



**HAL**  
open science

## A case of QARS1 associated epileptic encephalopathy and review of epilepsy in aminoacyl-tRNA synthetase disorders

Denise L Chan, Joëlle Rudinger-Thirion, Magali Frugier, Lisa Riley, Gladys Ho, Kavitha Kothur, Shekeeb Mohammad

### ► To cite this version:

Denise L Chan, Joëlle Rudinger-Thirion, Magali Frugier, Lisa Riley, Gladys Ho, et al.. A case of QARS1 associated epileptic encephalopathy and review of epilepsy in aminoacyl-tRNA synthetase disorders. *Brain and Development*, 2022, 44 (2), pp.142-147. 10.1016/j.braindev.2021.10.009 . hal-03554560

**HAL Id: hal-03554560**

**<https://hal.science/hal-03554560>**

Submitted on 3 Feb 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1

**TITLE PAGE****2 A case of *QARS1* associated epileptic encephalopathy and review**  
**3 of epilepsy in aminoacyl-tRNA synthetase disorders**

4

5 Denise L CHAN <sup>a</sup>, FRACP, Joëlle RUDINGER-THIRION <sup>b</sup>, PhD, Magali FRUGIER <sup>b</sup>, PhD,6 Lisa G RILEY <sup>c</sup>, PhD, Gladys HO <sup>d,e</sup>, PhD, Kavitha KOTHUR, PhD <sup>e</sup>, Shekeeb S7 MOHAMMAD, PhD <sup>e,f</sup>

8

9 <sup>a</sup> Neurology Department, Sydney Children's Hospital, Sydney, Australia; School of  
10 Women's and Children's Health, UNSW Medicine, UNSW Sydney, Australia11 <sup>b</sup> Université de Strasbourg, CNRS, Architecture et Réactivité de l'ARN, UPR 9002, F-67000  
12 Strasbourg, France.13 <sup>c</sup> Rare Diseases Functional Genomics, Kids Research, Sydney Children's Hospital Network &  
14 Children's Medical Research Institute, Sydney Children's Hospital Network, Sydney, NSW  
15 2145, Australia; Discipline of Child & Adolescent Health, Sydney Medical School,  
16 University of Sydney, Sydney, NSW 2006, Australia.17 <sup>d</sup> Sydney Genome Diagnostics, Western Sydney Genetics Program, The Children's Hospital  
18 at Westmead, Sydney, Australia; Discipline of Child & Adolescent Health; Discipline of  
19 Genetic Medicine, The University of Sydney, Sydney, Australia.20 <sup>e</sup> Children's Hospital at Westmead Clinical School, Faculty of Medicine and Health, The  
21 University of Sydney, Sydney, NSW, Australia22 <sup>f</sup> TY Nelson Department of Neurology and Neurosurgery, The Children's Hospital at  
23 Westmead, Sydney, NSW, Australia; Kids Neuroscience Centre, Kids Research, The  
24 Children's Hospital at Westmead, The University of Sydney, Sydney, NSW, Australia.

25

26

27 **Corresponding Author:** Dr Shekeeb S Mohammad, PhD, Clinical School, the Children's

28 Hospital at Westmead, Locked Bag 4001, NSW 2145 Australia

29 email: [shekeeb.mohammad@health.nsw.gov.au](mailto:shekeeb.mohammad@health.nsw.gov.au) T: +61298450000 F: +61298453389

30 Word Count – manuscript – 1548; abstract 250 words

31 References- 23

32 Tables - 2.

33 Figures – 1

34 Supplementary material : 1 file

35 Conflict of Interest: Nil

36

37

38

39 **STRUCTURED ABSTRACT**

40 *Introduction:* Mutations in *QARS1*, which encodes human glutaminyl-tRNA synthetase, have  
41 been associated with epilepsy, developmental regression, progressive microcephaly and  
42 cerebral atrophy. Epilepsy caused by variants in *QARS1* is usually drug-resistant and  
43 intractable. Childhood onset epilepsy is also reported in various aminoacyl-tRNA synthetase  
44 disorders. We describe a case with a milder neurological phenotype than previously reported  
45 with *QARS1* variants and review the seizure associations with aminoacyl-tRNA synthetase  
46 disorders.

47

48 *Case report:* The patient is a 4-year-old girl presenting at 6 weeks of age with orofacial  
49 dyskinesia and hand stereotypies. She developed focal seizures at 7 months of age. Serial  
50 electroencephalograms showed shifting focality. Her seizures were controlled after  
51 introduction of carbamazepine. Progress MRI showed very mild cortical volume loss without  
52 myelination abnormalities or cerebellar atrophy. She was found to have novel compound  
53 heterozygous variants in *QARS1* (NM\_005051.2): c.[1132C>T];[1574G>A],  
54 p.[(Arg378Cys)];[(Arg525Gln)] originally classified as “variants of uncertain significance”  
55 and later upgraded to “likely pathogenic” based on functional testing and updated variant  
56 database review. Functional testing showed reduced solubility of the corresponding *QARS1*  
57 mutants *in vitro*, but only mild two-fold loss in catalytic efficiency with the c.1132C>T  
58 variant and no noted change in tRNA<sup>Gln</sup> aminoacylation with the c.1574G>A variant.

