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# Defining and expanding the phenotype of *QARS*-associated developmental epileptic encephalopathy

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## Abstract

### Objective

The study is aimed at widening the clinical and genetic spectrum and at assessing genotype-phenotype associations in *QARS* encephalopathy.

### Methods

Through diagnostic gene panel screening in an epilepsy cohort, and recruiting through Gene-Matcher and our international network, we collected 10 patients with biallelic *QARS* variants. In addition, we collected data on 12 patients described in the literature to further delineate the associated phenotype in a total cohort of 22 patients. Computer modeling was used to assess changes on protein folding.

### Results

Biallelic pathogenic variants in *QARS* cause a triad of progressive microcephaly, moderate to severe developmental delay, and early-onset epilepsy. Microcephaly was present at birth in 65%, and in all patients at follow-up. Moderate (14%) or severe (73%) developmental delay was characteristic, with no achievement of sitting (85%), walking (86%), or talking (90%). Additional features included irritability (91%), hypertonia/spasticity (75%), hypotonia (83%), stereotypic movements (75%), and short stature (56%). Seventy-nine percent had pharmacoresistant epilepsy with mainly neonatal onset. Characteristic cranial MRI findings include early-onset progressive atrophy of

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## Glossary

**ABD** = anticodon-binding domain; **AED** = antiepileptic drug; **CATD** = catalytic domain; **CPI** = connecting peptide 1; **ID** = intellectual disability; **mRNA** = messenger RNA; **NTD** = N-terminal domain; **tRNA** = transfer RNA.

cerebral cortex (89%) and cerebellum (61%), enlargement of ventricles (95%), and age-dependent delayed myelination (88%). A small subset of patients displayed a less severe phenotype.

## Conclusions

These data revealed first genotype-phenotype associations and may serve for improved interpretation of new *QARS* variants and well-founded genetic counseling.

*QARS* (OMIM #615760) encodes a glutamyl-tRNA synthetase responsible for linking tRNA with amino acids.<sup>1</sup> This aminoacylation is essential for translation of mRNA to proteins.<sup>2</sup> *Drosophila* studies have shown that *QARS*-encoded synthetase is crucial for termination of fine branching structures at dendrites and axons during early brain development.<sup>1</sup> *qars*<sup>-/-</sup> zebrafish have smaller eyes and brains, progressive neural cell death, and exhibit uncoordinated movements.<sup>1</sup> Humans have 37 genes encoding different aminoacyl-tRNA synthetases. So far, pathogenic variants have been found in more than 22,<sup>3-9</sup> including *AARS* (encephalopathy, refractory early-onset epilepsy, microcephaly, growth retardation, and peripheral neuropathy)<sup>10</sup> and *VARS* (developmental delay, epileptic encephalopathy, and primary or progressive microcephaly).<sup>4,5</sup>

*QARS* was first connected to disease in 2014<sup>1</sup> in 4 individuals from 2 unrelated families with severe epilepsy of neonatal onset, severe intellectual disability (ID), and progressive microcephaly with cerebral-cerebellar atrophy. They were found to have compound heterozygous variants in *QARS*. Cellular in vitro studies of the disease-associated variants have shown a loss of function of the glutamyl-tRNA synthetase, probably due to misfolding and aggregation of the protein. Further reports confirmed both nonsense and missense variants in *QARS* as pathogenic.<sup>2,11-15</sup> The phenotype has been generally consistent with progressive microcephaly, cerebral atrophy, severe ID, and drug-resistant epilepsy. However, among the 12 published patients, a small subgroup with homozygous deleterious variants seems to display a milder phenotype. With this study, we aimed to further define the clinical spectrum associated with pathogenic *QARS* variants and assess first genotype-phenotype associations.

## Methods

New patients were ascertained through diagnostic screening of an epilepsy cohort, using the EPIDASD (Epilepsy, Intellectual disability and Autism Spectrum Disorders) gene panel at Amplexa Genetics A/S (NM\_005051.2) and whole-genome sequencing performed as part of the Undiagnosed Diseases Program—Western Australia.<sup>16</sup> Homozygous variants were reported when

present in heterozygous form in <100 controls and not seen in homozygous form in controls. All findings were confirmed by Sanger sequencing. All variants were detected in heterozygous state in the parents to prove for biallelic variants in the index patients. Further patients were recruited through an international network of clinical geneticists and physicians working with severe epilepsy and GeneMatcher.<sup>17</sup> Available cranial MRI data sets were reviewed by a single neuroradiologist (C.R.).

