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1

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

4

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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^{Gln} 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 3rd 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 (<3rd centile) and small
94 head circumference 42 cm (<10th 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, <3rd centile) and persisting low weight for stature (13 kg, 105

114 cm, <3rd 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 *QARS1* (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 *QARS1* [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 *QARS1* without epilepsy had
195 homozygous missense mutations in the catalytic domain [2]. Functional testing of the *QARS1*
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^{Gln} aminoacylation and may therefore contribute to the observation of some
207 milder phenotypic features.

208

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CONFLICT OF INTERST DISCLOSURES

The authors have no conflicts of interest to declare.

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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 (EIBE29/DEE29)	Slow background, excess sharp waves. Spasm with electrodecrement but no hypsarrhythmia	Progressive atrophy – cortical, white matter, cerebellar and brainstem, hypomyelination
<i>KARS</i> (<i>both 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,

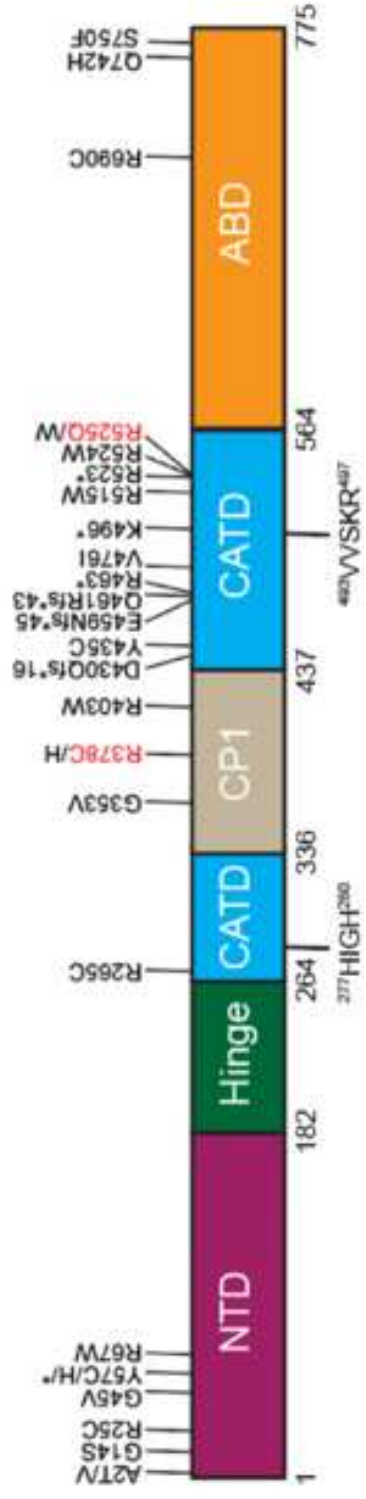
	hypotonia		multifocal, infantile DEE.	or focal	CC hypoplasia, simplified gyral pattern, thin corpus callosum
<i>CARS2</i> [15]	Developmental delay; cognitive decline, visual and hearing impairment, movement disorder	Infancy or childhood	Progressive myoclonic epilepsy, GTCS, infantile DEE	Multifocal discharges – 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 nd 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,

