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Expansion of the neurodevelopmental phenotype of individuals with *EEF1A2* variants and genotype-phenotype study

Citation for published version:

Paulet, A, Bennett-Ness, C, Ageorges, F, Trost, D, Green, A, Goudie, D, Jewell, R, Kraatari-Tiri, M, Piard, J, Coubes, C, Lam, W, Lynch, SA, Samuel, G, Ramond, F, Fluss, J, Fagerberg, C, Brasch Andersen, C, Varvagiannis, K, Kleefstra, T, Gérard, B, Fradin, M, Vitobello, A, Tenconi, R, Denommé-Pichon, A-S, Vincent-Devulder, A, Haack, T, Marsh, JA, Laulund, LW, Grimm, M, Riess, A, de Boer, E, Padilla-Lopez, S, Bakhtiari, S, Kruer, MC, Levy, J, Verloes, A, Abbott, CM & Ruaud, L 2024, 'Expansion of the neurodevelopmental phenotype of individuals with *EEF1A2* variants and genotype-phenotype study', *European Journal of Human Genetics*. <https://doi.org/10.1038/s41431-024-01560-8>

Digital Object Identifier (DOI):

[10.1038/s41431-024-01560-8](https://doi.org/10.1038/s41431-024-01560-8)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

European Journal of Human Genetics

Publisher Rights Statement:

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1 **Expansion of the neurodevelopmental phenotype of**
2 **individuals with *EEF1A2* variants and genotype-phenotype**
3 **study.**

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78 **Keywords:** *EEF1A2*, Intellectual disability, regression, epilepsy,
79 neurodevelopment.

80 **Abstract**

81 Translation elongation factor eEF1A2 constitutes the alpha subunit of
82 the elongation factor-1 complex, responsible for the enzymatic
83 binding of aminoacyl-tRNA to the ribosome. Since 2012, 21
84 pathogenic missense variants affecting *EEF1A2* have been described
85 in 42 individuals with a severe neurodevelopmental phenotype
86 including epileptic encephalopathy and moderate to profound
87 intellectual disability (ID), with neurological regression in some
88 patients.

89 Through international collaborative call, we collected 26 patients
90 with *EEF1A2* variants and compared them to the literature.

91 Our cohort shows a significantly milder phenotype. 83% of the
92 patients are walking (vs. 29% in the literature), and 84% of the
93 patients have language skills (vs. 15%). Three of our patients do not
94 have ID. Epilepsy is present in 63% (vs. 93%). Neurological
95 examination shows a less severe phenotype with significantly less
96 hypotonia (58% vs. 96%), and pyramidal signs (24% vs. 68%).
97 Cognitive regression was noted in 4% (vs. 56% in the literature).

98 Among individuals over 10 years, 56% disclosed neurocognitive
99 regression, with a mean age of onset at 2 years.

100 We describe 8 novel missense variants of *EEF1A2*. Modelling of the
101 different amino-acid sites shows that the variants associated with a
102 severe phenotype, and the majority of those associated with a
103 moderate phenotype, cluster within the switch II region of the protein
104 and thus may affect GTP exchange. In contrast, variants associated
105 with milder phenotypes may impact secondary functions such as
106 actin bundling. We report the largest cohort of individuals with
107 *EEF1A2* variants thus far, allowing us to expand the phenotype
108 spectrum and reveal genotype-phenotype correlations.

109 **Abbreviations:**

110 ACMG: American College of Medical Genetics

111 ADHD: Attention Deficit and Hyperactivity Disorder

112 ASD: Autism Spectrum Disorder

113 CADD: Combined Annotation Dependent Depletion

114 CLB: Clobazam

115 FISQ : Full-Scale Intelligence Quotient

116 ID: Intellectual Disability

117 IQ: Intellectual Quotient

118 KETO DIET: Ketogenic Diet

119 LAM: Lamotrigin

120 LORAZEP: Lorazepam

121 MICROPAK: Micropakine

122 MRI: Magnetic Resonance Imaging

123 OFC: Occipito-Frontal Circumference

124 SD: Standard Deviation

125 TIQ: Total Intellectual Quotient

126 VA: Valproic Acid

127 WISC: Wechsler Intelligence Scale for Children

128 WM and GM: White Matter And Grey Matter

129 WPPSI-IV: Wechsler Preschool and Primary Scale of Intelligence

130 Introduction

131 The eEF1 family of eukaryotic elongation factor genes, which
132 comprises the two paralog eEF1A proteins eEF1A1 and eEF1A2 and
133 the 3 subunits of the eEF1B complex (eEF1B α , eEF1B β , and eEF1B γ)
134 encodes integral components of the translation elongation factor
135 complexes whose function is delivery of aminoacyl tRNA to ribosome
136 during the elongation step of protein synthesis¹. The two eEF1A
137 proteins, the second most abundant protein in the cell², share 92%
138 identity. eEF1A binds aa-tRNAs in a GTP-dependent manner, relying
139 on its cognate guanine exchange factor (GEF), eEF1B, to recycle the
140 inactive eEF1A-GDP complex into the active GTP-bound form. *EEF1A1*
141 gene is expressed almost ubiquitously. *EEF1A2* is expressed mainly in
142 muscle (including cardiac muscle) and in neurons^{3,4}. During
143 development, eEF1A1 is down-regulated in muscle and neurons and
144 is undetectable in mouse muscle by 3 weeks post-natal^{5,6}.

