Expanding the spectrum of EEF1D neurodevelopmental disorders: Biallelic variants in the guanine exchange domain

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Abstract
Protein translation is an essential cellular process and dysfunctional protein translation causes various neurodevelopmental disorders. The eukaryotic translation elongation factor 1A (eEF1A) delivers aminoacyl-tRNA to the ribosome, while the eEF1B complex acts as a guanine exchange factor (GEF) of GTP for GDP indirectly catalyzing the release of eEF1A from the ribosome. The gene EEF1D encodes the eEF1Bδ subunit of the eEF1B complex. EEF1D is alternatively spliced giving rise to one long and three short isoforms. Two different homozygous, truncating variants in EEF1D had been associated with severe intellectual disability and microcephaly in two families. The published variants only affect the long isoform of EEF1D that acts as a transcription factor of heat shock element proteins. By exome sequencing, we...
identified two different homozygous variants in EEF1D in two families with severe developmental delay, severe microcephaly, spasticity, and failure to thrive with optic atrophy, poor feeding, and recurrent aspiration pneumonia. The EEF1D variants reported in this study are localized in the C-terminal GEF domain, suggesting that a disturbed protein translation machinery might contribute to the neurodevelopmental phenotype. Pathogenic variants localized in both the alternatively spliced domain or the GEF domain of EEF1D cause a severe neurodevelopmental disorder with microcephaly and spasticity.

KEYWORDS
cerebral palsy, EEF1D, neurodevelopmental disorders, optic atrophy, protein translation, rare disease

1 | INTRODUCTION

Pathogenic variants in genes required for protein translation have been linked to neurodevelopmental disorders highlighting the importance of protein translational homeostasis for neuronal development. A few genes that function in this elongation step of protein translation have been implicated in human disease and intellectual disability (ID).1 These include EFTUD2 (OMIM 610536), EEF2 (OMIM 609306), EEF1A2 (OMIM 616309, 616393), EIF5A (OMIM 619376), and EIF4A3 (OMIM 268305). The eukaryotic translation elongation factor 1A (eEF1A) is required for the delivery of aminoacylated-tRNA to the ribosome.2 The fast dissociation of eEF1A from the ribosome depends on GTP hydrolysis. After dissociation, the guanine exchange factor eEF1B speeds up the exchange of GDP for GTP by 1000 fold.3 EEF1D codes for one long and three short isoforms of eEF1Bβ. The long isoform contains a nuclear localization signal (NLS) and functions as a transcription factor of heat shock response genes.4

In a large study on recessive ID, Reuter et al identified homozygous, truncating variants in EEF1D (NM_001130053:c.69delG; p.[Glu24Serfs*26]) in three siblings with severe ID and microcephaly.5 Recently, Ugur Iseri et al reported the second family with three sisters presenting with ID caused by biallelic truncating variants in EEF1D (NM_001130053:c.948G>A; p.[Trp316*]).6 Both reported variants are localized in the alternatively spliced exon that is only retained in the long isoform. Therefore, it was suggested that a disturbed heat shock response pathway might cause neurodevelopmental impairment.

Herein, we report four individuals from two families with severe ID and microcephaly, seizures, failure to thrive, poor feeding, recurrent aspiration pneumonia and optic atrophy with biallelic variants in the guanine exchange domain (GEF) in EEF1D that is retained in both, the long and the short isoforms.

2 | MATERIALS AND METHODS

2.1 | Patients recruitment

Written informed consent for participation in this study and the publication of photographs was obtained from the legal guardians. The study was performed in accordance with the Declaration of Helsinki and approved by the local institutional review boards (SQU-MREC#1362, Göttingen: #3/2/16).

