Delineation of the *EEF1A2*-epileptic encephalopathy phenotypic spectrum

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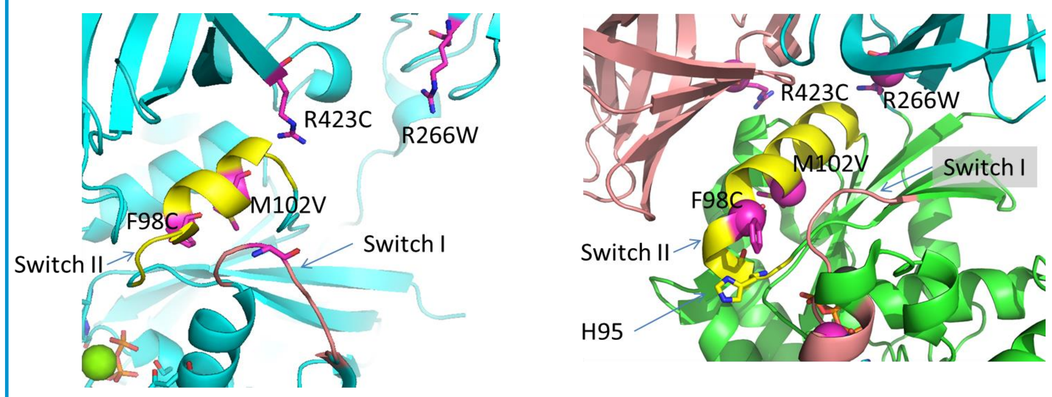
BACKGROUND

- *EEF1A2* encodes the eukaryotic translation factor alpha-2 protein, which transports aminoacyl tRNAs to the A-site of ribosomes, thereby playing a crucial role in protein synthesis.[1]
- *De novo* mutations in *EEF1A2* have been reported in 4 patients with complex phenotypes including epilepsy.[2-4]
- The phenotypic spectrum and functional consequences associated with mutations in *EEF1A2* remain unclear.
- We aimed to expand the phenotypic spectrum of *de novo EEF1A2* mutations and examine the predicted functional and structural consequences.

METHODS

- **Patients/study population:** Whole exome sequencing and next generation sequencing panel data of patients with epilepsy or neurodevelopmental disorders from Ambry Genetics and multiple international centers were reviewed to identify patients with mutations in *EEF1A2*.
- **Clinical phenotyping and data collection:** All patients were examined by neurologists or medical geneticists and available medical records including clinic notes, EEGs, and MRIs were reviewed.
- **Computational structural modeling:** The predicted structural consequences of the identified *EEF1A2* mutations were investigated using computational modeling. The structures of GDP bound form of eEF1A2 (PDB:4C0S) and the GTP bound form of eEF1A1 (PDB:3WXM) were used for analysis.[5-7] eEF1A1 and eEF1A2 are >95% identical, with all residues in the eEF1B-α binding site conserved.

FIG. 1: *EEF1A2* mutations located in switch II region



GDP-bound eEF1A | GTP-bound eEF1A

Figure 1. Mutations p.F98C, p.M102V, p.R266W, and p.R423C (magenta sticks) are shown, in the GDP-bound protein structure on the left and the GTP-bound protein structure on the right. Mg^{2+} is shown in the left as a green sphere. The entire structure of eEF1A with GDP is colored teal (left) and the eEF1A GTP is colored with domain I (green), domain II (teal), and domain II (peach) as a cartoon (right). eEF1A switch II region is labeled and colored yellow, and switch I region is labeled and colored peach.

RESULTS

- We report 6 new patients and review the phenotypes of 4 reported patients (Table). 5/6 newly reported patients had *de novo* mutations in *EEF1A2*; 1 patient had a variant of unknown inheritance.
- 9/10 individuals had an epileptic encephalopathy, with a median seizure onset of 4 months. Various seizure types were observed, which were often refractory to multiple anti-epileptic drugs.
- Movement disorders including choreoathetotic, other dyskinesias and ataxia were observed in 5/10 patients.
- 4/10 patients carried a recurrent c.208G>A (p.G70S) mutation.
- All mutations (except p.G70S in switch I) are located in or interact with the switch II region. These variants are predicted to destabilize this region and impact the orientation of several key residues including His95, an amino acid important for stabilizing the transition state of GTP hydrolysis (Fig. 1).
- The recurrent c.208G>A (p.G70S) mutation is located in switch I and is predicted to alter the position of residue Thr71, which coordinates the binding of GTP (Fig. 2).
- Structural modeling showed that all mutations occur within or interact with the switch I or switch II regions of the eEF1A2 protein, which are essential for GTP hydrolysis and dissociation from the ribosome. All mutations are predicted to impact GTP hydrolysis and exert a dominant-negative effect.

