abstract nor subheadings such as Introduction etc. The title should represent the major message. For more details on letters, see Instructions for Authors

*See comment above*

*For your further information, we have further updated the title of the manuscript, which we now find to better capture the complexity of the phenotype of this novel disorder. In addition, as we were able to add one additional case with detailed clinical information, we have added three additional authors to the list. Unfortunately, two additional cases could not yet be included due to struggle to obtain the*

*publication consent in a timely manner. Also these cases present with a similar disease phenotype, further substantiating our findings.*

[Click here to view linked References](#)

## Truncating mutations in *YIF1B* cause a progressive encephalopathy with various degrees of mixed movement disorder, microcephaly, and epilepsy

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Several intracellular proteins are involved in mediating vesicular transport of protein and lipid cargo from the endoplasmic reticulum (ER) to the Golgi apparatus (GA) in eukaryotic cells. Errors in membrane trafficking between ER and GA have been implicated in brain disorders [1, 7], showing that these processes are critical for neuronal biogenesis. An important protein in these processes is YIF1B, an intracellular 314-residue transmembrane protein. Hippocampal neurons from Yif1B knockout (KO) mice showed that Yif1B is implicated in anterograde trafficking and Golgi architecture [1], where depletion of Yif1b caused disorganization, fragmentation and volume reduction of the GA in pyramidal neurons.

Here we describe 6 patients from 5 unrelated families presenting with profound developmental and motor delay with dystonia, dysphagia, hypotonia, epilepsy and microcephaly, and homozygous truncating variants in *YIF1B* encountered by whole exome sequencing (WES), identifying *YIF1B* as a novel disease gene in humans (Figure 1, Supplementary Figure 1, Supplementary Table 1, online resource for detailed clinical information).

Clinical examinations revealed that all patients had an unremarkable pregnancy and birth, and no major dysmorphic features. Hypotonia and global developmental delay were noticed in infancy with smiling and partial babbling as their best achieved social and language skills. Motor development remained profoundly affected without head control, rolling or sitting. By age 2-3 years distal, limb choreiform movements started in 4 individuals which evolved into axial and limb dystonia with dyskinesia by the age of 4-8 years. Dystonia was unresponsive to levodopa or carbidopa but partially improved on trihexyphenidyl. Two individuals developed epileptic seizures at 6- to 8-month. EEG showed multiple bilateral epileptiform discharges and abnormal background indicating diffuse dysfunction. Microcephaly was found in all but one individual. There were no exaggerated startle, organomegaly or neurocutaneous stigmata. Hearing was normal. One case presented with cortical blindness. Repeated MRI brain imaging showed mild thinning of the corpus callosum in the majority of cases with (Figure 1f) brain atrophy in one case without other structural abnormalities. Other standard metabolic investigations in serum and liquor were unremarkable. Hence, all individuals with truncating *YIF1B* variants shared a similar phenotypical spectrum. All affected residues

encountered were highly conserved and identified variants (c.186dupT:p.Ala64fs; c.360\_361insACAT:p.Gly121fs; c.598G>T:p.Glu200\*) were absent from gnomAD and other databases of healthy individuals with no other homozygous loss-of-functions variants found (Supplementary Figure 1b online resource).

*YIF1B* is widely expressed at mRNA and protein levels in all tissues including neuronal cells, in particular raphe neurons (Supplementary Figures 2-4, online resource). Functional and network analyses identified 171 genes that are co-regulated with *YIF1B* in over 46,000 human RNAseq samples ( $r \geq 0.9$ ), that were enriched in genes involved in neurological and development disorders, and nervous system development and function (p-value <0.01) (Supplementary Figure 5a,b and Supplementary Tables 2,3, online resource).

In rat, the *YIF1B* ortholog interacts with serotonin receptor 1 (5-HT1AR) which is a Gi/Go protein-coupled receptor in dendrites of serotonin neurons of the raphe nuclei. Co-localization of Yif1B and 5-HT1AR was observed in intermediate compartment small vesicles, showing Yif1B involvement in transient intracellular trafficking and modulation of 5-HT1AR transport to dendrites [2, 3]. Hence, defects on Yif1B may lead to the impairment of the physiological functioning of 5-HT1AR [4]. *YIF1B* also interacts with the lysosomal protein TAPL in humans [5]. In HeLa cells, overexpressing truncated *YIF1B* reduced TAPL's lysosomal localization and co-localization with truncated *YIF1B* in the *cis*-Golgi [6]. In addition, Yif1B interacts with Rab6, a recycle trafficking protein. Therefore, alterations of this interaction may lead to aggregates accumulation in neurons, which has been reported to cause neurodegenerative diseases [1]. These findings show that to fulfill its biological function, *YIF1B* interacts with different proteins and controls their trafficking. Consequently, mutations affecting protein binding sites or subcellular localization of *YIF1B* are expected to cause trafficking deficiencies of its interaction partners.