59

60 *Conclusion:* We describe two *QARS1* variants associated with overall conserved tRNA  
61 aminoacylation activity but characterized by significantly reduced QARS protein solubility,  
62 resulting in a milder clinical phenotype. 86% of previous patients reported with *QARS1* had  
63 epilepsy and 79% were pharmaco-resistant. We also summarise literature regarding epilepsy

64 in aminoacyl-tRNA synthetase disorders, which is also often early onset, severe and drug-  
65 refractory.

66

67 **Key words:** *QARS1*, QARS, ARS, aminoacyl-tRNA synthetase, acquired microcephaly,  
68 DEE, seizures, epilepsy

69

## 70 **INTRODUCTION**

71 Mutations in aminoacyl-tRNA synthetases (aaRS) have emerged in association with diverse  
72 neurological presentations. Variants in *QARS1*, inherited as an autosomal recessive disorder,  
73 have been associated with a syndrome of “Microcephaly, progressive, with seizures and  
74 cerebral and cerebellar atrophy” (OMIM #615760). At the time of this publication, variants  
75 in *QARS1* (encoding glutaminyl-tRNA synthetase) were reported in 25 cases [1,2] with  
76 epilepsy affecting 86% including severe early infantile developmental and epileptic  
77 encephalopathy (DEE) and epilepsy of infancy with migrating focal seizures (EIMFS). 79%  
78 of reported *QARS1*-related epilepsy (15 of 19) are described as being pharmaco-resistant. We  
79 report a case with a milder epileptic phenotype and biallelic *QARS1* variants supported by  
80 functional findings in QARS1 activity and review the seizure associations with aaRS  
81 disorders.

82

## 83 **CLINICAL REPORT**

84 The patient is a now 4-year-old girl – the firstborn of a French father and Asian mother. She  
85 was born at 38 weeks’ gestation with intrauterine growth restriction (birthweight 2278 g,  
86 head circumference 32 cm, both 3<sup>rd</sup> centile). At 7 months, she presented with focal febrile  
87 status epilepticus lasting 1.5 hours in association with adenovirus, coronavirus and rhinovirus  
88 infections. She had a background of developmental delay, and orofacial dyskinesia –

89 intermittent mouthing and chewing movements and stereotypical hand-wringing movements  
90 starting from 6 weeks of age. There was no family history of epilepsy or developmental  
91 delay.

92

93 Initial examination at 7 months was remarkable for low weight 6.3 kg (<3<sup>rd</sup> centile) and small  
94 head circumference 42 cm (<10<sup>th</sup> centile). Peripheral tone was increased, becoming less  
95 evident as she grew older. She could not roll, sit or grasp objects. She could fix on but not  
96 track objects horizontally and had alternating esotropia. She was not cooing or babbling. EEG  
97 at 7 months showed right temporal discharges and brief electrographic seizures with  
98 generalised spike-and-waves. Orofacial movements and stereotypical hand movements did  
99 not have any EEG correlate. Following this, she had monthly seizures of febrile and afebrile  
100 type, with shifting focality on sequential EEGs, but migrating focality not noted in a single  
101 recording. Clinically, her seizures consisted of head and eye deviation to either side, mostly  
102 with altered awareness and sometimes with partial retention of awareness. Most seizures  
103 occurred in clusters and some were 15-20 minutes in duration. Levetiracetam and topiramate  
104 were ineffective. No seizures have been noted since initiation of carbamazepine at 17 months  
105 of age.

106

107 Her MRI at presentation showed a thin corpus callosum and enlarged ventricles  
108 (Supplementary figure 1, A and B). Progress MRI at 18 months showed progressive diffuse  
109 cerebral atrophy with prominent sulci, loss of white matter and possible delayed myelination  
110 (Supplementary figure 1, C and D).

111

112 At last follow up aged 3 years 2 months, she has stagnation of head growth representing  
113 acquired microcephaly (46 cm, <3<sup>rd</sup> centile) and persisting low weight for stature (13 kg, 105

114 cm, <3<sup>rd</sup> centile). She continues to make slow developmental progress. She can take a few  
115 steps independently and continues to babble. She can reach using both hands and transfer  
116 between hands but cannot perform fine motor movements such as a pincer grip. She is  
117 visually attentive to her surroundings and has intermittent, non-disabling motor stereotypies  
118 in the form of hand wringing and flapping movements.

119

## 120 **GENETIC AND FUNCTIONAL TESTING**

121 Biallelic *QARS1* variants were identified through whole exome sequencing (*QARS1*  
122 (NM\_005051.2): c.[1132C>T]:[1574G>A]). Parental segregation confirmed the variants to  
123 be on separate alleles (*trans*). The c.1132C>T variant, not reported at the time of testing (July  
124 2018) was subsequently reported in ClinVar (Variation ID: 598952). It has a population  
125 minor allele frequency (MAF) of 0.007% in gnomAD with highest MAF of 0.07% in the East  
126 Asian population (v2.1.1, gnomad.broadinstitute.org, last accessed 01/07/2021) and a CADD  
127 score of 31.0. The c.1574G>A variant has a MAF of 0.0004% in gnomAD and a CADD  
128 score of 25.8. Both variants are located in the catalytic domain of *QARS1*. At the time of  
129 reporting, both variants were considered to be variants of uncertain significance (VOUS)  
130 according to the American College of Medical Genetics and Genomics guidelines (ACMG;  
131 evidence used: PM2, PP3).[3] In view of the patient's milder phenotype compared to cases  
132 reported at the time of genetic results, functional testing was undertaken at CNRS in  
133 Strasbourg, France. The effect of the *QARS1* variants on tRNA aminoacylation activity was  
134 investigated as described in the supplementary material. This showed partial reduction in  
135 aminoacylation efficiency related to the *QARS1* p.Arg378Cys variant only but drastic  
136 reduction in solubility of both variants compared to wild-type QARS1 (supplementary figure  
137 2). Two previously reported *QARS1* variants located in the catalytic domain, p.Arg403Trp  
138 and p.Arg515Trp, also showed reduced solubility. [4] The variant classification for this