FIG. 2: Recurrent p.G70S mutation

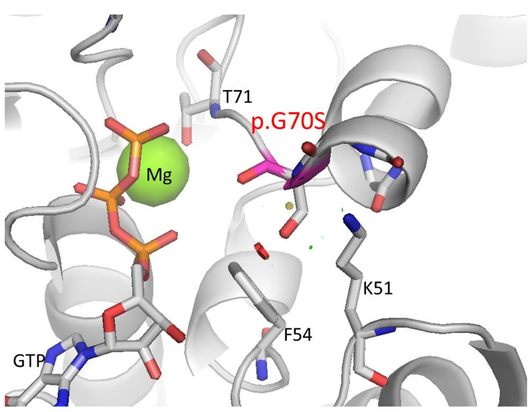


Figure 2. Crystal structure of eEF1A with bound GTP. The reference glycine at residue 70 provides flexibility to the switch I loop, allowing Thr71 to coordinate the catalytic Mg^{2+} (shown in green). The p.G70S mutation introduces mild clashes with Phe54 and Lys51. Binding of GTP will result in a shift of the switch I loop, likely altering the orientation of Thr71 and perturbing Mg^{2+} binding and catalysis.

TABLE: Clinical features of patients with *EEF1A2* mutations

	Newly Identified Patients						Previously Reported Patients			
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	de Ligt[2]	Veeramah[3]	Nakajima[4]	Nakajima[4]
Mutation	c.208G>A (p.G70S) *de novo*	c.208G>A (p.G70S) *de novo*	c.293T>G (p.F98C) *de novo*	c.304A>G (p.M102V) Unknown	c.796C>T (p.R266W) *de novo*	c.1267C>T (p.R423C) *de novo*	c.208G>A (p.G70S) *de novo*	c.208G>A (p.G70S) *de novo*	c.364G>A (p.E122K) *de novo*	c.754G>C (p.D252H) *de novo*
Sex	M	F	F	F	M	M	F	M	F	F
Age at study	Deceased 4y	7.5y	5y	8.5y	5m	4y	22y	14y	12y	8y
Seizure onset	18m	1m	No seizures	6m	4m	6m	4m	2m	4m	8y
Seizure types	Absence, GTCS, tonic, myoclonic	Atypical absence, myoclonic	-	FS, GTCS, hemiclonic	Absence, GTCS, hemiclonic	IS, myoclonic, tonic	Absence, GTCS, myoclonic	Atonic, GTCS, IS, myoclonic, tonic	IS	GTCS
Course	Refractory	Refractory	-	Ongoing	Controlled	Refractory	Unknown	Refractory	Controlled	Unknown
EEG findings	Burst suppression, GSSW	Multifocal, GSW, PPR	Multifocal	Unavailable	Focal discharges	Hyps, GSW	N/A	Burst suppression, hyps, GSSW	Multifocal	Multifocal
Movement disorder	Choreo-athetosis	No	Choreo-athetosis	No	No	Hyperkinetic movements	Not reported	Gait instability	Ataxic gait (onset 12y)	Not reported
Degree of ID	Severe, non-verbal	Moderate, non-verbal	Moderate, non-verbal	Moderate	Global devt delay	Moderate, non-verbal	Severe, non-verbal	Severe, non-verbal	Moderate, non-verbal	Moderate, non-verbal
Neuro Exam	Hypotonia, spasticity	Severe hypotonia	Hypotonia, dystonia	Normal	Hypotonia	Hypotonia	Hypotonia	Hypotonia, spasticity	Hypotonia	Hypotonia
Behavioral issues	Stereotypies	None	Stereotypies	None	None	None	Autistic features	None	Autistic features	Stereotypies
Brain MRI	Cerebral atrophy	Normal	Normal	Ventriculo-megaly	Thin corpus callosum	Thin corpus callosum, cerebral atrophy	N/A	Normal	Cerebral atrophy	Cerebral atrophy
Other features	respiratory failure				respiratory failure, microcephaly			microcephaly	dysmorphic, microcephaly	dysmorphic, microcephaly

FS: febrile seizures; GSW: generalized spike-wave; GSSW: generalized slow spike-wave; GTCS: generalized tonic-clonic seizures; IS: infantile spasms; Hyps: hypsarrhythmia; PPR: photoparoxysmal response

TAKE-HOME POINTS

- *EEF1A2*-encephalopathy is characterized by infantile onset of refractory seizures, severe hypotonia sometimes leading to spasticity, absent speech, and intellectual disability. Movement disorders and respiratory failure are also seen in some patients.
- p.G70S is a recurrent mutation found in 40% of our cohort.
- The mutations structurally perturb two catalytically essential regions with a predicted impact on GTP hydrolysis. We hypothesize that mutant eEF1A2 stalls on the ribosome, thereby inhibiting translation in a dominant-negative fashion.

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