Clinical information including data on phenotype, epilepsy, EEG, neuroimaging, and treatment response was evaluated for all the patients included in the article. Seizures and epilepsy syndromes were classified according to the International League Against Epilepsy.<sup>18,19</sup> A PubMed search using the search term “*QARS*” was performed to obtain information on all previously published cases. Additional information was added by the referring physicians on 2 previously reported patients.<sup>1</sup>

Variants were assumed pathogenic or likely pathogenic, if they were homozygous or compound heterozygous, non-synonymous, splice site altering, or frameshift changing. Variants were tested with prediction software (PolyPhen-2, SIFT, and MutationTaster) for probable pathogenicity. The gnomAD database was used to check allele frequency in healthy controls. Variants were classified pathogenic or likely pathogenic according to the recommendations for interpretation of sequence variants published by the American College of Medical Genetics and Genomics.<sup>20,21</sup>

Variants were modeled in silico, based on the crystal structure of the intact human GlnRS at 2.4 Å resolution.<sup>22</sup> The model was analyzed using the program COOT.<sup>23</sup> Point mutations were introduced in silico using the standard rotamer library of COOT and analyzed for the appearance of steric clashes or repulsive forces of side chains. Splice site variants gave an unknown protein product and could not be modeled. Figures were prepared using the program Pymol (pymol.org). Upon request, access to data will be given to those eligible for this.

(1) This study was approved by the local ethical authorities of University Hospital Medical Center Leipzig and University Center for Rare Diseases Leipzig. (2) Genetic analysis in the

patients reported in this study was performed through diagnostic screening. No additional experiments were performed. For retrospective analysis and genotype-phenotype assessment of genetic and clinical data, approval from the ethical standards committee was received. (3) All legal parents (all patients were minors) provided written informed consent for research. (4) Authorization has been obtained for disclosure (consent-to-disclose) of any recognizable persons in photographs or other information in this article. (5) This study is not included in a public trials registry. Data Availability Statement: All relevant clinical and genetic data of the patients in this study are shared and provided in the article, including supplemental material.

## Results

We collected a cohort of 22 patients (10 new patients and 12 published patients) from 18 families with recessive pathogenic biallelic variants in *QARS*. The cohort includes 11 female and 11 male patients; mean age at follow-up was 59 months (range 5 months to 10 6/12 years). Of note, patient 2 had a brother with a similar phenotype who died before genetic testing could be performed (for details of the brother, see supplemental data appendix e-1, [links.lww.com/NXG/A199](https://links.lww.com/NXG/A199)). Table 1 provides clinical, radiographic, and epilepsy characteristics of the new patients (for data on all 22 patients, see supplemental data table e-1, [links.lww.com/NXG/A195](https://links.lww.com/NXG/A195); for epilepsy phenotype, table e-2, [links.lww.com/NXG/A196](https://links.lww.com/NXG/A196)). Two patients (3 and 21) were children of consanguineous parents, 3 patients from 2 families (16, 17, and 18) were Ashkenazi Jews. The others were children of unrelated healthy parents.

### **QARS-associated phenotype in the cohort of 22 patients**

All patients were born after uncomplicated pregnancies. Microcephaly was present at birth in 65% (11/17 patients) and in all patients with available measurements (19/19 patients) at follow-up (range  $-2.1$  SD to  $-17.2$  SD). Five patients were diagnosed with microcephaly already in utero (1, 2, 7, 8, and 22). Regarding overall growth, 21% (3/14 patients) had short birth length, and 56% (9/16 patients) had short stature at follow-up (range  $-2.2$  SD to  $-5.6$  SD). At follow-up, 13% (2/15) showed dystrophy, and 8 patients were reported with a gastrostomy tube. All patients showed delay of motor and speech development. Seventy-three percent (16/22 patients) had severe developmental delay, and 14% (3/22 patients) had moderate delay. Eighty-five percent (17/20 patients) showed no achievement of unassisted sitting, 86% (18/21 patients) no achievement of unassisted walking, and 90% (18/20 patients) were reported with no speech at follow-up.

Hypotonia was reported in 83% (10/12 patients), mainly initially. Additional features included hypertonia/spasticity in 75% (6/8 patients) and stereotypic movements in 75% (6/8 patients). Ten patients displayed periods of intermittent

irritability/extreme agitation, with crying, increased sweating, and involuntary movements. Sixty-seven percent (8/12 patients) were reported with no visual interaction, and 6 patients had nystagmus.

Eighty-six percent (19/22 patients) were reported with seizures. Of 11 patients with epilepsy, EEG data were available. Eighty percent were reported with seizure onset within the first 4 days of life (total range 1st hour of life to 5.5 months). Epilepsy syndrome classification remained difficult due to the high variability of seizure types, duration, frequency, and EEG abnormalities. Often, seizures were reported as frequent and/or long lasting with variable seizure types including focal, multifocal, generalized, and myoclonic seizures. Seven patients had recurrent status epilepticus, some of which lasted several hours. At epilepsy onset, EEG showed various patterns, such as focal, bilateral, multifocal epileptic discharges, hypsarrhythmia, and even a “focal migrating seizure-like” pattern (Table 1, e-1, [links.lww.com/NXG/A195](https://links.lww.com/NXG/A195), and e-2, [links.lww.com/NXG/A196](https://links.lww.com/NXG/A196)). At follow-up, seizure semiology had not changed. Twenty-eight percent (5/18 patients) were reported with seizures sensitive to fever or infections, with increased seizure frequency under those circumstances. Several antiepileptic drugs (AEDs) were tried; 2 patients responded partly to vigabatrin, and 1 had a positive response to ketogenic diet. Seventy-nine percent of the patients had pharmacoresistant epilepsy (15/19 patients). Of interest, 27% (6/22 patients) were reported with no seizures or were seizure free at follow-up. Patient 3 and patients 16, 17, and 18 had no epilepsy. Patient 4 only had seizures during a course of meningoenzephalitis, and patient 21 was reported with febrile seizures only.<sup>11</sup>