145 No pathogenic *EEF1A1* variants have been described, presumably
146 because they would not be compatible with life⁷. In contrast, in 1972,
147 a deletion of 15.8 kb that abolishes expression of eEF1A2 (MIM_
148 01958) was discovered in mice that developed motor neuron
149 degeneration, muscle atrophy, gait abnormalities and then died by
150 four weeks^{3,5}. A trio-based exome in 2012 revealed a *EEF1A2* variant
151 in a patient with early onset epilepsy, severely delayed psychomotor
152 development, and autistic behavior⁸. Subsequent individual reports
153 and a series of 14 patients⁹ allowed to further delineate the
154 neurodevelopmental phenotype, combining moderate to severe

155 Intellectual Disability (ID), epilepsy, Autism Spectrum Disorder (ASD),
156 and sleep disorders neurodegeneration and movement disorders⁹⁻¹³.

157 Since 2012, 21 *EEF1A2* variants have been reported⁸⁻²⁷. All variants
158 are missense. In one pedigree, a variant was inherited from a parent
159 with less than 25% of mosaicism⁹. Other variants were *de novo*. To
160 date, no genotype-phenotype correlation has been studied.

161 Through an international collaboration, we collected data on 26
162 unreported *EEF1A2* patients, the largest cohort to date, and
163 investigated possible genotype-phenotype correlations.

164 **Material And Methods:**

165 **Individual ascertainment:**

166 Between December 2020 and December 2021, 26 individuals with
167 variants in *EEF1A2* were identified through European Reference
168 Network ERN-ITHACA (<https://ern-ithaca.eu/>) using its collaborative
169 call system. Patients were evaluated by a geneticist. Written
170 informed consents for DNA and data analyses were obtained from
171 individuals or their legal guardians.

172 **Cognitive assessment:**

173 Out of the 26 patients included in our study, we assessed cognitive
174 abilities for 13 individuals (Table 1): six underwent a Wechsler
175 Intelligence Scales for Children according to age (Scales IV and V: 6
176 years to 16 years 11 months old, or Wechsler Preschool and Primary
177 Scale of Intelligence III or IV: 4 years to 7 years 3 months old) and 7
178 patients were evaluated by clinicians (Table S1).

179 Among 6 patients which performed Wechsler tests, only 4 of the 6
180 patients had a computable Total Intelligence Quotient (TIQ). For the
181 remaining two, they had too heterogenous profiles to allow TIQ
182 calculation. We therefore utilized the available indices data (VCI, FRI,
183 VSI, WMI, PSI) to estimate their intellectual levels.

184 For the 7 patients evaluated by clinicians, cognitive assessments were
185 provided but we did not have quantitative data (Table S1).

186 Patients were divided into 4 categories such as “not ID” for $TIQ \geq 70$,
187 “mild ID” for $50 \leq IQ < 70$, “moderate ID” for $35 \leq IQ < 50$, and “severe ID”
188 for $IQ < 35$, according to their IQ scores especially TIQ. In total, we
189 classified 13 patients according to their level of intellectual efficiency
190 (Table 1).

191 We excluded 13 individuals of cognitive analysis because they were
192 too young at the last follow-up appointment or they have been lost
193 to follow-up.

194 To categorize individuals from the literature when Weschler’s FSIQ
195 was not available, we took into account the authors' assigned
196 category ID for each individual or we used the information on
197 individual developmental milestones (walking age, capacity for
198 language, verbal abilities...) to estimate the developmental delay as
199 “mild”, “moderate” or “severe” phenotype (Table S2). The individuals
200 whose information was insufficient to be to be classified into these
201 categories were not scored.

202 **Genetic investigations:**

203 DNA was extracted from the peripheral blood leukocytes of the
204 patients and parents (whenever possible) using standard procedures.

205 In 24/26 patients, genotyping was performed by exome sequencing
206 (ES) (single or trio) using routine methods. Confirmation and
207 segregation of variants in single ES were carried out by Sanger
208 sequencing. Variant prioritization was conducted according to the
209 transmission mode (de novo, autosomal recessive and X-linked), and
210 the frequency of the variants in the gnomAD database. Those variants
211 were classified according to American College of Medical Genetics
212 (ACMG) (ACMG and Combined Annotation Dependent Depletion
213 (CADD) score in Supplementary data, Table S3). There were no other
214 pathogenic variants in ClinVar and HGMD or loss-of-function variants
215 which could explain the patient phenotypes. Two variants were
216 identified on a NGS panel of 119 ID genes using standard NGS
217 procedures.

218 **Protein Modelling:**

219 The structures of eEF1A2*GDP (4C0S), eEF1A γ *eEF1B α (pdb: 1IJF),
220 aEF1A*GTP (pdb: 3AGJ), and EF-TU*EF-TS (pdb:1EFU) were used for
221 analysis using Pymol (The PyMOL Molecular Graphics System, Version
222 1.2r3pre, Schrödinger, LLC.) and CCP4MG²⁸. Structure-based variant
223 predictions made using Missense 3D²⁹ and dimer predictions using
224 FoldX³⁰. Briefly, eEF1A2 (pdb: 4C0S) was repaired, then the protein

225 stability was estimated for each amino acid change in both
226 monomeric and the dimeric eEF1A2.