139 patient was subsequently revised to “likely pathogenic” (ACMG PS3\_supporting; PM2, PM3  
140 and PP3).

141

## 142 **DISCUSSION**

143 Thirty-seven genes encode the family of aaRS, of which 17 are responsible for catalysing  
144 attachment of amino acids to their cognate tRNA in the cytoplasm, 17 in the mitochondria  
145 and 3 act across both cellular locations (*QARS1*, *KARS1* and *GARS1*). Of these, 34 genes are  
146 associated with human disease, which include all 3 aaRS servicing both cellular locations and  
147 all 17 servicing the mitochondria. In addition to *QARS1*, early infantile DEE is described in  
148 association with variants in *AARS1*, *FARS2*, *RARS2* and *VAR2* and seizure onset in infancy  
149 is noted in most other aaRS disorders. Neuroimaging and clinical features of aaRS associated  
150 with epilepsy are summarized in Table 1. Non-specific cortical atrophy associated with  
151 acquired microcephaly is noted in many disorders including *QARS1*, while distinct MRI  
152 changes are noted in some disorders such as those with *KARS1* and *EARS2* variants (Table 1).  
153 The phenotypes of different aaRS deficiencies show significant overlap and the central  
154 nervous system is most often affected, along with impaired hearing or vision, failure to thrive  
155 and feeding difficulties being other common themes [5].

156

157 The first description of *QARS1*-associated disease in two unrelated families described a very  
158 severe phenotype of neonatal onset epileptic encephalopathy, progressive neurological  
159 decline with delayed myelination, cortical and cerebellar atrophy [4]. Detailed phenotypic  
160 information for 22 cases including those previously published was recently summarised by  
161 Johannsen et al [1].

162



163 Overall, epilepsy associated with *QARSI* variants is thought to be more severe than other  
164 aaRS disorders, often with seizure onset on the first day of life (Table 2) and latest seizure  
165 onset at 5.5 months in reported cases. Our patient only started having seizures at 7 months of  
166 age which is later than previously described. In Johannesen's series, 28% of QARS patients  
167 reported increased seizure frequency with fever or infections similar to some episodes in our  
168 patient [1]. A migrating EEG pattern as noted in EIMFS was not noted in a single epoch in  
169 our patient but has been described in previous cases [4]. Her seizures have been remarkably  
170 responsive to carbamazepine, whereas most previous cases (15/19) had drug-refractory  
171 epilepsy, in some cases despite carbamazepine use [6], thus implying that response to  
172 carbamazepine may reflect the overall milder disease course in our patient rather than a drug-  
173 specific effect. No specific antiepileptic strategy has been highlighted as particular  
174 efficacious in previously reported *QARSI* encephalopathy cases apart from one report of  
175 benefit from the ketogenic diet in a severe, early onset case with multi-drug resistant epilepsy  
176 [6]. New research suggests supplementation of the deficient amino acid in aaRS deficiencies  
177 may be beneficial [7], although oral glutamine supplementation has been reported to worsen  
178 seizure control in one *QARSI* patient, for whom memantine was subsequently effective [2].

179

180 Neuroimaging features associated with *QARSI* encephalopathy include thin corpus callosum,  
181 delayed myelination and cortical atrophy. Our patient was only noted to have mild cortical  
182 atrophy and no cerebellar atrophy or immaturity of myelination on progress MRI at 18  
183 months of age. Few previous cases have noted the lack of posterior fossa abnormalities but  
184 cortical atrophy and hypomyelination (88%) have been noted in the majority of cases (Table  
185 2).

186

187 Our case is novel and adds to literature as a milder neurological phenotype associated with  
188 *QARSI* (Table 2) in terms of lack of developmental regression, controlled epilepsy, and  
189 subtle neuroimaging changes. The variants in our case are both missense mutations located  
190 in the catalytic domain (Figure 1), but disease-causing variants have also been reported in the  
191 N-terminal domain and the anti-codon binding domain of *QARSI* [8]. One previously  
192 reported patient with the p.(Arg378Cys) variant in combination with a null variant had a  
193 more severe epilepsy phenotype than our patient [1]. Another patient was homozygous for  
194 p.(Arg378His) but had no seizures. Five reported cases of *QARSI* without epilepsy had  
195 homozygous missense mutations in the catalytic domain [2]. Functional testing of the *QARSI*  
196 variants in most prior reports have noted either complete disruption or marked reduction of  
197 glutamine aminoacylation activity as well as reduced solubility of QARS protein with  
198 preserved protein expression, thus implying an adverse impact on protein folding and  
199 aggregation [4,6]. QARS protein associated with the p.Arg378Cys and p.Arg525Gln variants  
200 was also largely insoluble, while the soluble protein showed only mild effects on  
201 aminoacylation. Interestingly, similar to our case, one prior case series [9] reported patients  
202 without epilepsy and with a less progressive course along with functional analysis showing a  
203 milder reduction in glutamine aminoacylation (67% reduction). However, aminoacylation  
204 activity in aaRS does not always correlate with disease severity [10]. We hypothesise that the  
205 low solubility of the QARS protein in our patient is likely to result in partial reduction of the  
206 level of tRNA<sup>Gln</sup> aminoacylation and may therefore contribute to the observation of some  
207 milder phenotypic features.