Cranial MRI was performed in almost all patients (21/22 patients with data). Figure 1 displays characteristic neuroimaging features in the patients. Characteristic features include cortical atrophy (89%, 16/18 patients) and cerebellar atrophy (61%, 11/18 patients), cortical structural anomalies (56%, 10/18 patients) including simplified gyral pattern and pachygyria, hypoplasia of the corpus callosum (79%, 15/19 patients), delayed myelination (88%, 15/17 patients), ex vacuo enlargement of internal and external liquor spaces, and widening of subarachnoid spaces (95%, 18/19 patients). No abnormalities of the brain stem were reported. Progressive atrophy is most evident in cranial imaging of patient 2 with almost 9-year timespan to follow-up. In this patient, progressive atrophy is evident in the supratentorial brain regions with reduced white matter bulk and pachygyria and of the corpus callosum, which is described as hypoplastic at age 14 weeks and completely atrophic at age 9 years. One reported patient (patients 21)<sup>11</sup> was described with no structural defaults on MRI.

Additional features included hepatosplenomegaly or elevated liver enzymes in 2 patients and ventricular septum defect, hydronephrosis with unilateral hydroureter, and tracheomalacia as single reports. The patients showed mild facial dysmorphism, including long nose with upturned nose tip,

**Table 1** Genetic and clinical data of 10 new patients with biallelic variants in *QARS*

Patient ID	1	2	3	4	5	6	7	8	9	10
Family ID	1	2	3	4	5	6	7	8	9	10
Sex	Female	Female	Female	Male	Female	Male	Female	Female	Female	Male
Ethnicity	European	European	Turkish	n.r.	European	n.r.	American/Hispanic	European	European	European
<b>Measurements at birth</b>										
Weeks of gestation	38	38 + 2	38	38 + 2	37 + 6	39	36 + 4	36 + 2	38	39
Weight	-2.3 SD	-1.2 SD	1.0 SD	-2.0 SD	0.7 SD	-0.9 SD	-1.9 SD	-1.9 SD	-0.4 SD	-2.0 SD
Length	-1.9 SD	-3.6 SD	1.1 SD	n.r.	0.2 SD	n.r.	-3.5 SD	-2.3 SD	-0.2 SD	-0.4 SD
OFC	-4.9 SD	-3.0 SD	-2.5 SD	n.r.	-0.9 SD	n.r.	-3.2 SD	-3.4 SD	-3.3 SD	-2.5 SD
<b>Measurements at follow-up</b>										
Age at follow-up	—	9 4/12 y	10 6/12 y	5 1/12 y	4 6/12 y	2 6/12 y	2 6/12 y	2 2/12 y	5 mo	9 y
Weight	—	-0.8 SD	-0.6 SD	-4.3 SD	Mean	0.5 SD	1.7 SD	-3.2 SD	-0.8 SD	-4.4 SD
Length	—	-2.8 SD	-1.3 SD	-4.2 SD	-2.2 SD	0.4 SD	0.3 SD	-4.3 SD	-0.7 SD	-3.5 SD
BMI	—	0.9 SD	0.2 SD	-1.2 SD	1.7 SD	0.3 SD	2.0 SD	-0.2 SD	-0.5 SD	-2.1 SD
OFC	—	-11.1 SD	-2.4 SD	-3.1 SD	-2.4 SD	-3.5 SD	-6.4 SD	-11.9 SD	-10.3 SD	-7.0 SD
<b>Neurologic features</b>										
Motor development	Severe delay	Severe delay	Severe delay	Mild delay	Mild delay	Severe delay	Severe delay	Severe delay	Severe delay	Severe delay
Sitting (at age)	No	No	No	n.r.	10 mo	21 mo	No	No	Too young	No
Walking (at age)	No	No	No	2 y	4 2/12 y	No	No	No	Too young	No
Hypotonia	n.r.	Initially hypotonia	n.r.	n.r.	No	n.r.	Severe	Initially hypotonia	Yes	No
Hypertonia/spasticity	n.r.	At 9 y spasticity	n.r.	n.r.	No	n.r.	At 2 y contractures	At 2 y severe spasticity	No	Hypertonia
Speech development	No speech	No speech	No speech	Speaks sentences	Vocalizes, no words	No speech	No speech	No speech	Too young	No speech
Stereotypies/involuntary movements	n.r.	Yes	n.r.	n.r.	Yes	n.r.	Yes	No	No	Dystonia At 3 y
Episodes of irritability	Intermittent irritability	Intermittent irritability	n.r.	n.r.	Rare	n.r.	Intermittent severe irritability	Intermittent severe irritability	Intermittent irritability	No