227 **Statistical analysis**

228 We compared our patients to the previous reported ones. We used
229 Student Test with bilateral hypothesis, have tolerated a risk of error
230 of less than 5% (*p-value* <0.05).

231 **Literature review:**

232 PubMed was searched for peer-reviewed articles published in English
233 using the following keywords: « *EEF1A2* », « epileptic
234 encephalopathy gene », « *EEF1A* » «phenotype of *EEF1A2* » « *EEF1A2*
235 with no intellectual disability » « epilepsy gene ».

236 **Results**

237 In our series, the mean age at last examination is 10.67 years vs. 9.6
238 years for literature patients (Table S4). The mean Occipito-frontal
239 circumference (OFC) is -0.67 SD (vs. -1.33 SD) (Table S4). Most
240 patients can walk on independently. The mean age for acquiring
241 walking is 30 months. For the 4 individuals in the literature this mean
242 is 39 months, *p-value*= 0.41 (Table S4).

243 Among 18 individuals aged more than 2 years, 83% are verbal (Figure
244 1) with a mean age of first words at 29 months (Table S4) (data not
245 available for published cases). The average age at onset of epilepsy is
246 3.5 years (Table S4) (data not available for previously reported
247 patients).

248 Neuropsychologist assessments were performed in six patients
249 (Table 1). For the 4 patients for whom a TIQ could be calculated, the
250 Full-Scale Intelligence Quotient (FSIQ) ranged from 40 to 77 (mean=
251 60.2; median= 58.5) (Table S1).

252 Patient 1 has a FSIQ in the low normal range (77) and the two others
253 (patients 6 and 19) had several indexes > 70 despite their
254 heterogeneous profile did not allow to compute FSIQ. Patient 19 has
255 a FSIQ of 69, but with low normal verbal comprehension index (76)
256 and fluid reasoning (71) (Table S1).

257 In our cohort of 13 for whom we have developmental information, 4
258 patients have a phenotype described as severe (31%) compared to

259 those from the literature (76%) p -value=0.008 (-0.77;-0.13) (Table
260 1). Among published patients, 5/33 (15%) had profound ID (Table 2),
261 whereas there was no patient classified as having profound ID in our
262 cohort. There was no difference observed concerning mild and
263 moderate ID (Table 1).

264 The individuals of this series are more ambulant than previous
265 reported ones: 82% (18/22) vs. 29% (10/34), p -value=0.00004 (0.29;
266 0.76). They are more likely to have acquired language (83% vs. 14%
267 p -value=10E-7 (0.47;0.91)) (Figure 1).

268 In our cohort, less patients had hypotonia and pyramidal syndrome
269 (Figure 1). There was no difference in the incidence of ataxia and ASD.
270 A majority of our patients have sleep disorders (11/24), with limited
271 effectiveness of melatonin treatment (Table S1). Half of our patients
272 (53%-9/17) had ADHD (Table S1). More than a half of our patients
273 (14/25) have gross motor issues with mainly unstable walk, and 77%
274 (17/22) have fine motor issues particularly concerning coordination
275 (Table S1).

276 We also compared our patients to the previously described ones
277 regarding several items. The percentage of patients with epilepsy
278 refractory to therapy, the percentage with epileptic encephalopathy
279 were compared so as our series brain MRI features. The results are
280 presented in Table 2.

281 We report 8 new *EEF1A2* variants (T24M, R96H, D97N, T104R, G356S,
282 D362N, P420L, V437F) with detailed phenotyping. Phenotypes

283 observed varies from mild to severe (Figure 2a). The patients with
284 variants T24M and R96C have no ID but are symptomatic: have
285 epilepsy (R96C) or speech delay with firsts sentences at age 6 (T24M).
286 For patients with P420L and V437F we could not document ID
287 because of the lack of psychometric evaluation (Table S1).

288 **Molecular Modelling**

289 We explored the impact of the variants through 3D-modelling of
290 eEF1A2, comparing the GDP-bound form of eEF1A2 (pdb:4C0S) with
291 eEF1A γ *eEF1B α (pdb:1IJF), aEF1A*GTP (pdb:3AGJ), and EF-TU*EF-TS
292 (pdb:1EFU). All variants have CADD scores above 20 (Table S3).

293 Mapping of the variants onto the structure of eEF1A2 (Figure 2b)
294 showed that several of the variants cluster around the switch II
295 region, a flexible motif key for GTP hydrolysis and GEF-mediated GDP
296 dissociation. R96C, R96H, and D97N are found in a loop in the Switch
297 II region, whilst T104R, R381W, and V437F are situated nearby. These
298 variations are anticipated to contribute towards switch II
299 destabilization and interfere with GTP recycling, whilst another
300 variant, T24M, is located at the GTP-binding pocket, and is predicted
301 to directly disrupt nucleotide binding (Supplementary modelling in
302 Supplemental data).