208

## 209 **ACKNOWLEDGEMENTS**

210 Part of this work, conducted at Kids Research, was supported by the Luminesce Alliance –  
211 Innovation for Children’s Health, a not-for-profit cooperative joint venture between the

212 Sydney Children's Hospitals Network, the Children's Medical Research Institute, and the  
213 Children's Cancer Institute. It has been established with the support of the NSW Government  
214 to coordinate and integrate paediatric research. Luminesce Alliance is also affiliated with the  
215 University of Sydney and the University of New South Wales Sydney. Functional studies  
216 were supported by the CNRS and the Université de Strasbourg. S.S.M. acknowledges support  
217 from Cerebral palsy alliance, Allambie Heights, Sydney

## **CONFLICT OF INTERST DISCLOSURES**

The authors have no conflicts of interest to declare.

## REFERENCES

1. Johannesen KM, Mitter D, Janowski R, Roth C, Toulouse J, Poulat AL, et al. Defining and expanding the phenotype of QARS-associated developmental epileptic encephalopathy. *Neurol Genet.* 2019;5:e373.
2. Shen YW, Zou LP. Correspondence on “Aminoacyl-tRNA synthetase deficiencies in search of common themes” by Fuchs et al. *Genet Med.* 2021;23:587–8.
3. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17:405–24.
4. Zhang X, Ling J, Barcia G, Jing L, Wu J, Barry BJ, et al. Mutations in QARS, encoding glutaminyl-tRNA synthetase, cause progressive microcephaly, cerebral-cerebellar atrophy, and intractable seizures. *Am J Hum Genet.* 2014;94:547–58.
5. Fuchs SA, Schene IF, Kok G, Jansen JM, Nikkels PGJ, van Gassen KLI, et al. Aminoacyl-tRNA synthetase deficiencies in search of common themes. *Genet Med.* 2019;21:319–30.
6. Datta A, Ferguson A, Simonson C, Zannotto F, Michoulas A, Roland E, et al. Case Report: QARS deficiency and favorable outcome following treatment of seizures with ketogenic diet. *J Child Neurol.* 2017;32:403–7.
7. Kok G, Tseng L, Schene IF, Dijsselhof ME, Salomons G, Mendes MI, et al. Treatment of ARS deficiencies with specific amino acids. *Genet Med.* 2021, in press. doi: 10.1038/s41436-021-01249-z.

8. Ognjenović J, Wu J, Matthies D, Baxa U, Subramaniam S, Ling J, et al. The crystal structure of human GlnRS provides basis for the development of neurological disorders. *Nucleic Acids Res.* 2016;44:3420–31.
9. Leshinsky-Silver E, Ling J, Wu J, Vinkler C, Yosovich K, Bahar S, et al. Severe growth deficiency, microcephaly, intellectual disability, and characteristic facial features are due to a homozygous QARS mutation. *Neurogenetics.* 2017;18:141–6.
10. González-Serrano LE, Chihade JW, Sissler M. When a common biological role does not imply common disease outcomes: Disparate pathology linked to human mitochondrial aminoacyl-tRNA synthetases. *J Biol Chem.* 2019;294:5309–20.
11. Meyer-Schuman R, Antonellis A. Emerging mechanisms of aminoacyl-tRNA synthetase mutations in recessive and dominant human disease. *Hum Mol Genet.* 2017;26: R114–27.
12. Simons C, Griffin LB, Helman G, Golas G, Pizzino A, Bloom M, et al. Loss-of-function alanyl-tRNA synthetase mutations cause an autosomal-recessive early-onset epileptic encephalopathy with persistent myelination defect. *Am J Hum Genet.* 2015;96:675–81.
13. Ardisson A, Tonduti D, Legati A, Lamantea E, Barone R, Dorboz I, et al. KARS-related diseases: Progressive leukoencephalopathy with brainstem and spinal cord calcifications as new phenotype and a review of literature. *Orphanet J Rare Dis.* 2018;13:45.
14. Friedman J, Smith DE, Issa MY, Stanley V, Wang R, Mendes MI, et al. Biallelic mutations in valyl-tRNA synthetase gene VARS are associated with a progressive neurodevelopmental epileptic encephalopathy. *Nat Commun.* 2019;10:1–10.