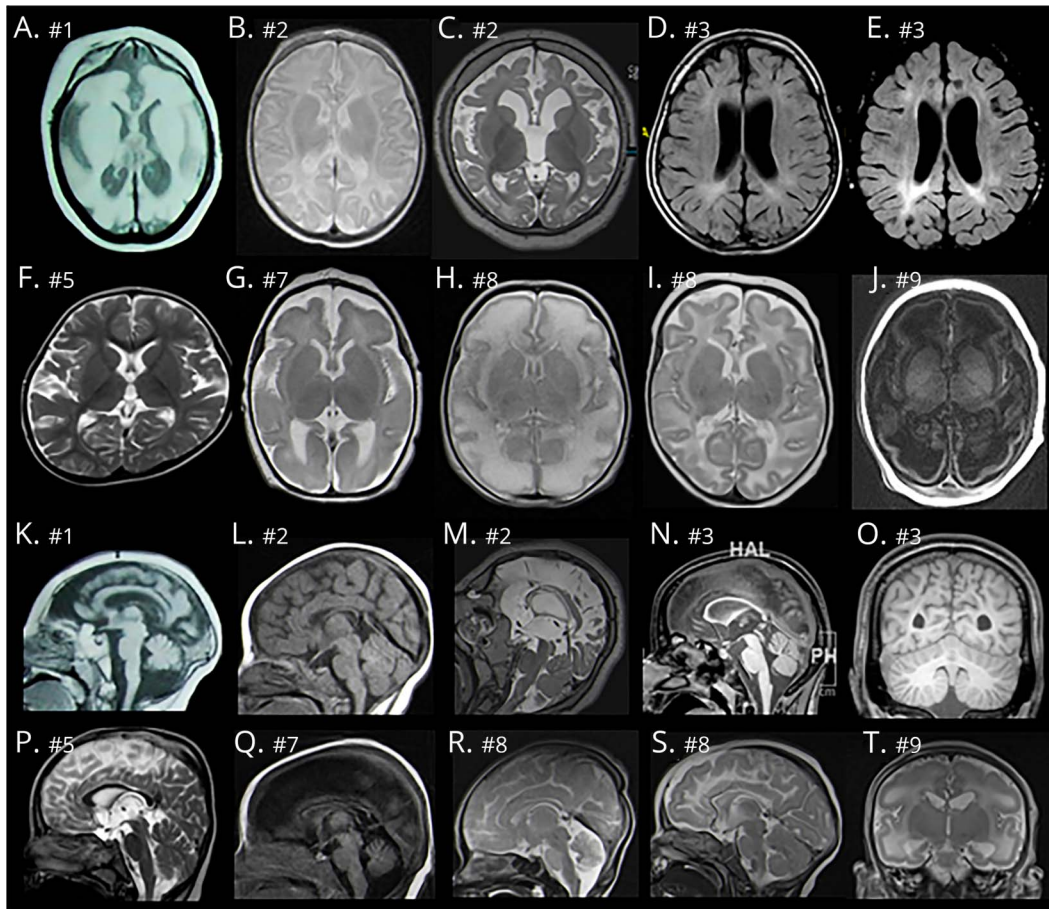
Continued

**Table 1** Genetic and clinical data of 10 new patients with biallelic variants in *QARS* (continued)

<b>Visual interaction</b>	n.r.	No	n.r.	n.r.	Yes	n.r.	No	No	No	No
<b>Epilepsy</b>										
<b>Onset</b>	1 h after birth	1 h after birth	No epilepsy	n.r.	5.5 mo	2.5 mo	2 h after birth	3 h after birth	1 h after birth	4th day of life
<b>Type</b>	Myoclonus, tonic-clonic seizures	Focal seizures with generalization	—	Encephalopathy during meningoencephalitis, seizure free at follow-up	Focal seizures and spasms	Tonic seizures	Myoclonic seizures	Focal and generalized seizures	Myoclonic seizures, status epilepticus	Multifocal clonic and tonic seizures
<b>Effect of antiepileptic therapy</b>	Pharmacoresistant	Pharmacoresistant	—	—	Pharmacoresistant	Pharmacoresistant	Pharmacoresistant	Pharmacoresistant	Pharmacoresistant	Pharmacoresistant
<b>cMRI</b>										
<b>Age at cMRI</b>	1 8/12 y	14 wk/9 4/12 y	10 y	n.r.	6 mo/27 mo	n.r.	0 wk/5 wk	1 wk/19 wk	5 d	18 mo/+ follow-up
<b>Corpus callosum anomalies</b>	No	At 14 wk hypoplasia, at 9 y complete atrophy	Hypoplasia	Hypoplasia	No	Hypoplasia	Hypoplasia	Hypoplasia	No	Hypoplasia
<b>Delayed myelination</b>	No	Hypomyelination	Hypomyelination	Hypomyelination	No	Hypomyelination	Too young	Hypomyelination	No myelination central and subcortical	Hypomyelination
<b>Cortical anomalies</b>	White matter atrophy, cortical dysplasia	Progressive atrophy of supratentorial brain, reduced white matter, pachygyria	White matter atrophy	n.r.	Moderate atrophy	White matter atrophy	Progressive atrophy of supratentorial brain, reduced white matter, pachygyria	Mild atrophy of cerebral cortex, reduced white matter, pachygyria	Symmetric cortical pachygyria	Progressive atrophy of the cerebral cortex
<b>Cerebellar anomalies</b>	Atrophy of the vermis cerebelli	Mild atrophy of cerebellum	Normal	n.r.	Moderate atrophy of the vermis cerebelli	Atrophy of the vermis cerebelli	Normal	Normal	Normal	Normal
<b>Brain stem</b>	Normal	Normal	Normal	n.r.	Normal	Normal	Normal	Normal	Normal	Normal
<b>Enlarges cerebral ventricles</b>	Yes	Yes	Yes	n.r.	Yes	Yes	Yes	Yes	Yes	Yes