303 Further from the GDP-binding or switch regions, several variations
304 (E297K, G356S, D362N, and P420L) are located near the actin binding
305 region (Figure 2b) and are anticipated to disrupt actin-related
306 functions (Supplementary modelling). Three of these variants, E297K,
307 D362N and P420L are located near the tRNA-binding site, and may
308 affect eEF1A2 interactions with aminoacyl-tRNA. Given the overlap
309 between the binding sites for actin and aa-tRNAs and the eEF1B-
310 binding region, some of these variants may also impact eEF1B
311 binding. Additionally, the presence of variants T104R and D362N on
312 the dimer interface, and the change in amino acid charge, suggest

313 they may influence eEF1A2 dimer formation (Supplementary
314 modelling).

315 **Discussion**

316 **Widening *EEF1A2*-related spectrum**

317 Our results show that patients with *EEF1A2* variants can have efficient
318 cognitive abilities. Indeed, in our cohort, 3 individuals do not have an
319 intellectual disability (IQ > 70), while in the literature, no patients are
320 reported with preserved intellectual efficiency. In addition, within our
321 cohort, the severity of ID is lower, with a majority of patients with
322 mild to moderate ID.

323 In terms of development, the majority of our individuals have access
324 to language and walking, contrary to what has already been
325 described.

326 Taken together, the phenotypic spectrum associated with *EEF1A2*
327 variants is wider than previously suggested. All previously reported
328 patients had intellectual disabilities and the majority (93%) were
329 epileptic and nonverbal. We have shown that individuals with *EEF1A2*
330 variants may have milder phenotypes and that nearly half of them do
331 not have epilepsy at time of report. Epilepsy can be late-onset, as
332 illustrated by patient 7 (Ref. 20) with the D252H variant who did not
333 develop epilepsy until age 8 and the average age in the cohort was
334 10.7 years.

335 Young individuals are difficult to assess using the WPPSI-IV or WISC
336 scales. There are 13 individuals who have received cognitive
337 evaluation but only 4/13 have a valid TIQ because profiles are too
338 heterogenous. For this study, we chose to take the heterogenous

339 profiles into account because even if partial Weschler 'scales remain
340 an objective way to evaluate individuals' cognitive capacities.

341 To date, the only reported patient of autosomal dominant inherited
342 *EEF1A2* variant was from a mother with <25% of mosaicism⁹ but there
343 was no clinical information available. We newly report 2 patients
344 (patient 6 (whose father is patient 7) and patient 20) with inherited
345 variants from an asymptomatic parent (in terms of
346 neurodevelopmental disorder) which suggests an incomplete
347 penetrance of these variants (G356S and R96C). G356S is not
348 reported in the database and the position of the residue is highly
349 conserved. For G356S, the asymptomatic parent has a history of
350 irregular heartbeat, and he has at least 3 relatives who had seizures
351 (the relatives have not been tested for the variant yet). One individual
352 (patient 6) has the variant R96C inherited from his affected father
353 (patient 7), this variant had been shown to be associated with
354 (Genetic Generalized epilepsies) GGE²⁷. No mosaicism detected in
355 unaffected parents.

356 These two examples of inherited *EEF1A2* variants strongly suggest
357 incomplete penetrance. Although only 3 patients (including our 2) are
358 reported so far, incomplete penetrance should be considered in
359 *EEF1A2* variants. More individuals are needed to confirm this
360 hypothesis and we recommend to test proband's parents even if
361 there are apparently not affected. This incomplete penetrance is
362 relevant to genetic counselling.

363 We can notice a fairly variability of expression in patients with the
364 same variation. For example, in our series, patients 15, 16 and 17
365 have the same E124K variant already reported by Kaur et al¹⁵.
366 Whereas patients 15,16 and Kaur et al patient acquired independent
367 walk before 2 years with good language abilities and epilepsy, our
368 patient 17 acquired walk at 4 years and had no epilepsy.

369 We also report 2 patients (11 and 5) of de novo *EFF1A2* variant
370 mosaicism which did not cause milder phenotypes. Patient 11
371 presents epileptic encephalopathy and has TIQ of 52 at 3y. Patient 5
372 can speak in sentences. Nevertheless, they have similarities: they
373 both have white and grey matter abnormalities on brain MRI and
374 focal epilepsy, controlled with antiepileptic treatment. Concerning
375 patient 5, the variation D91N has been reported three times in the
376 literature in patients with severe to profound ID and early onset
377 epilepsy^{9,15,18}, whereas our patient is obviously less severe affected
378 than the three others. This mosaicism (estimated to 23%) could have
379 caused bias in comparison because this milder phenotype is possibly
380 only a result of mosaicism. So, mosaicism state should be taken into
381 account while considering an *EFF1A2* variant, although the
382 impossibility to estimate percentage of mosaicism in brain makes the
383 phenotype difficult to be predicted.

384 Ten patients^{9-10,12,22-23} have been reported to have regression in
385 childhood. In our cohort, only patient 22 (R381W) has shown
386 regressive traits which began in her third decade (but she is also the
387 only individual to have reached this age). She has started to show less

388 interest in activities she used to like such as writing, swimming and
389 she lost some abilities. This individual suggest that regression may
390 appear later than we thought while reviewing the literature patients
391 (where most of patients showed regression in infancy⁹). It's clear that
392 patients must be followed up and supported to prevent
393 complications during time.