15. Coughlin CR, Scharer GH, Friederich MW, Yu HC, Geiger EA, Creadon-Swindell G, et al. Mutations in the mitochondrial cysteinyl-tRNA synthase gene, CARS2, lead to a severe epileptic encephalopathy and complex movement disorder. *J Med Genet*. 2015;52:532–40.
16. Steenweg ME, Ghezzi D, Haack T, Abbink TEM, Martinelli D, Van Berkel CGM, et al. Leukoencephalopathy with thalamus and brainstem involvement and high lactate “LTBL” caused by EARS2 mutations. *Brain*. 2012;135:1387–94.
17. Almannai M, Wang J, Dai H, El-Hattab AW, Faqeih EA, Saleh MA, et al. FARS2 deficiency; new cases, review of clinical, biochemical, and molecular spectra, and variants interpretation based on structural, functional, and evolutionary significance. *Mol Genet Metab*. 2018;125:281–91.
18. Seaver LH, DeRoos S, Andersen NJ, Betz B, Prokop J, Lannen N, et al. Lethal NARS2-related disorder associated with rapidly progressive intractable epilepsy and global brain atrophy. *Pediatr Neurol*. 2018;89:26–30.
19. Mizuguchi T, Nakashima M, Kato M, Yamada K, Okanishi T, Ekhilevitch N, et al. PARS2 and NARS2 mutations in infantile-onset neurodegenerative disorder. *J Hum Genet*. 2017;62:525–9.
20. Nishri D, Goldberg-Stern H, Noyman I, Blumkin L, Kivity S, Saitsu H, et al. RARS2 mutations cause early onset epileptic encephalopathy without pontocerebellar hypoplasia. *Eur J Paediatr Neurol*. 2016;20:412–7.
21. Begliomini C, Magli G, Di Rocco M, Santorelli FM, Cassandrini D, Nesti C, et al. VARS2-linked mitochondrial encephalopathy: Two case reports enlarging the clinical phenotype. *BMC Med Genet*. 2019;20:1–6.
22. Theisen BE, Rumyantseva A, Cohen JS, Alcaraz WA, Shinde DN, Tang S, et al. Deficiency of WARS2, encoding mitochondrial tryptophanyl tRNA synthetase,

causes severe infantile onset leukoencephalopathy. *Am J Med Genet Part A*.  
2017;173:2505–10.

23. Wortmann SB, Timal S, Venselaar H, Wintjes LT, Kopajtich R, Feichtinger RG, et al. Biallelic variants in *WARS2* encoding mitochondrial tryptophanyl-tRNA synthase in six individuals with mitochondrial encephalopathy. *Hum Mutat*.  
2017;38:1786–95.



## FIGURE LEGENDS

**Figure 1** : Schematic diagram of QARS1 (NP\_005042.1) showing location of reported coding variants (this case in red), conserved HIGH and VVSKR motifs, and functional domains. NTD, N-terminal domain; CATD, catalytic domain; CP1, connecting peptide 1 within the catalytic domain; ABD, anticodon-binding domain[8].

**Table 1 : Aminoacyl-tRNA synthetase disorders associated with epilepsy - typical phenotype and epilepsy subtype**

Gene	Typical Phenotype [11]	Age of seizure onset	Seizure description/epilepsy syndrome	EEG features	Neuroimaging
<i>AARS1</i> * [12]	Progressive microcephaly, spasticity, hypomyelination, absent reflexes, extrapyramidal features	Infancy	Refractory myoclonic seizures: infantile DEE (EHEE29/DEE29)	Slow background, excess sharp waves. Spasm with electrodecrement but no hypsarrhythmia	Progressive atrophy – cortical, white matter, cerebellar and brainstem, hypomyelination
<i>KARS</i> (both <i>mitochondrial and cytosolic</i> ) [13]	Developmental delay, visual loss, non-syndromic hearing loss, progressive microcephaly, CMTN	Infancy to adolescence	Febrile seizures, status epilepticus, NCSE, West syndrome, infantile DEE	Poorly organised background, fronto-centro-temporal spikes, multifocal discharges	Leukodystrophy with long tract signal change, calcification maybe noted in the brain and spinal cord
<i>QARS1</i> [1]	Developmental delay, progressive microcephaly, cerebral atrophy, PCH	Neonatal to infancy	Intractable seizures, frequent and prolonged. Multiple seizure types – focal, multifocal, generalised, myoclonic. Can be associated with fever or infections. Infantile DEE.	Various: focal, bilateral, multifocal, hypsarrhythmia, “focal migrating seizure-like” pattern	Progressive cerebral and cerebellar atrophy, cortical structural abnormalities (simplified gyri, CC hypoplasia), delayed myelination, enlarged subarachnoid spaces
<i>FAKSI</i> [14]	Developmental delay, seizures, progressive microcephaly, axial	Infancy	Generalised, myoclonic, focal, infantile spasms,	EEG hypsarrhythmia, multifocal discharges,	Progressive cortical atrophy, white matter volume loss,