2.2 | Genetic workup

After DNA isolation, DNA was barcoded and exons were enriched using hybrid capture technology (SureSelect All exons-v6, Agilent Technologies). Prepared DNA libraries of individuals #1–4 were sequenced on a HiSeq2000 (family 1) or HiSeq4000 (family 2) sequencer (200X coverage, minimal coverage 10x in >95%). Variant filtration for novel or rare variants (≤0.1%) was performed using public (1000 Genomes, Exome Variant Server, gnomAD) and in-house exome databases. Sanger sequencing was used for confirmation. Parents were tested by Sanger sequencing. For primer sequences and microarray see Supplementary text.

3 | RESULTS

3.1 | Clinical presentation

All four female individuals (Family 1: Individuals #1 and #2; Family 2: Individuals #3 and #4) presented with severe ID, spastic quadriparesis, severe microcephaly, and failure to thrive (Figure 1).

At birth, weight and body length were normal and head circumference ranged between microcephalic (individual #1) and normal (Table 1). Within the first year of life, all individuals developed severe microcephaly (−4.4 to −8.1 SD) and all were noticed to have muscular hypotonia of the trunk, stagnation of motor development, convergent strabismus and spontaneous nystagmus. After the age of 1 year, all developed spasticity of the limbs with joint contractions. Spontaneous cloni were observed. Individuals #1 and #2 had intermittent bruxism and psychomotor restlessness. Individuals #1 and #3 had a weak suck requiring nasogastric tube feeding in the neonatal period and gastric tube feeding later on. Individual #2 was able to swallow mashed food. All four individuals had absent speech, were unable to follow simple demands, sit, crawl or walk independently and all were...
**FIGURE 1** Photographs and cranial MRI. (A + B) Pedigrees (C) Photographs of #1 (15 years), (D) of #2 (3.5 years), (E) of #3 (10 years) (F) Updated photographs of one male from family MR065 (Reuter et al.) (15 years). (G) Brain MRI of #2 (7 months) (i) T2-weighted, (ii) T1-weighted sequences depicting a global brain atrophy, hypoplastic corpus callosum, hypomyelination (T2 hyperintense and T1 hypointense signal of the white matter), an arachnoid cyst and wide lateral ventricles. (H) Brain MRI of #3 (6 months) (i) T2-weighted, (ii-iii) T1-weighted. (I) Fundoscopy of #2: Pale optic discs on both sides. [Colour figure can be viewed at wileyonlinelibrary.com]
**Table 1: Clinical presentation**