394 **Molecular insights**

395 Plotting the patients' variants and those previously described on the
396 surface of the eEF1A2 protein (Figure 2b) suggests that variants,
397 resulting in severe ID are generally clustered around the switch I and
398 switch II regions, or nucleotide/GEF binding sites for GTP and GTP
399 exchange factor eEF1B. The growing cluster of variants around the
400 switch II region or GDP-binding site (Figure 2b) suggests that
401 disruption of GDP-binding and GEF-induced GDP dissociation may be
402 a key mechanism for the NDD-causing *EEF1A2* missense variants,
403 adding to the evidence from Carvill et al⁹. Four of the 19 variants
404 classified as severe directly coincide with defined eEF1B binding sites
405 (Figure 2b).

406 Many of the milder variants map further away but could affect tRNA
407 or actin binding (Figure 2b). There is evidence that E295K, the
408 equivalent E297K mutation in yeast, affects translational fidelity.
409 Variants affecting translational fidelity might be less likely to affect
410 neurodevelopment, but would be anticipated to lead to
411 neurodegeneration, which is observed in a subset of individuals with
412 *EEF1A2* variants. There is also the possibility that these variants are
413 not pathogenic and the cause of the individual phenotype was
414 misunderstood.

415 **Conclusion**

416 We have described a cohort of individuals with a less severe
417 phenotype, though they share characteristics such as developmental
418 delay (especially speech delay), mild to severe intellectual disability,
419 ASD, ADHD, early onset epilepsy, hypotonia, ataxia and sleep disorder
420 which are concordant with the features related to variants in this
421 gene in the literature. We suggest the existence of incomplete
422 penetrance of certain variants which was not described so far with
423 *EEF1A2*. Our series illustrate how the evolution of diagnostic
424 strategies may lead to redefine the phenotypic spectrum of known
425 genes that have been initially reported with a homogeneous and
426 usually “severe” phenotype. There was probably an ascertainment
427 bias in older patients that were more likely to be reported because
428 they were severe, and were perhaps selected on this basis. The
429 widespread adoption of the whole genome and whole exome
430 sequencing, which results in an agnostic pan-genomic evaluation lead
431 to the diagnosis of patients that would not be sent to targeted panel
432 diagnosis. The first genotype-phenotype correlations are emerging,
433 but new patients will be necessary to confirm these correlations.

434 To conclude, we expanded the phenotype spectrum and described
435 new *EEF1A2* variants.

436 All anonymized data and related documentation from this study are
437 available on reasonable request.

438 Declaration to public database: All variants are reported and
439 annotated in ClinVar website (accession number SCV004171535-
440 SCV004171551): <https://www.ncbi.nlm.nih.gov/clinvar/>

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566 **Figure Legends:**

567 **Table 1. Cognitive abilities of patients in our series compared to**
568 **those previous reported.**

569 This table shows comparison in terms of cognitive abilities between
570 our patients and the previously reported ones. The 4 categories
571 classification strategy has been explained in the Material and Method
572 section. The number of patients are in parentheses. * means the
573 comparison is statistically significant, p-value <0.05. CI : Confidence
574 Interval.

575 **Table 2. Cohort and literature comparison regarding epilepsy and**
576 **brain MRI characteristics**

577 This table shows comparison between our cohort and the previous
578 reported cases regarding epilepsy and brain MRI features. *P-value*
579 and CI 95% are mentioned. CI : Confidence Interval. * when
580 significant.

581 **Figure 1. Our series main features compared with the patients**
582 **from the literature.**

583 The figure 1 shows the proportion of our patients (in black) in
584 comparison to those from the literature (featured in grey) according
585 to the main criteria studied. Walking abilities is represented by the
586 motor category. Concerning the speech abilities, “verbal” means the
587 patient can speak and be understandable, “words” means he can
588 express himself in words but not in sentences, “sentences” stands
589 for the ability of the patient to make proper sentences. On the right

590 part of the diagram, the neurological features are shown with the
591 presence of hypotonia, pyramidal syndrome, epilepsy and the
592 presence of regression. * Stands for statistical significance $p < 0.05$.
593 *P-values* are framed at the top.

594 **Figure 2. Distribution of pathogenic *EEF1A2* variants.**

595 Figure 2a: distribution of variants from our cohort (up) and the
596 previously reported ones (down). Novel variants are underlined.
597 Variants are classified by their associated phenotype in terms of ID,
598 classification is as described as above with associated symbols (circle
599 for not deficient, triangle for mild ID, square for moderate ID and star
600 for severe). Blank when the variant is not clearly classified (for
601 example when associated with 2 ID categories). Figure 2b: variants
602 mapped onto the crystal structure of GDP-bound eEF1A2 (PDB:4C0S).
603 New variants are shown in black, with labels in bold, previously
604 described mutations are in white. T24M and V437F are buried. The
605 binding site of eEF1B is highlighted in white, and the GTP binding site
606 in dark grey.