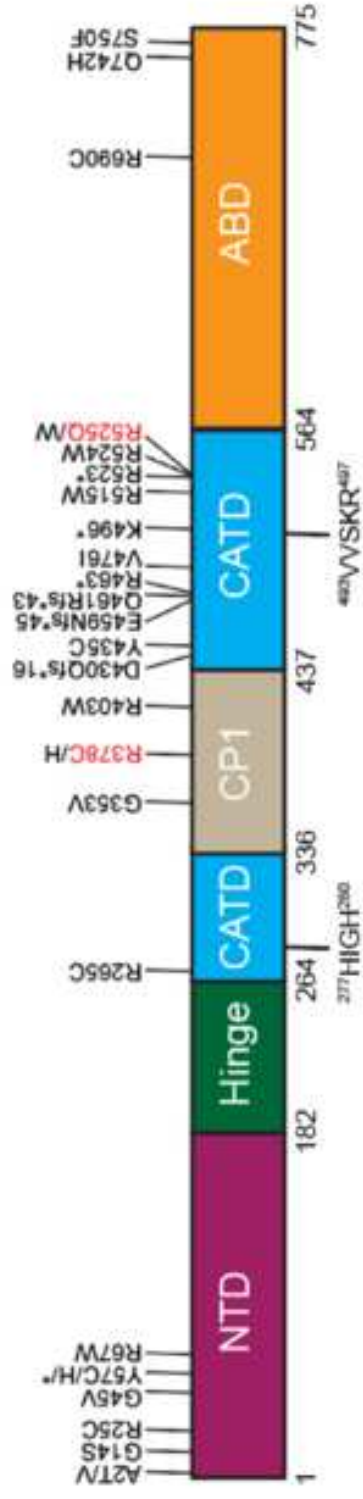
	hypotonia		multifocal, infantile DEE.	or focal	CC hypoplasia, simplified gyral pattern, thin corpus callosum
<i>CARS2</i> [15]	Developmental delay; cognitive decline, visual and hearing impairment, movement disorder	Infancy or childhood	Progressive myoclonic epilepsy, GTCS, infantile DEE	Multifocal discharges – posterior predominant, high amplitude	Progressive cortical and cerebellar atrophy (white matter loss), T2 hyperintensity in cortex and white matter, CC hypoplasia.
<i>EARS2</i> [16]	Leukoencephalopathy with thalamus and brainstem involvement, elevated lactate, respiratory chain complex defects. Psychomotor delay spasticity. A milder phenotype in some.	Infancy	Drug refractory seizures, infantile DEE. Seizures can improve after 2 <sup>nd</sup> year of life	EEG can be normal, background slow and disorganised	T2 hyperintensities in a typical distribution – “Leukoencephalopathy with thalamus and brainstem involvement and high lactate on spectroscopy” (LTBL). MRI changes can improve with time in milder group. CC agenesis
<i>FARS2</i> [17]	Global developmental delay, Alpers syndrome, hereditary spastic paraplegia	Infancy	Focal seizures, infantile spasms, infantile DEE, status epilepticus, epilepsia partialis continua	Hypsarhythmia. Focal discharges. PLEDs in one subject, alternating asymmetry between hemispheres in another	Cortical atrophy, sometimes with cystic change, thin CC and T2 hyperintensities in the dentate nuclei
<i>NARS2</i>	Developmental delay,	Infancy to early	Absence, myoclonic,	Focal, electrographic	Rapid cortical atrophy,

