SHORT COMMUNICATION

Mutations in the glutaminyl-tRNA synthetase gene cause early-onset epileptic encephalopathy

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Aminoacylation is the process of attaching amino acids to their cognate tRNA, and thus is essential for the translation of mRNA into protein. This direct interaction of tRNA with amino acids is catalyzed by aminoacyl-tRNA synthetases. Using whole-exome sequencing, we identified compound heterozygous mutations [c.169T>C (p.Tyr57His) and c.1485dup (p.Lys496*)] in *QARS*, which encodes glutaminyl-tRNA synthetase, in two siblings with early-onset epileptic encephalopathy (EOEE). Recessive mutations in *QARS*, including the loss-of-function missense mutation p.Tyr57His, have been reported to cause intractable seizures with progressive microcephaly. The p.Lys496* mutation is novel and causes truncation of the QARS protein, leading to a deletion of part of the catalytic domain and the entire anticodon-binding domain. Transient expression of the p.Lys496* mutant in neuroblastoma 2A cells revealed diminished and aberrantly aggregated expression, indicating the loss-of-function nature of this mutant. Together with the previous report, our data suggest that abnormal aminoacylation is one of the underlying pathologies of EOEE.

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INTRODUCTION

Aminoacylation is the process of attaching amino acids to their cognate tRNA. This direct interaction is catalyzed by the aminoacyl-tRNA synthetase (ARS) family of enzymes.¹ In humans, 37 nuclear genes encode ARS enzymes, and to date, 22 of 37 ARS genes were shown to be mutated in human diseases.^{2–6}

QARS encodes glutaminyl-tRNA synthetase. Two families with autosomal recessive primary microcephaly and intractable seizures harboring compound heterozygous *QARS* mutations have been recently reported.⁷ In this study, we present the detailed molecular and clinical analysis of a Japanese family with two siblings affected with early-onset epileptic encephalopathies (EOEEs) in which whole-exome sequencing confirmed the presence of *QARS* mutations.

MATERIALS AND METHODS

Subjects

We investigated two affected siblings (II-1 and II-3) and their parents and unaffected two siblings. Clinical information and peripheral blood samples were obtained from family members after obtaining their written informed consent. A summary of the clinical features of two patients along with four patients previously reported⁷ is presented in Table 1, and their case reports are shown in Supplementary Information. Both patients developed intractable seizures during the neonatal period, and showed severe developmental delays, involuntary movements and intellectual disabilities. Electroencephalogram demonstrated epileptic activities, and also temporarily showed hypersarrhythmia

or suppression-burst patterns (Figures 1a and b). Brain magnetic resonance imaging revealed progressive cerebral atrophy with white matter volume loss, myelination delay, ventriculomegaly and a thin corpus callosum in both individuals (Figures 1c–f). They exhibited signs of excitement, such as crying, bending backward, excessive sweating, hypertonicity and sleep disturbance. These occurred from 2.5 to 6 years of age in individual II-1, and from 5 to 10 years of age (at present day) in individual II-3. Both individuals also experienced a variety of seizures, mainly partial seizures, which worsened with fever. At birth, their head circumferences were normal (II-1: 32.0 cm (−0.9 s.d.s); II-3: 33.0 cm (−0.2 s.d.s)), but acquired microcephaly occurred in both: the head circumference of II-1 was 44 cm (−3.2 s.d.) at 2 years and 8 months, whereas that of II-3 was 35 cm (−2.9 s.d.) at 3 months. This study was approved by the Institutional Review Board of the Yokohama City University School of Medicine.

RESULTS AND DISCUSSION

We conducted whole-exome sequencing to identify the genetic cause (s) of the two patients (Figure 2a). Genomic DNA was captured using the SureSelect Human All Exon Kit v5 (Agilent Technologies, Santa Clara, CA, USA) and sequenced on an Illumina HiSeq2000 (Illumina, San Diego, CA, USA) with 101 bp pair-end reads. Exome data processing, variant calling and variant annotation were performed as previously described.⁸

The average mean read depth of the protein-coding regions in RefSeq ranged from 133 to 136, and approximately 96.7% of target coding sequences were covered by 10 or more reads (Supplementary

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Table 1 Clinical features of individuals with QARS mutations