607 **Table S1. Cohort's patients characteristics**

608 The table S1 shows the characteristics of our 26 patients ordered by
609 the position of their variation along the *EEF1A2* gene. Each column
610 represents a criterion of interest. "M" means Male, "F" means
611 Female. The ages at last examination are presented in years "y". The
612 ages at first steps achievement without falling and first
613 comprehensive words achievement are presented in months "m".
614 Concerning the languages abilities at last examination, classification

615 into 3 categories (absent/words/sentences) are presented, with
616 details of language level. About the neuropsychological assessment
617 column, Total Intellectual Quotients (TIQ) are presented when they
618 can be calculated. In case the TIQ could not be calculated the different
619 QI indicators are presented with their respective scores: VCI, Verbal
620 Comprehension Index, VSI, Visual Spatial Index, FRI, Fluid Reasoning
621 index, WMI, Working Memory Index, PSI, Processing Speed Index.
622 The age of the tests were carried out are in parentheses (“NA” when
623 non available), followed by the type of test performed: WISC-V or
624 WPPSI-IV. For the therapy and efficacy column, the drugs used are
625 in parentheses. Regarding the weight, height and Occipito-frontal
626 circumference (OFC) columns; the results are presented in standard
627 deviation (SD). The gross motor feature indicates walking disorder.
628 The fine motor features entail coordination disorder. The proportion
629 of patients are in parentheses.

630 Y: Yes // N: No // NA: Non available // NC: Non concerned //ADHD:
631 Attention Deficit Hyperactivity Disorder // VA: Valproic Acid // LAM:
632 Lamotrigin // CLB: Clobazam // Lorazep: Lorazepam // Micropak:
633 micropakine // Keto diet: Ketogenic Diet // TIQ: Total Intellectual
634 Quotient.

635 **Table S2. Literature patients characteristics**

636 The table S2 shows the characteristics of the 42 patients reported in
637 literature. Each column represents a criterion of interest. The ages at
638 last examination are presented in years. “y” stands for “years”, “m”
639 for “months”. “M” means Male, “F” means Female. “NA”: Non

640 available. “Y” stands for yes, “N” stands for no. Regarding the
641 Occipito-frontal circumference (OFC) at examination column, the
642 results are presented in SD. The proportion of patients for each
643 category are in parentheses.

644 **Table S3. ACMG classification and CADD Score**

645 WES :Whole Exome sequencing; ACMG: American College of Medical
646 Genetics; CADD: Combined Annotation Dependent Depletion. This
647 table presents the classification according to ACMG guidelines and
648 the CADD score for each variant ordered by their position along the
649 *EEF1A2* gene. The aim of ACMG classification is to divide the variants
650 into 5 categories, classes “1” and “2” are “benign classes”. The class
651 “3” is for “unknown significance variants”, the class “4” represents
652 “probably pathogenic variants” and the 5th is for “pathogenic
653 variants”. The CADD score evaluates the pathogenicity : if it is > 20, it
654 supports the pathogenicity of the variant. The “mosaic state” column
655 indicates if there is mosaicism for the variant and the associated
656 percentage found in the patient’s blood sample in case of mosaicism.

657 **Table S4. Our series and previously reported patients description**

658 This table shows the number of patients and the mean age at last
659 examination (in years), the mean age at first steps achievement
660 without falling (in months), the mean age at onset of epilepsy and the
661 mean occipito-frontal circumference (OFC) in standard deviation (SD).
662 The comparison, when possible, was not significant, *p-value* <0.05.

663 **Supplementary modelling figures:**

664 **Figure S1. Variations in the switch II loop**

665 Crystal structure of eEF1A2:GDP (4C0S), with wild-type residues in
666 blue, and variant residues in red. Variants R96C, R96H, D97N, and
667 R381W highlighted in the switch II region.

668 **Figure S2. R96H and R96C disrupt hydrogen bond network in**
669 **eEF1B α -bound form.**

670 Crystal structure of eEF1A_y*eEF1B α (1IJF), with wild-type residues in
671 blue and mutant residues in red. R96 forms hydrogen bonds with
672 E132 and E135 of helix C in eEF1B-bound form. Both variants, R96C
673 and R96H, orient away from helix C, likely preventing hydrogen bond
674 formation and potentially destabilising disrupting eEF1B α -mediated
675 release of GDP.

676 **Figure S3. Variants impact eEF1A2 dimerisation.**

677 Crystal structure of eEF1A2 homodimer (4C0S), with chain A in blue
678 and chain B in gold. WT residues shown in blue, mutant residues in
679 red. T104 interacts with Thr71 from chain B on the dimer interface.
680 Modelling the T104R variant suggests that the bulky Arg residue will
681 clash with chain B and disrupt dimer formation. D362 forms a salt
682 bridge with K62 across the dimer interface, and the loss of charge in
683 the D362N variant suggests that this interaction will be lost.

684 **Figure S4. Variant at the nucleotide-binding site.**

685 Crystal structure of eEF1A2:GDP (4C0S), showing the GDP-binding
686 site. The T24M variant is depicted, adjacent to the GDP-binding

687 residues. The Thr residue is buried within a helix, so the Met
688 substitution disrupts side-chain hydrogen bonds and sterically
689 clashes. The variant likely destabilises the helix and displaces Lys20,
690 disrupting GDP-binding.