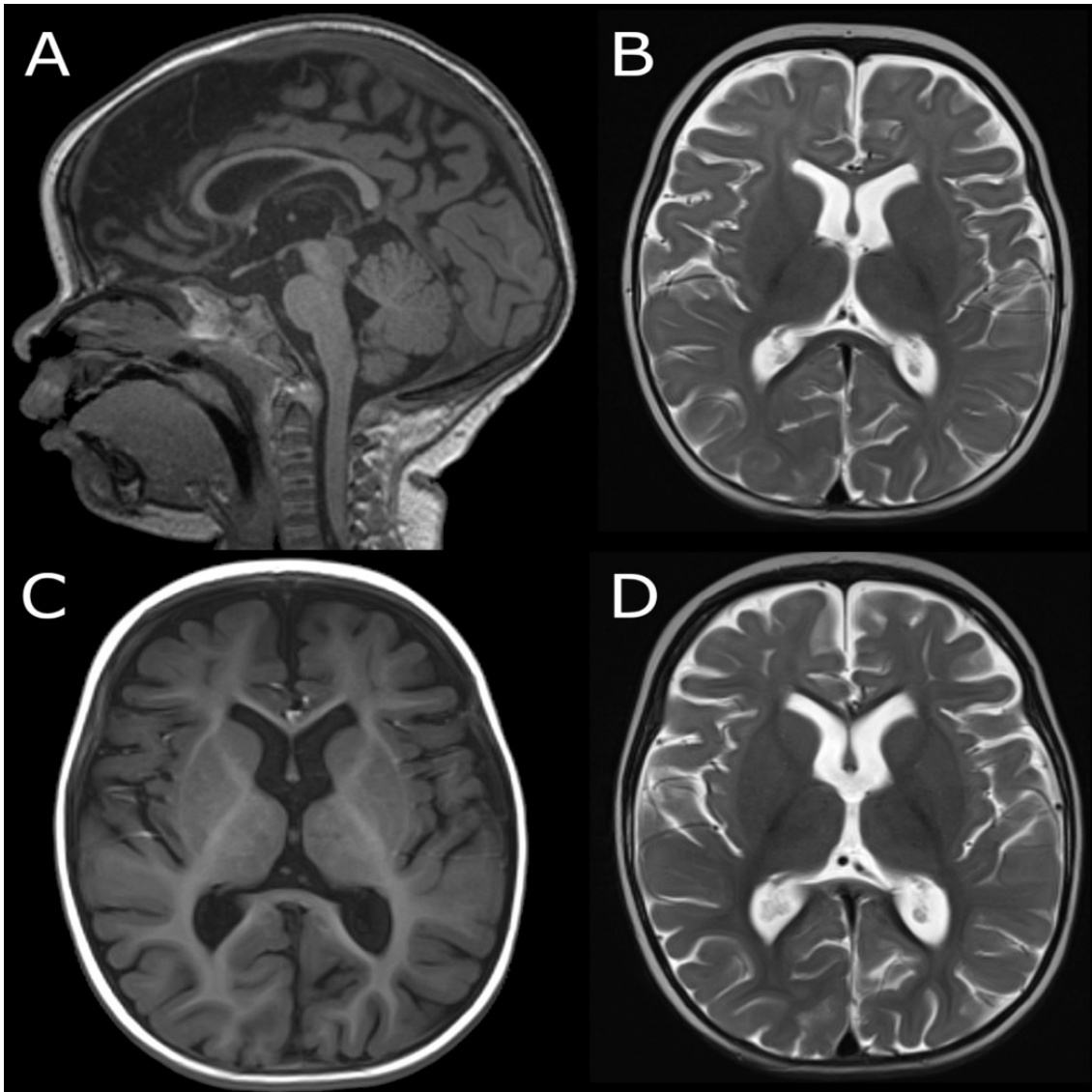
[18,19]	cerebellar atrophy, Leigh Syndrome, Alpers Syndrome, myopathy	childhood	GTCS, status epilepticus, hemiconvulsion–hemiplegia–epilepsy	seizures, hypersarrhythmia, burst-suppressed	leukodystrophy, Leigh syndrome like changes
<i>PAKS2</i> [19]	Developmental delay, progressive microcephaly, Alpers syndrome	Infancy	Infantile spasm, focal seizures, infantile DEE	EEG showing hypersarrhythmia, independent occipital discharges, multifocal	Cortical atrophy, hypomyelination and elevated lactate on spectroscopy; cerebellar atrophy in some cases.
<i>RARS2</i> [20]	Developmental delay, developmental regression, lactic acidosis	Infancy	Infantile DEE. Eye blinking and clonic movements, myoclonic and multifocal seizures	EEG showing temporal focus, then diffuse polyspike and slow	Diffuse cortical atrophy, hypomyelination. Pontocerebellar hypoplasia is not universal
<i>FAKS2</i> [21]	Hypertrophic non-obstructive cardiomyopathy, mitochondrial encephalopathy, hypertonnia, lactic acidosis	Infancy	Focal seizures (facial muscle clonus and apnoea), infantile DEE	EEG burst suppression	Thin CC, cerebellar hypoplasia, signal change in the brainstem, insula and dentate nuclei
<i>WARS2</i> [22,23]	Early developmental delay, ataxia, microcephaly, lactic acidosis	Neonatal to infancy	West syndrome, infantile DEE, seizures causing childhood death	Background slowing, multifocal spikes, intermittent rhythmic delta	Cortical atrophy, delayed myelination, patchy white matter hyperintensities

*Abbreviations used in Table 1 CC: Corpus Callosum, MTN: Charcot-Marie-Tooth neuropathy, Early infantile DEE: Early infantile developmental and epileptic encephalopathy, GTCS: Generalised tonic-clonic seizures, NCSE: Nonconvulsive status epilepticus, PCH: Pontocerebellar hypoplasia, PLEDs: Paroxysmal lateralised epileptiform discharges. \* Dominant mutations in AARS1 are associated with Charcot-Marie-Tooth neuropathy whereas diallelic autosomal recessive changes are described with EIEE29*

**Table 2 : Features of *QARSI*-associated epileptic encephalopathy**

Clinical features	In our case	In literature [1]
<b>NEUROLOGICAL FEATURES</b>		
Motor delay	Walk independently at 3 years	86% (18/21) Not walking
Speech	Babbling at 3 years	90% (18/20) No speech
Visual interaction	Fixes, follows horizontally	67% (8/12) No visual interaction
Stereotypies	Hand stereotypies at 6/52	75% (6/8) Hand stereotypies
<b>GROWTH PARAMETERS</b>		
Birth weight	2278 g (0.1 centile, -2.2 SD)	15% (3/20) Weight < -2 SD
Birth head circumference	32 cm (0.1 centile, -3.0 SD)	65% (11/17) HC < -2 SD
<b>EPILEPSY</b>		
Antiepileptic medications	Levetiracetam ineffective. Well controlled on carbamazepine and clobazam	79% (15/19) Pharmacoresistant epilepsy
Seizure onset	7 months	60% (9/15) Onset D1 life; Onset not recorded in 3/15.
Seizure type	Febrile and afebrile focal seizures Status epilepticus	Multiple seizure types reported in literature: myoclonic, GTCS, tonic, spasms, focal with secondary generalisation, focal seizures, epileptic encephalopathy
<b>MRI NEUROIMAGING</b>		
Thin corpus callosum	Yes	79% (15/19) Corpus callosal hypoplasia
Cortical atrophy	Yes	89% (16/18) Cortical atrophy
Cerebellar atrophy	No	61% (11/18) Cerebellar atrophy
Hypomyelination	No	88% (15/17) Hypomyelination





**Supplementary Figure 1.** Magnetic Resonance Imaging of QARS1 case performed at 7 months (A, B) and 18 months of age (C, D). Thin corpus callosum seen on midline sagittal T1-weighted imaging (A). T2 axial imaging showing progressive cerebral atrophy, prominent sulci and ventricular dilation (B, D). Corresponding T1 axial image is also shown (C).