Abbreviations: BMI = body mass index; cMRI = cranial MRI; n.r. = not reported; OFC = occipitofrontal head circumference.

**Figure 1** Spectrum of structural brain anomalies in *QARS*-associated microcephaly



MRI data sets of the new patients 1 (1a,b), 2 (2a,c at 14 weeks; 2b,d at 94/12 years), 3 (3a at 6 years; 3b,c,d at 10 years), 5 (5a,b at 27 months), 7 (7a,b at 5 weeks), 8 (8a,c at 1 week; 8b,d at 19 weeks), and 9 (9a,b). Cortical structural anomalies: axial magnetic resonance (MR) images show generalized atrophic supratentorial cerebral volume loss and enlargement of ventricles with progressive atrophy (2 and 8), severe pachygyria (1, 2, 7, 8, and 9), and moderate pachygyria (3 and 5). Trim and flair sequences in patient 3 (3a,b) demonstrate progressive cerebral demyelination. Corpus callosum: sagittal MR images show a spectrum of anomalies of the corpus callosum with relative thinning (3, 6, 7, and 8) and complete atrophy (2, 2d at follow-up).

but overall no recognizable dysmorphic facial phenotype (figure 2).

### **QARS genotype**

Patients in this cohort were either compound heterozygous (17/22) or homozygous (5/22 patients) for *QARS* variants. Most variants located in the N-terminal domain (NTD) and the catalytic domain (CATD), and only 2 variants located in the anticodon-binding domain (ABD). Three variants were recurrent: the variant p.(Gly45Val) was detected in patient 5 and siblings 13/14, the variant p.(Tyr57His) was seen in 2 pairs of siblings (11/12, 19/20), and the splice variant c.2084+2\_2084+3del was seen in patients 6 and 22. Two hot spots were found at position 57 (p.(Tyr57\*), p.(Tyr57Cys), p.(Tyr57-His)) and at position 378 (p.(Arg378Cys), p.(Arg378His)). All other variants were single reports (figure 3).

In silico models of the effects of the variants at the protein level were performed (figure e-1, [links.lww.com/NXG/A198](https://links.lww.com/NXG/A198)). In patient 1, p.(Arg67Trp), a surface variant, may interrupt so far

unknown interactions, for instance with protein ligands. The crystal structure of this variant is unknown. The variant p.(Gln461Argfs\*43) truncates *QARS*, and thus, the protein lacks part of the CATD and the entire ABD, making tRNA binding impossible. For patient 2, the variant p.(Arg378Cys) is involved in stabilizing the fold of the loop in the connecting peptide 1 (CP1) domain, which is involved in interaction with tRNA. This amino acid replacement of arginine to cysteine likely causes minor folding or interaction defects in the CP1 domain. In the variant p.(Arg523\*), the ABD is missing, which affects tRNA binding. In patient 3, the same p.(Arg378His) change observed as in patient 2 was detected in homozygous state, causing minor folding or interaction defects in the CP1 domain. For patient 4, the variant p.(Glu459Asnfs\*45) truncates *QARS*, causing it to lack part of CATD and the entire ABD, and tRNA binding is abolished, resulting in a clear loss of function. The p.(Tyr435Cys) variant is involved in a number of hydrophobic interactions and hydrogen bonds stabilizing the fold of the surface loop at the border between CP1 domain and CATD, which are involved in interaction with tRNA. Replacement of