Individuals	II-1	II-3	I-1 ⁷	I-2 ⁷	II-1 ⁷	II-2 ⁷
Gender	Male	Male	Male	Male	Male	Female
Diagnosis	EOEE	EOEE	MCPH	MCPH	MCPH	MCPH
Mutation	c.169T>C (p.Tyr57His) c.1485dup (p.Lys496*)	c.134G>T (p.Gly45Val) c.1207C>T (p.Arg403Trp)	c.169T>C (p.Tyr57His) c.1543C>T (p.Arg515Trp)			
Birth head circumference	32 cm (−0.9 s.d.)	33 cm (−0.2 s.d.)	27.9 cm (−3.5 s.d.)	31.1 cm (−2.1 s.d.)	−1 s.d.	32 cm (−1 s.d.)
Microcephaly	44 cm (−3.2 s.d.) at 2.7 years; 45 cm (−3.6 s.d.) at 4.5 years	35 cm (−2.9 s.d.) at 3 months; 45 cm (−3.9 s.d.) at 5 years	34 cm (−10.4 s.d.) at 21 months	34 cm (−7.8 s.d.) at 7 months	−3 s.d. at 5.5 years	−2.5 s.d. at 3 years
Seizure onset	Fourth day of life	First day of life	First hour of life	First day of life	First hour of life	One-month old
Type of seizures	Perioral twitch → Tonic seizure with cyanosis → Eye deviation and head turning	Tonic seizure with cyanosis → Eye deviation and head turning	Generalized tonic or focal clonic seizure	ND	Clonic movements with cyanosis	Clonic seizure with apnea and cyanosis
Interictal EEG	Rt hemispheric spike → Hypsarrhythmia → Multifocal spike, spike & wave	Multifocal sharp, spike, spike and wave → Suppression-burst → High-voltage delta and multifocal spike	ND	ND	ND	ND
Seizure control	Intractable	Intractable	Intractable	ND	Intractable	Intractable
Development	Severe motor and intellectual disability;	Severe motor and intellectual disability;	Profound delay	Profound delay	Psychomotor delay	Psychomotor delay
Muscle tone	Hypotonia	Hypotonia	Mixed hypotonia and hypertonia	High tone with brisk reflexes	Severe hypotonia	Severe hypotonia
Involuntary movement	+	+ (Pedaling-like)	ND	ND	ND	ND
Brain images	Mild cerebral atrophy with left posterior horn enlargement (7 days); diffuse cerebral atrophy with white matter volume loss, ventriculomegaly, myelination delay, focal atrophy with signal abnormality in bilateral occipital lobes (7 months)	Enlargement of lateral ventricle, thin corpus callosum (11 days); diffuse cerebral atrophy with white matter volume loss, myelination delay, ventriculomegaly, thin corpus callosum (7 months); progression of ventriculomegaly (2 years)	Severely simplified gyral patterns, decreased contrast of white and gray matter, microcephaly, enlarged subarachnoid space, enlarged lateral ventricles, thin corpus callosum, atrophy in cerebral cortex, and cerebellar vermis	Microcephaly, enlarged subarachnoid space, enlarged lateral ventricles, thin corpus callosum, atrophy in cerebral cortex, and cerebellar vermis		

Abbreviations: EEG, electroencephalogram; EOEE, early-onset epileptic encephalopathy; MCPH, autosomal recessive primary microcephaly; ND, not described; Rt, right.

Figure 1 Electroencephalogram (EEG) and brain magnetic resonance imaging (MRI) of individuals with *QARS* mutations. (a) The EEG of II-1 showed hypsarrhythmia at 10 months. (b) The EEG of II-3 showed multifocal sharp, spike and spike-and-wave complexes at 6 days of age. (c–f) T2-weighted axial (c, e) and sagittal (d, f) images of II-1 (c, d) and II-3 (e, f). Diffuse cerebral atrophy with severe white matter volume loss, hypomyelination, hypoplastic corpus callosum, enlargement of the lateral ventricles and cerebellar atrophy were observed in both individuals. II-1 showed T2 elongation in the occipital lobes (arrows).

Table 1). We filtered out common variants in dbSNP135, variants in dbSNP137NonFlagged data ('clinically associated' variants are excluded in this data) and variants found in more than 2 of our 575 in-house control exomes. As a result, we identified 152 and 127 rare protein-altering and splice site variants in individuals II-1 and II-3, respectively (Supplementary Table 1).