<table>
<thead>
<tr>
<th>Family 1</th>
<th>Family 2</th>
<th>Reuter et al 2017 (N = 3)</th>
<th>Ugur Iseri et al 2019 (N = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>#2</td>
<td>#3</td>
<td>#4</td>
</tr>
<tr>
<td><strong>EEF1D variants</strong></td>
<td>c.1780T&gt;C, p.(Trp594Arg) homozygous</td>
<td>c.1905+1G&gt;A homozygous</td>
<td>c.69del, p.(Glu24Serfs*26) homozygous</td>
</tr>
<tr>
<td>Consanguinity in parents</td>
<td>Second cousins</td>
<td>Double first cousins</td>
<td>+</td>
</tr>
<tr>
<td>Relation of affected individuals</td>
<td>Siblings</td>
<td>Double first cousins</td>
<td>Siblings</td>
</tr>
<tr>
<td>Origin</td>
<td>Turkey</td>
<td>Oman</td>
<td>Syria</td>
</tr>
<tr>
<td>Gender</td>
<td>F</td>
<td>F</td>
<td>1F, 1M</td>
</tr>
<tr>
<td>Intellectual disability</td>
<td>Severe</td>
<td>Severe</td>
<td>Severe</td>
</tr>
<tr>
<td>Age at last follow up</td>
<td>22 yr.</td>
<td>6 yr.</td>
<td>10 yr.</td>
</tr>
<tr>
<td>Gestational age</td>
<td>42 weeks</td>
<td>38 weeks</td>
<td>39 weeks</td>
</tr>
<tr>
<td>Birth weight, kg, SD</td>
<td>2.8, –1.8</td>
<td>2.6, –1.4</td>
<td>2.7, –1.34</td>
</tr>
<tr>
<td>Birth size, cm, SD</td>
<td>48, –2.0</td>
<td>48, –1.0</td>
<td>48, –0.6</td>
</tr>
<tr>
<td>OFC at birth, cm, SD</td>
<td>32, –2.6</td>
<td>35, +0.5</td>
<td>33, –1.1</td>
</tr>
<tr>
<td>Age of last follow up of body measurements</td>
<td>18 yr.</td>
<td>3.5 yr.</td>
<td>10 yr.</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>Congenital</td>
<td>Acquired</td>
<td>Acquired</td>
</tr>
<tr>
<td>OFC, cm, SD</td>
<td>44.5, –8.1</td>
<td>45, –4.5</td>
<td>45.5, –5</td>
</tr>
<tr>
<td>Failure to thrive</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Weight, kg, SD</td>
<td>17, –12</td>
<td>13.5, –2</td>
<td>13, –4</td>
</tr>
<tr>
<td>Body length, cm, SD</td>
<td>112, –9</td>
<td>65, –3.2</td>
<td>n/r</td>
</tr>
<tr>
<td>Sat/walked independently</td>
<td>–/–</td>
<td>–/–</td>
<td>–/–</td>
</tr>
<tr>
<td>First words</td>
<td>No speech</td>
<td>No speech</td>
<td>No speech</td>
</tr>
<tr>
<td>Vision problems</td>
<td>Optic atrophy (pallor of optic disc)</td>
<td>Suspicion of optic atrophy</td>
<td>Mild temporal optic pallor, very poor VEP bilaterally</td>
</tr>
<tr>
<td>Hearing</td>
<td>Normal</td>
<td>Normal</td>
<td>Not formally assessed</td>
</tr>
<tr>
<td>Neurological findings</td>
<td>Quadriparesis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Muscular hypotonia of trunk</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Ataxia</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(Continues)
<table>
<thead>
<tr>
<th></th>
<th>Family 1</th>
<th>Family 2</th>
<th>Reuter et al 2017 (N = 3)</th>
<th>Family MR065</th>
<th>Ugur Iseri et al 2019 (N = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nystagmus</strong></td>
<td>+</td>
<td>+</td>
<td></td>
<td>n/r</td>
<td>–</td>
</tr>
<tr>
<td><strong>Spasticity of limbs</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>n/r</td>
<td>3/3</td>
</tr>
<tr>
<td><strong>Contractures of limbs</strong></td>
<td>+</td>
<td>+</td>
<td>+ (mild)</td>
<td>n/r</td>
<td>n/r</td>
</tr>
<tr>
<td><strong>Seizures</strong></td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>n/r</td>
<td>3/3</td>
</tr>
<tr>
<td><strong>Seizure type</strong></td>
<td>Tonic clonic</td>
<td>–</td>
<td>–</td>
<td>NA</td>
<td>Generalized</td>
</tr>
<tr>
<td><strong>Seizure frequency</strong></td>
<td>Twice a year, last seizure at 3 yrs</td>
<td>–</td>
<td>–</td>
<td>n/r</td>
<td>Once a month</td>
</tr>
<tr>
<td><strong>EEG</strong></td>
<td>Sporadic spike and wave complexes, left frontotemporal area</td>
<td>Left centroparietal slowing, focal epileptic activity with secondary generalization during sleep</td>
<td>Comb-like theta rhythm over central areas</td>
<td>n/r</td>
<td>2/3 Generalized epileptiform discharges</td>
</tr>
<tr>
<td><strong>Gastrointestinal problems</strong></td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>n/r</td>
<td>–</td>
</tr>
<tr>
<td><strong>Recurrent vomiting</strong></td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>n/r</td>
<td>–</td>
</tr>
<tr>
<td><strong>Gastric tube feeding</strong></td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>n/r</td>
<td>–</td>
</tr>
<tr>
<td><strong>Recurrent aspiration pneumonias</strong></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>n/r</td>
<td>–</td>
</tr>
<tr>
<td><strong>Head MRI</strong></td>
<td>Arachnoid cyst, cerebellar atrophy, hypoplastic corpus callosum, delayed myelination, reduced diameter of optic nerve</td>
<td>Arachnoid cyst, hypoplastic corpus callosum, delayed myelination, reduced diameter of optic nerve</td>
<td>Cerebral atrophy, hypoplastic corpus callosum, abnormal myelination</td>
<td>n/r</td>
<td>2/2 Hypoplastic corpus callosum</td>
</tr>
</tbody>
</table>