tyrosine by cysteine likely affects folding and hence tRNA binding. In patient 5, p.(Gly45Val) is a surface variant that may interrupt other, yet unknown, interactions, for instance with protein ligands. The NTD fold might be locally affected.<sup>22</sup> In patient 6, the variant p.(Arg265Cys) is located in the interface between NTD, CATD and the Hinge domain. Local structural rearrangement may be the consequence of this variant. For patient 7, the p.(Gly14Ser) variant is unlikely to affect folding or tRNA interaction; however, as it is a surface variant, it may interrupt other still unknown interactions, possibly with protein ligands, as is the case with p.(Gly45Val). The variant p.(Arg525Trp) might result in a changed orientation of ABD and thus affect tRNA binding. For patient 8, the variant p.(Arg524Trp) is expected to lead to full protein expression, but misfolding and changed orientation of ABD may affect tRNA binding. The variant p.(Arg690Cys) may affect the local folding of human GlnRS. However, how this influences tRNA binding is unknown. In patient 9, variant p.(Tyr57\*) is located in the N-terminal part of the NTD, which causes only the first third of the domain to be expressed and a large C-terminal portion missing. No functional protein is expressed. Regarding the variant p.(Tyr57Cys), it is unlikely for a single amino acid variation to cause drastic conformational changes, but according to Ognjenović et al.,<sup>22</sup> variants in the NTD affect tRNA positioning and binding and decrease catalytic activity and stability of human GlnRS. These mutants will be unable to form a productive complex with tRNAGln. In patient 10, the variant p.(Arg403Trp) is located in the CP1 domain. The hydrophilic side chain of arginine is involved in a number of hydrogen bonds with the surrounding residues, stabilizing the fold of the CP1 domain. Its replacement by the larger aromatic tryptophan will cause local disturbance of interactions and likely misfolding. This might affect interaction with potential protein ligands. The surface variant p.(Ala2Thr) in the NTD is unlikely to affect fold or tRNA interaction but may interrupt other, yet unknown, interactions.

We also modeled previously published variants. In siblings 11 and 12,<sup>1</sup> the variant p.(Tyr57His) was described for patient 9. The variant p.(Arg515Trp) is expected to lead to full protein expression, but folding defects and changed orientation of ABD may affect tRNA binding. In patients 16, 17, and 18,<sup>14</sup>

the homozygous p.(Val476Ile) variant, located in the CATD, may cause local disturbance of interactions and minor misfolding. In addition, the p.(Gly353Val) variant in patient 21 is likely to cause minor effects on folding, which might affect protein-protein interaction. This variant is located in the solvent exposed loop of the CP1 domain.

## Discussion

In this work, we expand the known population of 12 individuals with biallelic pathogenic variants in *QARS* by 10 new patients and further delineate the *QARS*-associated clinical spectrum. Although most patients show a homogeneous phenotype, some patients (mainly the patients with a homozygous variant) display a milder presentation with better outcome.

Microcephaly was present in utero, at birth, or became evident during the early infantile period in all patients, being extremely severe with up to  $-17$  SD and progressive. It seems that progression is worst in early infancy. Patients below or around 2 years of age have progression of their microcephaly with 4 to 7 SD per year. Older patients have a moderate progression, albeit severe, around 2 to 4 SD per year. Part of this, or all, will probably be due to the fact that the normal brain grows the most during early childhood. In addition to microcephaly, some dysmorphic facial features can be present, including a long nose with upturned nose tip and dystopia canthorum. Developmental delay is evident in all patients, with severe delay in the patients with compound heterozygous variants (except patients 4 and 5) and mild to moderate delay in those with homozygous variants who also display mild progression in their speech and motor development, with some even learning to walk (patient 16 at age 10 years). Neurologically, hypotonia is a common feature, not only in the neonatal period but also in childhood. Furthermore, feeding difficulties seem to be a common problem, with several patients requiring gastrointestinal tube feeding. Of interest periods of irritability or agitation with autonomic features were reported in several patients. The background for these episodes is not clarified, and might be

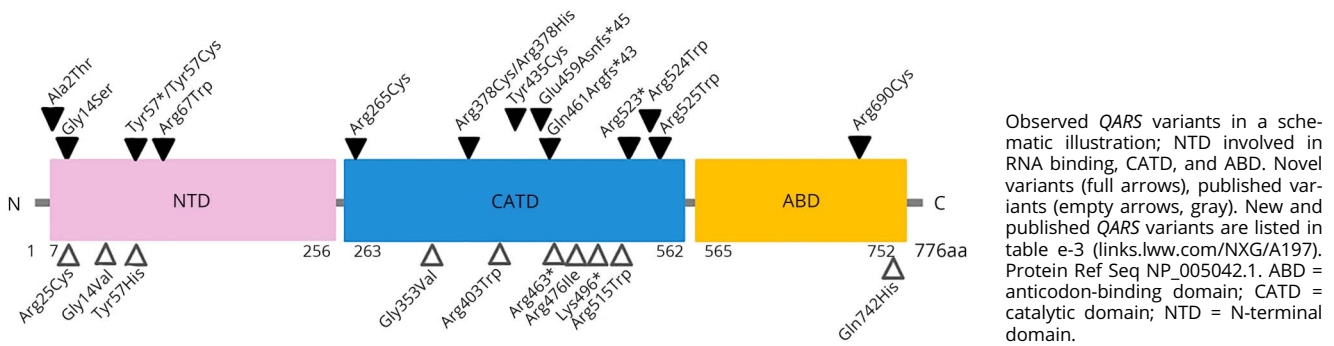
**Figure 2** Photographs of patients 2, 7, 9, and 10



Patients 2, 7, 9, and 10 show mild facial dysmorphism, including narrow foreheads, as would be expected in children with microcephaly, sparse eyebrows, long nose with upturned tip of the nose, dystopia canthorum, dysconjugate gaze, full cheeks, and downturned corners of the mouth.