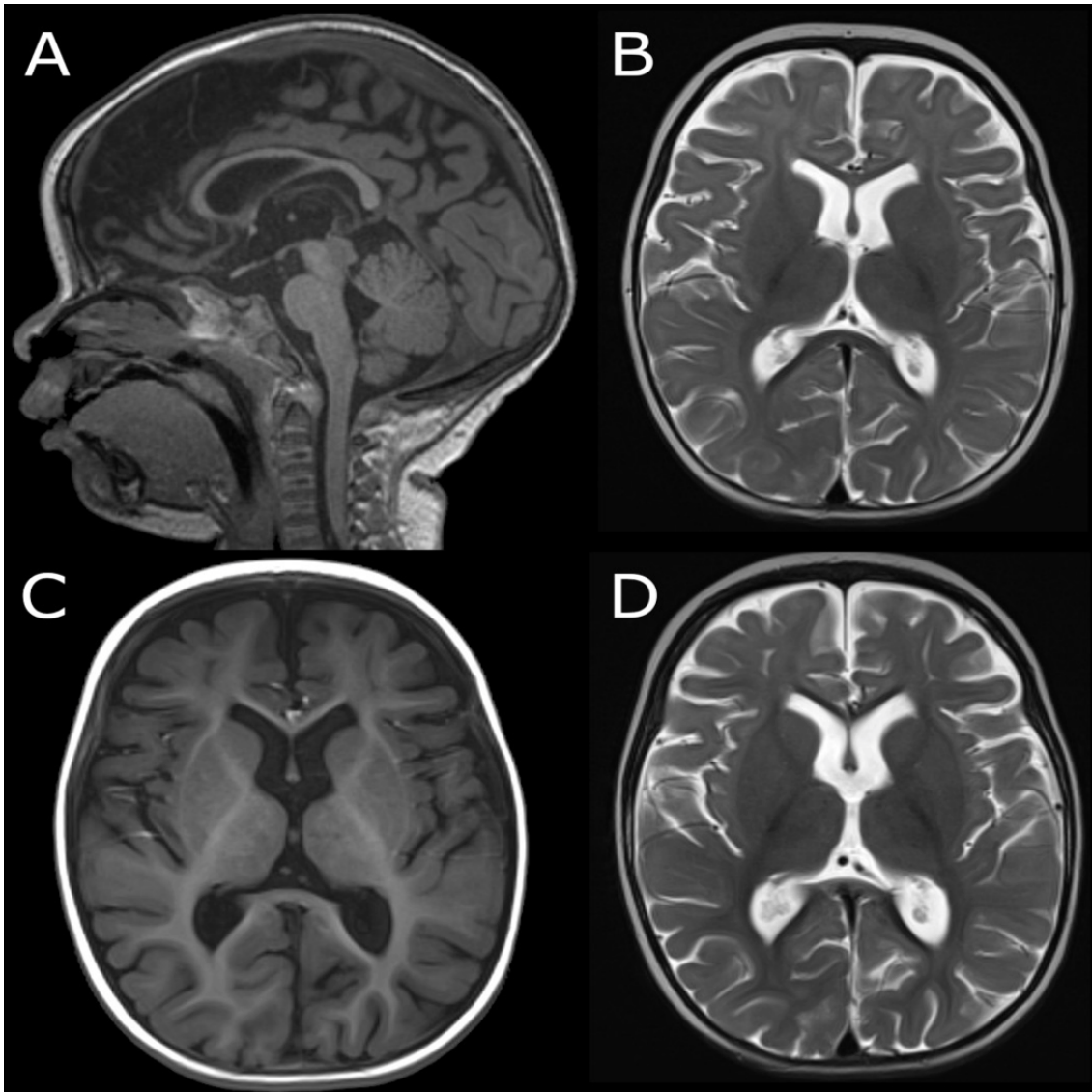
[18,19]	cerebellar atrophy, Leigh Syndrome, Alpers Syndrome, myopathy	childhood	GTCS, status epilepticus, hemiconvulsion–hemiplegia–epilepsy	seizures, hypersarrhythmia, burst-suppressed	leukodystrophy, Leigh syndrome like changes
<i>PAKS2</i> [19]	Developmental delay, progressive microcephaly, Alpers 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, hypertonia, 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]
NEUROLOGICAL FEATURES		
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
GROWTH PARAMETERS		
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
EPILEPSY		
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
MRI NEUROIMAGING		
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

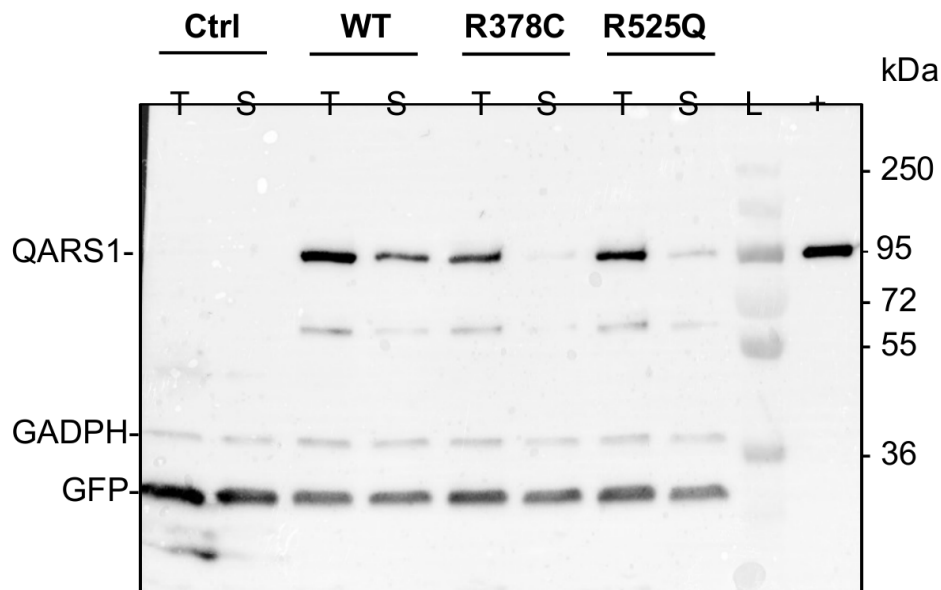
by the incorporation of [¹⁴C]-glutamine into the cognate tRNA^{Gln} substrate as described by Zhang [4]. QARS1 p.Arg378Cys showed no more than a two-fold reduction in aminoacylation efficiency compared to the wild-type QARS1, mainly due to a modest reduction in catalytic rate (k_{cat}), indicating the variant slightly hinders the transfer of the glutamyl 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^{Gln} transcripts by wild-type (WT) and variant QARS1.

QARS variant	K_m (μM)	k_{cat} (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 μM tRNA^{Gln} 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.