**Abbreviations:** n/r, not reported; VEP, visual evoked potentials.
dependent on wheelchair. Individuals #1–3 developed optic atrophy (Figure 1I). Fundoscopy of individual #4 showed retinal pigmentary changes and electroretinogram showed cone and rod dysfunction.

Individual #1 had epileptic seizures twice a year until the age of 3 years. Individuals #2 and #3 had epileptic findings on electroencephalography, but no reported seizures.

All individuals had failure to thrive. The deficit in weight and height aggravated with age (−2 SD-youngest; −12 SD-oldest).

Individual #1 suffered from an episode of acute pancreatitis. Individual #4 had recurrent aspirations and chest infections. At the age of 5 years, she died of an aspiration pneumonia. Cranial MRI showed reduced brain volume, hypoplastic corpus callosum, and abnormal, delayed myelination. Individuals #1 and #2 additionally had unilateral arachnoid cysts, but no compression of other brain structures, no midline shift indicating normal intracranial pressure. All had large, low set ears, but no distinct dysmorphic facial features (Figure 1C–E). For detailed case reports see Supplementary text.

Microarray analysis revealed normal results for family 2. Individual #2 has a heterozygous 0.5-Mb duplication of Xq28 (arr[hg19] Xq28 (154,118,619-154,560,375), hg19/GRCh37) that is not shared by the sister #1 (Figure S1). Int22h1/int22h2-mediated Xq28 duplications are associated with mild to moderate ID, increased weight, recurrent infections, and dysmorphic features in males. Female carriers are
largely unaffected or show only mild cognitive impairment. The healthy mother of #1 and #2 also carries the Xq28 duplication, and therefore, the duplication does not explain the severe neurodevelopmental disorder of individual #2.

3.2 Exome sequencing and identification of homozygous variants in EEF1D

By exome sequencing we found that individuals #1 and #2 harbor a homozygous missense variant c.1780T>C, p.[Trp594Arg]) in EEF1D (NM_001130053.4, exon 9). Trp594 is highly conserved among different species and the variant resides in a highly conserved stretch in the GEF domain (Figure 2B). The variant was absent from databases, and an alternative heterozygous variant at position 594 (p.[Trp594Leu]) was detected once in gnomAD. In silico tools predict a likely damaging effect (CADD 32).

In individuals #3 and #4 a homozygous canonical splicing variant c.1905+1G>A: [p.?] (NM_001130053.4) in EEF1D was detected. The variant was found once in a European heterozygous carrier in gnomAD. After the initial visit the family was not available for collecting a new blood sample for a transcript analysis, therefore unfortunately, a transcript analysis was not possible. This variant affects the canonical transcript, and it is located within the canonical splicing site (-1), which is considered a high-impact variant and predicted to cause aberrant splicing.

 Parents carrier status was confirmed by sanger sequencing (Figure 1A). According to the ACMG/AMP guidelines 2015 both variants were rated to be likely pathogenic (PM1, PM2, PP1, PP3) and disease-causing.

As the four affected individuals stem from consanguineous families, we quantified the total percentage of homozygosity (referred to the human genome size of 3,000,000,000 bp): Individuals #1: 9.5%, #2 5.7%, #3: 8.2%, #4: 5.0%. The EEF1D variant is located within the first 19th largest regions of homozygosity in all four individuals.