#### **DETAILED DESCRIPTION OF FUNCTIONAL TESTING**

QARS1 wild-type (WT), c.1132C>T, p.(Arg378Cys) and c.1574G>A, p.(Arg525Gln) variants were expressed in *E. coli* from a pET-15b plasmid with a N-terminal 6-His tag to aid purification. *In vitro* aminoacylation activity of purified recombinant QARS1 p.Arg378Cys and p.Arg525Gln was measured

by the incorporation of [<sup>14</sup>C]-glutamine into the cognate tRNA<sup>Gln</sup> substrate as described by Zhang [4]. QARS1 p.Arg378Cys showed no more than a two-fold reduction in aminoacylation efficiency compared to the wild-type QARS1, mainly due to a modest reduction in catalytic rate ( $k_{cat}$ ), indicating the variant slightly hinders the transfer of the glutaminy moiety to the tRNA (supplementary table 1). QARS1 p.Arg525Gln did not have a significant effect on *in vitro* aminoacylation. However, the solubility of QARS1 p.Arg525Gln and p.Arg378Cys were drastically reduced compared to WT QARS1 in a mammalian expression system (Supplementary figure 2). While 24% of WT QARS1 was found in the soluble fraction, about 3% of p.Arg378Cys and 4% of p.Arg525Gln could be detected in the soluble fraction. This observation suggests that the amount of mutant proteins would be reduced about 8-fold compared to the WT in cells.

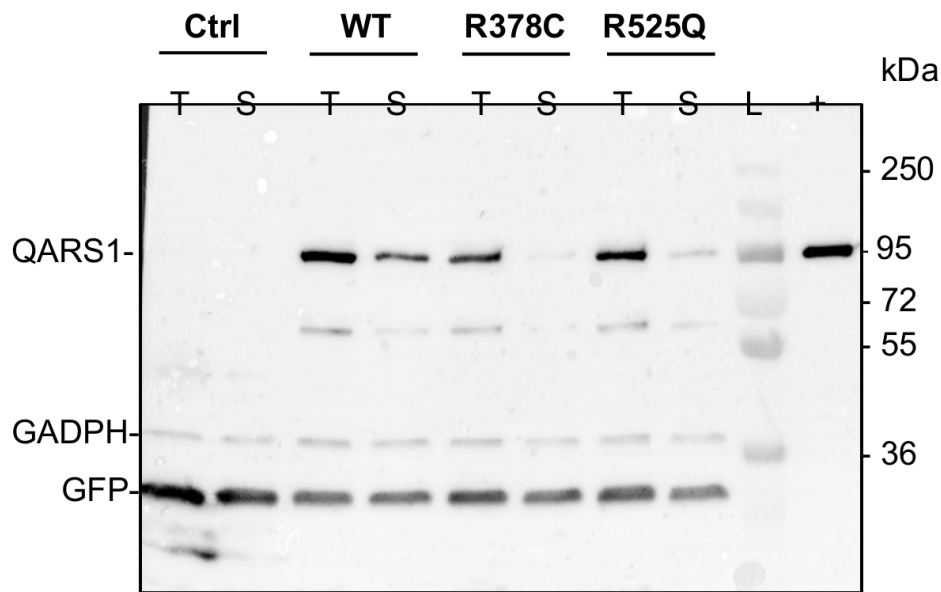
**Supplementary table 1: Kinetic parameters for aminoacylation of human cytosolic tRNA<sup>Gln</sup> transcripts by wild-type (WT) and variant QARS1.**

QARS variant	$K_m$ ( $\mu\text{M}$ )	$k_{cat}$ ( $\text{min}^{-1}$ )	$k_{cat}/K_m$ ( $\text{min}^{-1}\mu\text{M}^{-1}$ )	Loss of catalytic efficiency*
catalytic efficiency				
WT	1.5 (0.1)	11.2 (1.2)	7.5 (0.5)	1.00
p.Arg378Cys	1.7 (0.3)	6.4 (1.0)	3.8 (0.4)	2.00
p.Arg525Gln	1.5 (0.1)	10.5 (0.3)	7.1 (0.2)	1.05



\*Loss of catalytic efficiency calculated as a fold change relative to WT. 0.25 to 2.5  $\mu\text{M}$  tRNA<sup>Gln</sup> transcripts were tested in the presence of 10 to 20 nM WT or variant QARS1. Values are shown as mean (SEM).

**Supplementary figure 2 : Effect of individual variants on QARS1 solubility.**



Immunoblot showing the soluble QARS1 fraction (S) obtained after centrifugation of the total protein extract (T): Mammalian Cos7 cells were co-transfected with an empty pcDNA3.1 vector (Ctrl), pcDNA3.1 encoding wild-type QARS1 (WT) or variant QARS1 (R378C and R525Q), and GFP. The membrane was probed with both anti-His Tag (QARS1) and anti-GFP antibodies (GFP). GADPH was used as a loading control. (L) shows the protein ladder, (+) shows a positive control recombinant QARS1 purified from *E. coli* cells. Image is representative of four individual immunoblots.