691

692 **Acknowledgements:**

693 We want to acknowledge AnDDI-Rares and the families for their
694 participation. In addition, the collaboration in this study were
695 facilitated by ERN ITHACA, one of the 24 European Reference
696 Networks (ERNs) approved by the ERN Board of Member States,
697 cofounded by European Commission. [EU Framework Partnership
698 Agreement ID: 3HP-HP-FPA ERN-01-2016/739516]

699 The aims of this study contribute to the Solve-RD project, which has
700 received funding from the European Union's Horizon 2020 research
701 and innovation program under grant agreement number 779257.

702 **Author Contributions:**

703 **Conceptualization:** AP, LR, CA, AV.

704 **Formal Analysis:** AP.

705 **Investigation:** AP, FA, LR, MCK, SB, JL, DT, SB, MCK, AV, RT, CBA,
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708 **Visualization:** AP, CA, CBN.

709 **Writing - Original Draft:** AP, LR, CA, CBN.

710 **Writing - Review & Editing:** AP, LR, CA, CBN, AV, FM, CC, RF, JP, SJ,
711 CF, LW, JF, AV, ASDP, TH, JM, SAL, WL, RJ, MK, DG, AG, EDB, JL, DT,
712 SB, MCK, AV, RT, CBA, MG, BG, SP.

713 **Conflicts of interest:**

714 The authors declare no conflicts of interest.

715 **Ethical Approval:**

716 Ethical approval was not required because it was a retrospective
717 observational study and patients have already signed consent for
718 genetics analyses for diagnosis. Every patient has been anonymized
719 by the clinician before collecting data (a number has been assigned
720 to each patient).

721 **Funding:**

722 The authors received no financial support for the research,
723 authorship, and/or publication of this article.

Cognitive Evaluation	Cohort (13 patients)	Literature (33 patients)	<i>P-value</i>	CI 95%
No ID	23% (3)	0%	0.082	[-0.03 ; 0.49]
Mild	31% (4)	3% (1)	0.063	[-0.02 ; 0.57]
Moderate	15% (2)	6% (2)	0.418	[-0.14 ; 0.33]
Severe*	31% (4)	76% (25)	0.008	[-0.77 ; -0.13]
Profound*	0	15% (5)	0.023	[-0.28 ; -0.02]

		Cohort	Literature	<i>p-value</i>	CI 95%
Epilepsy	Epilepsy refractory to therapy	25% 3/12	50% 15/30	0.13	[-0.58 ; 0.08]
	Epileptic encephalopathy	7% 1/15	24% 9/38	0.085	[-0.36 ; 0.02]
Brain MRI	Normal	50% 11/22	47% 15/32	0.8258	[-0.25 ; 0.31]
	Thin corpus callosum	14% 3/22	17% 5/30	0.767	[-0.23 ; 0.17]
	Delayed myelinization	9% 2/22	13% 4/30	0.636	[-0.22 ; 0.13]
	White and grey matter abnormalities	27% 6/22	9% 3/32	0.114	[-0.04 ; 0.40]
	Cerebellar and cortical atrophy*	9% 2/22	34% 11/32	0.0207*	[-0.46 ; -0.04]

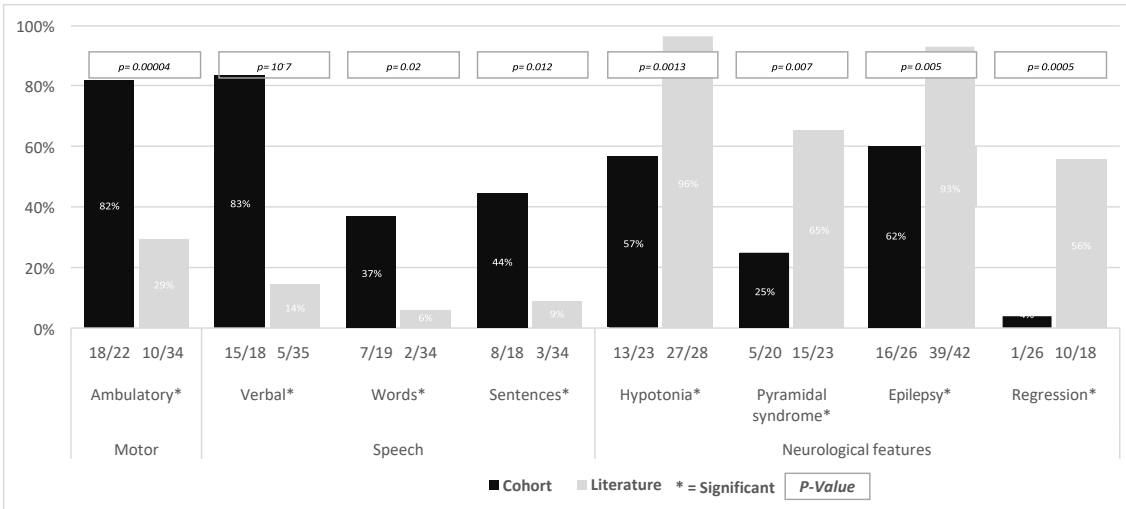
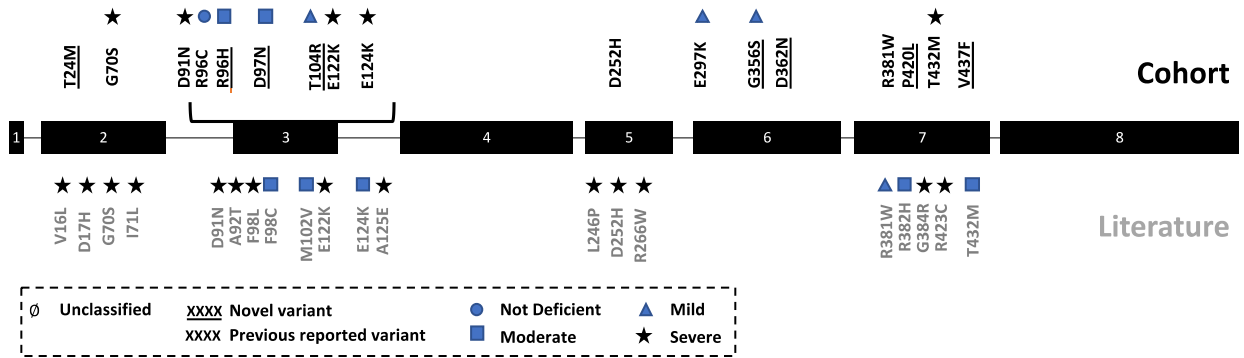