Computational protein modelling of *YIF1B* predicted that the first ~68 residues form a cytosolic disordered region, and indicated the presence of transmembrane helices in the remaining ~250

residues (Figure 1e, Supplementary Figure 6a, b, online resource). The p.Gly121fs variant leads to the insertion of 31 non-related residues which are predicted to be unstructured (Supplementary Figure 6a, b, c online resource). Although we cannot rule out that this variant might retain some capacity to insert into membrane, its biological function is most likely lost. The p.Ala63fs variant leads to the introduction of twelve unrelated residues following Pro62 before the stop codon, resulting in truncation before the first helix, indicating a complete loss of function. The variant p.Glu200\* has 3.5 transmembrane helices missing, which is therefore predicted to be non-functional.

Therefore, the reported disease phenotypes are corresponding to the effects of a complete loss-of-function of *YIF1B*. Collectively, our data provide a demonstration of the importance of *YIF1B* in humans and argue that this gene is involved in a novel neurogenetic disorder. Further functional studies are required to unravel the precise pathogenetic mechanisms of this novel disease entity.

#### **Conflict of interest**

MSC, PB and ABA are employees of CENTOGENE AG. AB is an employee of GeneDx. The other authors declare no conflict of interest.

#### **Author contribution**

NK conceived and designed the experiments. RAM, AAH, JH, JAS, LAQ and AB performed experiments. DC, MS, PB, ABA, AB analyzed data. DC performed bioinformatics, pathway and network analyses. SA performed protein modeling. MAM, LAQ collected specimen. MAM handled biopsies, undertook patient care and management, collected clinical data, and delineated the patients' phenotype (F1, F2, and F3). AC helped MAM to recruit one patient. TB, MSC, PB, AB-A, JL, PK collected clinical data (F4, F5). NK, TSB, EMS, MAM, DC, JH, IHK wrote the paper.