**Figure 3** QARS domains and distribution of variants



underestimated, because those who displayed them were in their early childhood, and the mean age of this cohort is still fairly low. Agitation is a cluster of behaviors that can be found in a number of psychiatric disorders<sup>24</sup> and have overlap with hyperactivity and restlessness. The agitation described in the patients of this cohort could be classified as aggressive physical and verbal. The underlying mechanisms of agitation are not yet clarified, but it is suggested that agitation runs through the same neurologic systems as movement disorders. This could be part of the explanation of why patients with pathogenic QARS variants experience these agitated episodes.

Epilepsy associated with deleterious variants in QARS is in most cases extremely severe. Most patients have seizure onset within the first few days and often within the first few hours of life. Focal seizures seem to be the predominant seizure type, often described as multifocal, and often, seizure frequency is extremely high. Patients, who experienced status epilepticus, often had very long status episodes lasting several hours. At follow-up, epilepsy remained relentless and uncontrolled despite of different combinations of AEDs. As of now, the mechanisms underlying an epilepsy so severe are unknown, but a crucial function of the glutaminyl-tRNA synthetase can be postulated, and the in silico modeling indicated that most of the variants will damage the protein folding and expression. Furthermore, follow-up times are very short, and thus, developmental evolution in adolescence and adulthood in affected patients is still unknown.

Our study shows that some patients with QARS deleterious variants had a milder phenotype compared with the overall cohort. Patient 3 is microcephalic (−2.4 SD at 10 6/12 years) and severely disabled but does not have epilepsy or experienced seizures. Patient 4 is microcephalic (−3.1 SD at 5 1/12 years), but shows only mild ID. He has gained the ability to walk and even speaks short sentences, albeit expressive language is delayed. Furthermore, he only experienced seizures as part of an episode of meningoencephalitis and has been seizure free since. Patient 5 is microcephalic (−2.4 SD

at 4 6/12 years), but did not have seizure onset before age 5.5 months, and so far, the phenotype looks milder than expected with a seizure frequency of 1 seizure in 3 months at follow-up. He is currently classified with mild ID. Of interest, one of his variants only shows minor structural changes by in silico modeling, which may help explain the milder phenotype. Patient 21 is reported to be microcephalic (no measurements available) and has experienced febrile seizures only that resolved spontaneously. His MRIs were reported to be normal, and he was classified with mild ID.<sup>11</sup> His homozygous variant showed only minor effects on folding when modeled, which could support the milder outcome. The variant is seen one time as heterozygous in gnomAD. Siblings 16 and 17, and patient 18, all Ashkenazi Jews, have mild to moderate ID and have not developed seizures at the last follow-up (at age 10 years, 7 6/12 years, and 3 years, respectively).<sup>14</sup> It should be noted that the heterozygous form of the variant p.(Val476Ile) has a high incidence (allele count 443) in gnomAD, and furthermore, one healthy homozygous carrier is listed, which might suggest that this variant could be a benign polymorphism. However, functional analyses in the original study<sup>14</sup> showed a 67% decrease in QARS activity, and in silico modeling performed in this study supports a disease-causing function of the variant. Thus, we classified the variant as “likely pathogenic.”<sup>18</sup> One can speculate that ethnicity-based differences might attribute to the differential phenotypic expression as the homozygous carrier listed in gnomAD is of south Asian origin.

Of note, 5 of the above discussed 7 patients with a milder phenotype carry a homozygous pathogenic missense variant in QARS. Thus, it somehow seems that homozygosity is protective for the risk of developing epilepsy. This phenomenon has not been seen in the other bifunctional tRNA synthetase KARS.<sup>25</sup> However, it could be speculated that harboring compound heterozygous variants is worse because it interferes with protein building in two different spots compared with homozygous variants that alter the protein in a milder and alike way. Why patients 4 and 5, with compound heterozygous variants, also display a less severe phenotype remains unknown. Another explanation might be that

compound heterozygosity with one protein truncated (null allele) is more deleterious and that homozygous truncating variants in *QARS* are in fact lethal. Therefore, viable homozygous missense variants may be hypomorphic variants, which result in a milder phenotype compared with compound heterozygous variants with one null allele and another hypomorphic allele.

In most patients with pathogenic variants in *QARS*, the clinical picture is uniform and consists of the triad of primary or secondary progressive microcephaly, moderate or severe ID, and severe pharmaco-resistant epilepsy. However, our cohort shows that the phenotype is broader than expected with milder phenotypes in some patients. Nonetheless, the presence of the core triad—microcephaly, developmental disability, and early-onset refractory epilepsy—in a young child should elicit a search for biallelic variants in *QARS*, either using a gene panel, because this triad is also common in other epilepsies, or when evaluating exome data. Longer follow-up and the addition of more patients to this exclusive cohort are necessary to gain a better understanding of *QARS* functioning and the associated phenotype.