Figure 1. Our series main features compared with the patients from the literature. The figure 1 shows the proportion of our patients (in black) in comparison to those from the literature (featured in grey) according to the main criteria studied. Walking abilities is represented by the motor category. Concerning the speech abilities, “verbal” means the patient can speak and be understandable, “words” means he can express himself in words but not in sentences, “sentences” stands for the ability of the patient to make proper sentences. On the right part of the diagram, the neurological features are shown with the presence of hypotonia, pyramidal syndrome, epilepsy and the presence of regression. * Stands for statistically significance $p<0.05$. *P-values* are framed at the top.

2a



2b

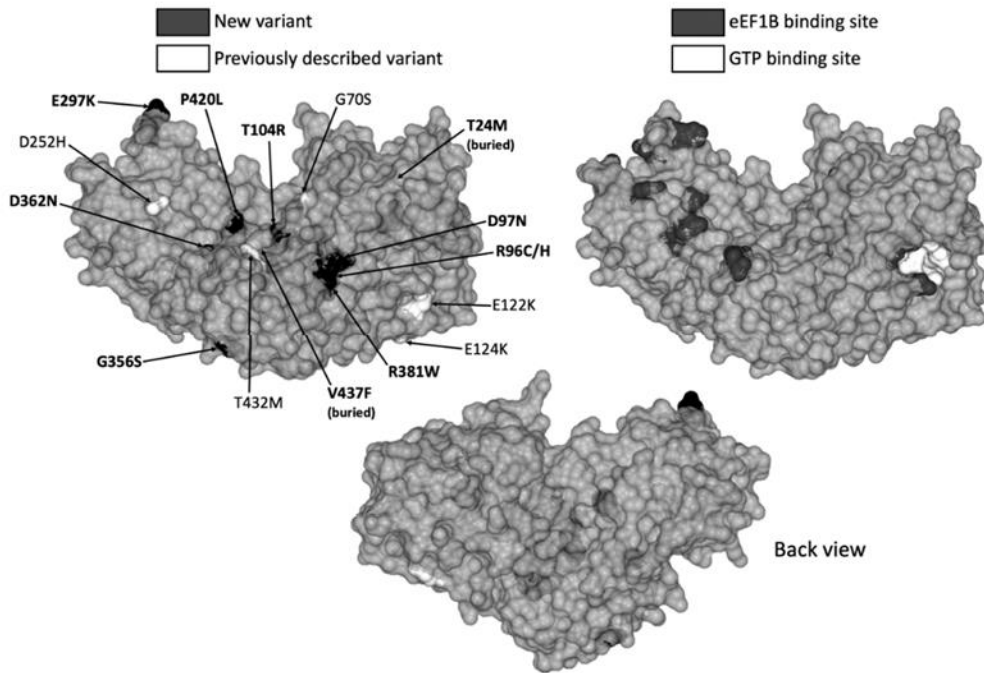


Figure 2. Distribution of pathogenic *EEF1A2* variants. Figure 2a: distribution of variants from our cohort (up) and the previous reported ones (down). Novel variants are underlined. Variants are classified by their associated phenotype in terms of ID, classification is as described as above with associated

symbols (circle for not deficient, triangle for mild ID, square for moderate ID and star for severe). Blank when the variant is not clearly classified (for example when associated with 2 ID categories). Figure 2b: variants mapped onto the crystal structure of GDP-bound eEF1A2 (PDB:4C0S). New variants are shown in black, with labels in bold, previously described mutations are in white. T24M and V437F are buried. The binding site of eEF1B is highlighted in white, and the GTP binding site in dark grey.