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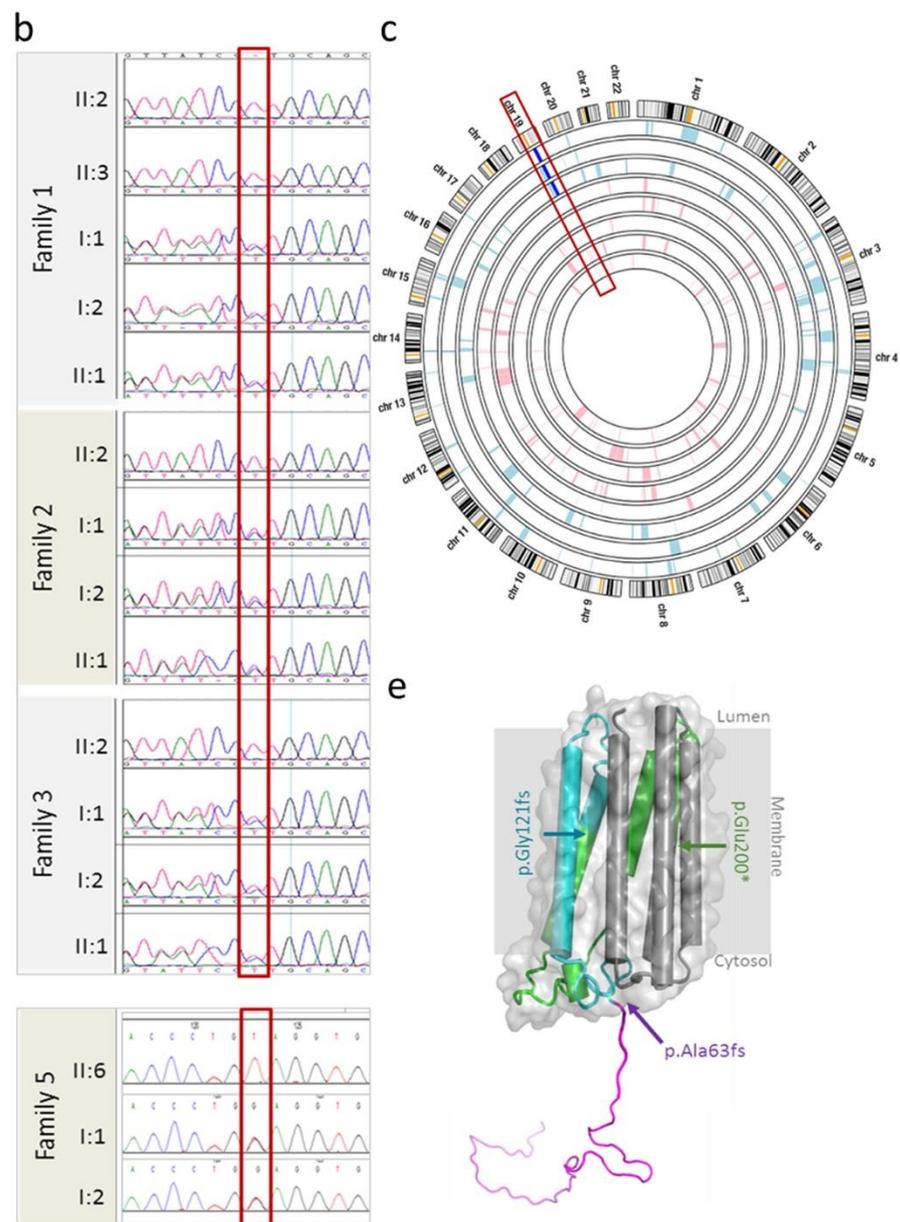
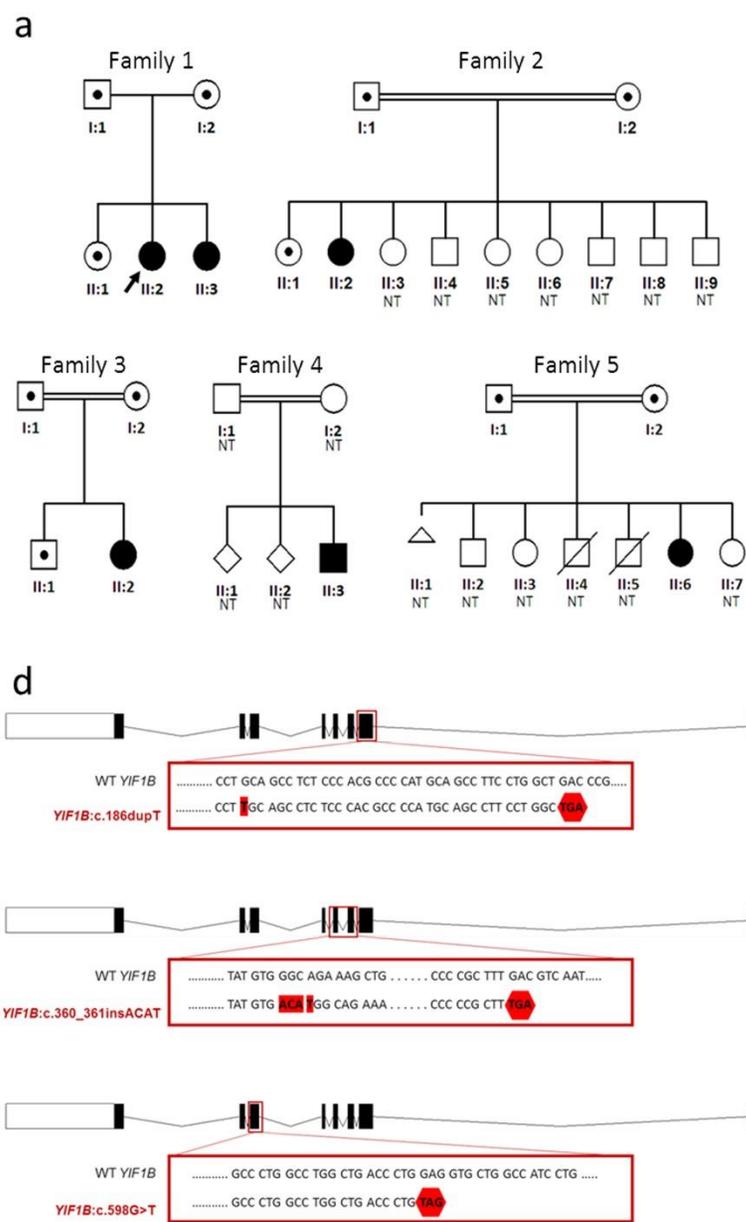
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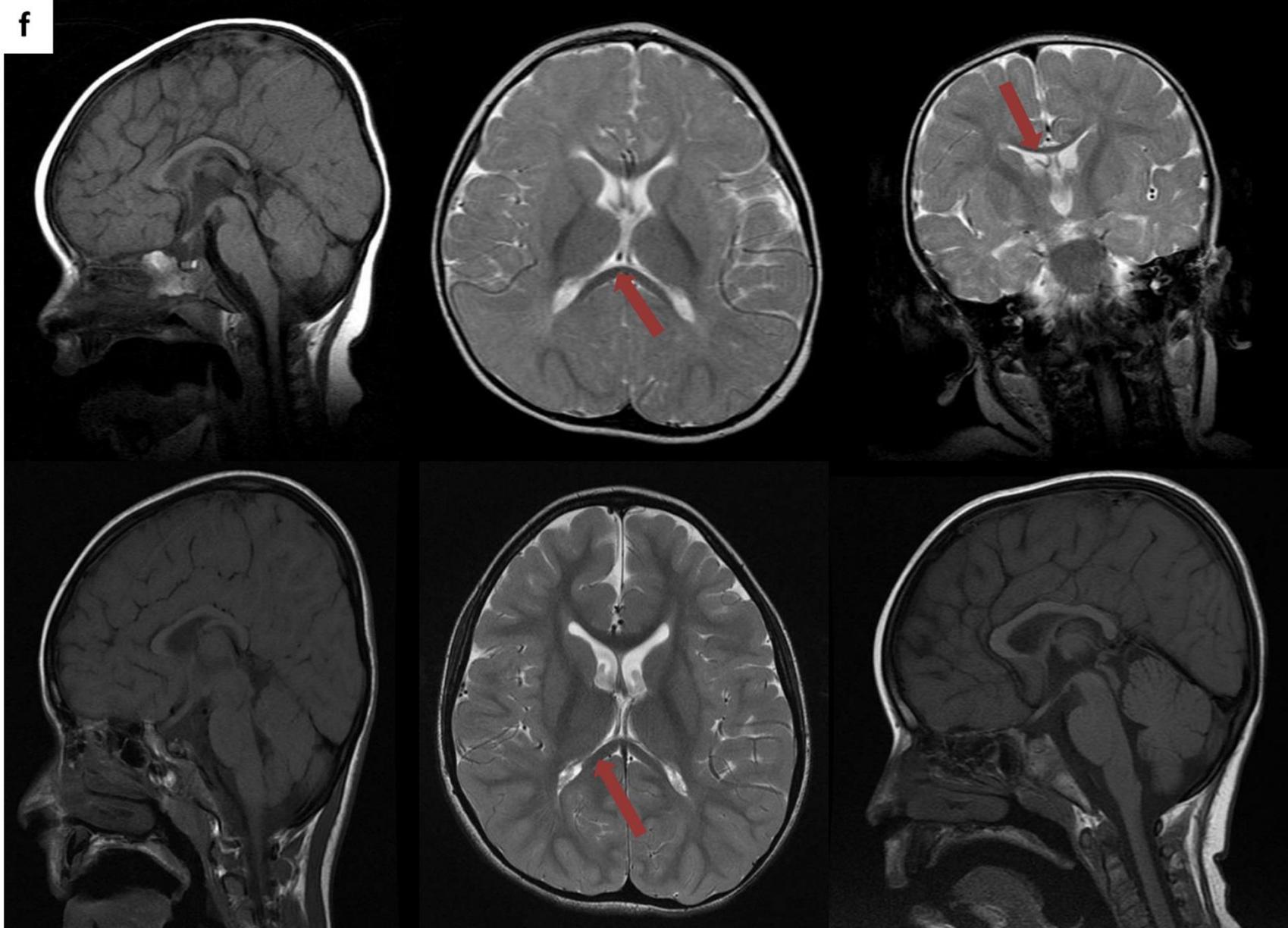
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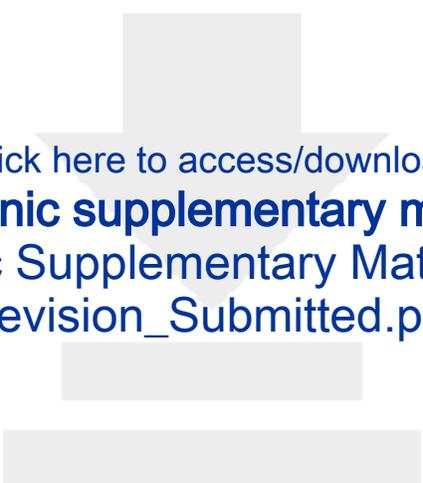
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## FIGURE LEGEND

**Figure 1.** a). Pedigrees of six affected individuals from five families b). Sanger sequencing reveals segregation of the variants in the tested families. c). An ROH was detected on chromosome 19 including *YIF1B*. d). Scheme of *YIF1B* transcripts, affected exons, and resulting truncations. e). 3D structural model for YIF1B. Helices are shown as cylinders, and the predicted molecular surface of the structured transmembrane portion of YIF1B is indicated. The flexible cytosolic region is shown in an arbitrary conformation. Protein regions retained in the variants are shown in magenta (p.Ala63fs) and magenta/cyan (p.Gly121fs) and magenta/cyan/green (p.Glu200\*). The residues resulting from the frame shifts were not included (see Supplementary Figures 2 and 3). f). Brain MRI. Mild thinning of the corpus callosum was noted in all patients with the founder mutation.







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