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## Disclosure

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## References

1. Zhang X, Ling J, Barcia G, et al. Mutations in *QARS*, encoding glutamyl-tRNA synthetase, cause progressive microcephaly, cerebral-cerebellar atrophy, and intractable seizures. *Am J Hum Genet* 2014;94:547–558.
2. Koderia H, Osaka H, Iai M, et al. Mutations in the glutamyl-tRNA synthetase gene cause early-onset epileptic encephalopathy. *J Hum Genet* 2015;60:97–101.
3. Abbott JA, Francklyn CS, Robey-Bond SM. Transfer RNA and human disease. *Front Genet* 2014;5:158.
4. Friedman J, Smith DE, Issa MY, et al. Biallelic mutations in valyl-tRNA synthetase gene *VARS* are associated with a progressive neurodevelopmental epileptic encephalopathy. *Nat Commun* 2019;10:707.
5. Siekierska A, Stamberger H, Deconinck T, et al. Biallelic *VARS* variants cause developmental encephalopathy with microcephaly that is recapitulated in vars knockout zebrafish. *Nat Commun* 2019;10:708.
6. Konovalova S, Tyynismaa H. Mitochondrial aminoacyl-tRNA synthetases in human disease. *Mol Genet Metab* 2013;108:206–211.
7. Wolf NI, Salomons GS, Rodenburg RJ, et al. Mutations in *RARS* cause hypomyelination. *Ann Neurol* 2014;76:134–139.
8. Casey JP, McGittigan P, Lynam-Lennon N, et al. Identification of a mutation in *LARS* as a novel cause of infantile hepatopathy. *Mol Genet Metab* 2012;106:351–358.
9. Sofou K, Kollberg G, Holmström M, et al. Whole exome sequencing reveals mutations in *NARS2* and *PARS2*, encoding the mitochondrial asparaginyl-tRNA synthetase and prolyl-tRNA synthetase, in patients with Alpers syndrome. *Mol Genet Genomic Med* 2015;3:59–68.
10. Simons C, Griffin LB, Helman G, 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;96:675–681.
11. Alabdullatif MA, Al Dhaibani MA, Khassawneh MY, El-Hattab AW. Chromosomal microarray in a highly consanguineous population: diagnostic yield, utility of regions of homozygosity, and novel mutations. *Clin Genet* 2017;91:616–622.
12. Datta A, Ferguson A, Simonson C, et al. Case report. *J Child Neurol* 2017;32:403–407.
13. Fuchs SA, Schene IF, Kok G, et al. Aminoacyl-tRNA synthetase deficiencies in search of common themes. *Genet Med* 2019;21:319–330.
14. Leshinsky-Silver E, Ling J, Wu J, et al. Severe growth deficiency, microcephaly, intellectual disability, and characteristic facial features are due to a homozygous *QARS* mutation. *Neurogenetics* 2017;18:141–146.
15. Salvarinova R, Ye CX, Rossi A, et al. Expansion of the *QARS* deficiency phenotype with report of a family with isolated supratentorial brain abnormalities. *Neurogenetics* 2015;16:145–149.
16. Baynam G, Broley S, Bauskis A, et al. Initiating an undiagnosed diseases program in the Western Australian public health system. *Orphanet J Rare Dis* 2017;12:83.
17. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. *Hum Mutat* 2015;36:928–930.
18. Scheffer IE, Berkovic S, Capovilla G, et al. ILAE classification of the epilepsies: position paper of the ILAE Commission for Classification and Terminology. *Epilepsia* 2017;58:512–521.
19. Fisher RS, Cross JH, French JA, et al. Operational classification of seizure types by the International League Against Epilepsy: position paper of the ILAE Commission for Classification and Terminology. *Epilepsia* 2017;58:522–530.
20. Richards S, Aziz N, Bale S, 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;17:405–424.
21. Nykamp K, Anderson M, Powers M, et al. Sherloc: a comprehensive refinement of the ACMG-AMP variant classification criteria. *Genet Med* 2017;19:1105–1117.
22. Ognjenović J, Wu J, Matthies D, et al. The crystal structure of human GlnRS provides basis for the development of neurological disorders. *Nucleic Acids Res* 2016;44:3420–3431.
23. Emsley P, Lohkamp B, Scott WG, Cowtan K. Features and development of Coot. *Acta Crystallogr D Biol Crystallogr* 2010;66:486–501.
24. Lindenmayer JP. The pathophysiology of agitation. *J Clin Psychiatry* 2000;61(suppl 14):5–10.
25. McMillan HJ, Humphreys P, Smith A, et al. Congenital visual impairment and progressive microcephaly due to Lysyl-transfer ribonucleic acid (RNA) synthetase (*KARS*) mutations: the expanding phenotype of aminoacyl-transfer RNA synthetase mutations in human disease. *J Child Neurol* 2015;30:1037–1043.

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