Based on an autosomal or X-linked recessive model, we focused on homozygous or compound heterozygous autosomal variants, and hemizygous variants on the X chromosome that were commonly seen in the two siblings. This analysis revealed only one autosomal candidate gene, *QARS* (NM_005051.2; Supplementary Table 2). Sanger sequencing confirmed that a novel *QARS* nonsense mutation, c.1485dup (p.Lys496*), was inherited from their father, and a missense mutation, c.169T>C (p.Tyr57His), was inherited from their mother (Figure 2a). Two other unaffected siblings only carried the c.1485dup (p.Lys496*) mutation. Neither mutation was found in the 6500 exomes of the National Heart, Lung and Blood Institute Exome Sequencing Project Variant Server, whereas c.169T>C was not found in any of our in-house 575 Japanese control exomes, and c.1485dup was only found in one of these control exomes.

Four online protein prediction programs (SIFT, Polyphen-2, PhyloP and MutationTaster; URLs listed in Supplementary Information) predicted the p.Tyr57His mutation to be damaging (Supplementary Table 2). The p.Tyr57His mutation occurs at an amino acid that is highly evolutionarily conserved among mammals, vertebrates, flies, nematodes and plants (Figure 2b). In addition, the p.Tyr57His mutation found in our family was previously identified in another family with autosomal recessive primary microcephaly.⁷ The p.Tyr57His mutation was demonstrated to decrease *QARS* aminoacylation activity to less than 10% that of the wild-type protein, indicating that it is a loss-of-function mutant.⁷ On the other hand, the p.Lys496* is predicted to cause truncation of *QARS*, involving loss of part of the

catalytic domain and the entire anticodon-binding domain, registered as IPR020058 and IPR020059 in InterPro, respectively, suggesting that the function of the mutant protein would be severely impaired (Figure 2b). To examine the effect of the two *QARS* mutations, we performed transient expression experiments in neuroblastoma 2A cells. The p.Tyr57His mutant showed a similar expression pattern to wild type; however, expression of the p.Lys496* mutant was severely diminished or aggregated in most of the cells (Figure 2c). In addition, the c.1485dup mutant transcript possessing a premature stop codon is considered to undergo nonsense-mediated decay in patient cells. Therefore, it is very likely that the c.1485dup mutation would lead to loss-of-function of *QARS* by diminished expression both at mRNA and protein levels.

The clinical features of our two siblings are consistent with those of previous cases, including intractable seizures starting in the early neonatal period, postnatal microcephaly, cerebral atrophy with reduced white matter, hypomyelination, severe developmental delay and intellectual disability. Notably, our cases showed excited states similar to individual I-1 and -2 in Zhang's report.⁷ These episodes associated with excitement may be a key feature for suspecting *QARS* mutations. In addition, seizures and hypomyelination are most commonly observed in disorders with abnormality of *ARS* genes.^{4,6–7,9–14} Therefore, abnormal aminoacylation is likely to be an underlying cause in EOE, especially when hypomyelination and/or white matter abnormalities were seen.

In conclusion, we have described two siblings with EOE who harbor compound heterozygous mutations in *QARS*. Further genetic analysis of the aminoacylation process is needed to obtain a comprehensive causal view of epileptic encephalopathy.

The following supporting information is available for this article: Supplementary case reports, and Materials and Methods.

Figure 2 Familial pedigree, QARS mutations and their protein localization. **(a)** Familial pedigree and QARS mutations in familial members. **(b)** Schematic QARS proteins with InterPro domains (<http://www.ebi.ac.uk/interpro/>). Locations of each mutation are depicted. Previously reported mutations are shown in black and mutations identified in this study in red. **(c)** Localization of AcGFP1-tagged QARS proteins in neuroblastoma 2A cells. AcGFP1-tagged wild-type (WT) QARS (top panels, green) and the p.Tyr57His mutant (middle panels, green) were localized in the cytosolic compartment. Expression of the p.Lys496* mutant (bottom panels, green) was severely diminished or aggregated. The nucleus was stained with 4',6-diamidino-2-phenylindole (DAPI; blue). Scale bars, 10 μm.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (<http://www.nature.com/jhgc>)