4 DISCUSSION

EEF1D codes for the eEF1B6 that functions as a guanine exchange factor (GEF) and is required for the efficient release of eEF1A from the ribosome during protein translation.\(^2\) The long isoform (eEF1B6L) has an alternatively spliced exon that contains a nuclear localization signal (NLS).\(^4\) In the nucleus, eEF1B6L acts as a transcription factor for heat shock element-containing genes. After heat exposure, the expression of the short, canonical eEF1B6 isoforms is downregulated and the long eEF1B6L is upregulated indicating a role cellular stress response.\(^4\)

While the two variants reported by Reuter and Ugur Iseri et al are loss-of-function variants that affect the alternatively spliced exon of eEF1B6L, this is the first study reporting homozygous variants in the highly conserved C-terminal GEF domain in association with a severe neurodevelopmental disorder.\(^5\)\(^,\)\(^6\)

All published individuals and all individuals from this study have microcephaly and thinning of the corpus callosum (Table 1). All individuals of whom data were available, had short stature, muscular hypotonia and spasticity. While none of the individuals from this study or in the study by Ugur Iseri et al were able to sit, stand or walk independently, one individual reported by Reuter et al walked with assistance at the age of 15 years (Figure 1F). Additional clinical signs that have not been reported to date are optic atrophy, poor feeding, and recurrent aspiration pneumonia. Of note, the progressive deficit of head circumference, weight and height over time – that is also observed in EFF1A2 and EFTUD2-associated disorders - suggests a degenerative disorder, however, long-term clinical follow-up is needed to draw a final conclusion on a potentially progressive course.

Ugur Iseri et al proposed that an altered heat shock transcriptional response - rather than a dysfunctional protein elongation - might be the underlying disease mechanism of the EEF1D-associated neurodevelopmental disorders.\(^6\) In cell culture, the overexpression of either wildtype, Lys646Ala or Lys646Arg mutant eEF1B6L upregulated the transcription of heat-shock element (HSE)-containing genes.\(^3\) The Lys646 is one of the many highly conserved amino acids within the highly conserved GEF domain. This allows the hypothesis that the N-terminal GEF domain that includes both variants of this study might be dispensable for heat shock response.

The early neuronal development appears to be particularly sensitive to disturbed protein homeostasis. Many genes that are essential for proper protein translation have been implicated with neurodevelopmental disorders.\(^1\) In mice, EEF1D short and long isoforms were expressed in the brain at all stages of development, but the expression of the short isoforms was significantly upregulated in the early fetal and neonatal stages, pointing to a pivotal role in the early infantile brain development.\(^10\) The variants of family 1 and 2 are localized in the C-terminal GEF domain and likely affect the canonical protein translation elongation function of EEF1D.

Based on our clinical findings, we can only speculate on the disease mechanism, and functional studies are required to delineate the damaging effect of mutant EEF1D long and short isoforms. As many genes that are involved in protein translation (e.g. YARS1) have acquired diverse non-canonical functions during the evolution, not yet identified functions should also be considered as potential disease mechanisms.\(^11\)

This is the first report of a neurodevelopmental disorder associated with biallelic variants in the C-terminal GEF domain of EEF1D. Variants in the C- and N-terminal domains cause a similar phenotype including severe ID, microcephaly, spasticity, seizures and failure to thrive.

AUTHOR CONTRIBUTIONS

Abou Jamra, and Almundher Al-Maawali. Funding acquisition: Bernd Wollnik, Dagmar Wieczorek, and Almundher Al-Maawali. Writing-original draft and visualization: Luisa Averdunk. Writing-review & editing: Khalid Al-Thihli, Almundher Al-Maawali, and Dagmar Wieczorek.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1111/cge.14290.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT
The study was approved by the Institutional Ethical Review Boards (SQU-MREC#1362, Göttingen; #3/2/16).

PHOTO CONSENT STATEMENT
The legal guardians gave consent to the publication of photographs.

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SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.