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Case Report

A case of early onset life-threatening epilepsy associated with a novel *ATP1A3* gene variant

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Abstract

Introduction: Mutations of the *ATP1A3* gene are associated with a wide spectrum of neurological disorders including rapid onset dystonia-parkinsonism and alternating hemiplegia of childhood (AHC). The genotype-phenotype correlations in these cases remain unclear however. We here report a pediatric case of catastrophic early life epilepsy, respiratory failure, postnatal microcephaly, and severe developmental disability associated with a novel heterozygous *ATP1A3* mutation.

Subject: A boy with a normal birth to nonconsanguineous parents was transferred to the NICU due to postnatal respiratory failure at 2 days. He showed extreme hypotonia, episodic oculomotor abnormality and tachycardia, and frequent epileptic seizures. Mechanical ventilation was required but his epileptic seizures were intractable to multiple antiepileptic drugs, including extremely high doses of phenobarbital.

Methods and Results: Whole exome sequencing analysis of the case and his parents identified a de novo heterozygous mutation in the *ATP1A3* gene (c.2736_2738CTTdel, p.Phe913del).

Discussion: The Phe913 residue in the ATP1α3 protein that is deleted in our case is highly conserved among vertebrates. Notably, an amino acid deletion in the same transmembrane domain of this protein, p.Val919del, has been reported previously in typical AHC cases, suggesting that p.Phe913del is a pathogenic mutation. Several reported cases with severe symptoms and very early onset epilepsy harbor ATP1α3 mutations at structural positions in this protein that differ from that of Phe913. Further functional studies are required to clarify the relationship between the loss of Phe913 and the very distinct resulting phenotype.

Keywords: *ATP1A3*; Catastrophic early life epilepsy; Respiratory failure; Postnatal microcephaly; Whole exome sequencing

1. Introduction

Rapid onset dystonia-parkinsonism (RDP, DYT12) is a movement disorder characterized by an abrupt onset of dystonia usually accompanied by signs of parkinsonism [1]. Alternating hemiplegia of childhood (AHC) is another rare neurological disorder characterized by transient episodes of alternating hemiplegia/hemiparesis, dystonic attacks, paroxysmal abnormal ocular movements, epileptic seizures, episodes of autonomic dysfunction and intellectual disability [2]. These are distinctive autosomal-dominant disorders with variable expressivity and reduced penetrance caused by mutations in the *ATP1A3* gene [1,3,4]. It has been recently

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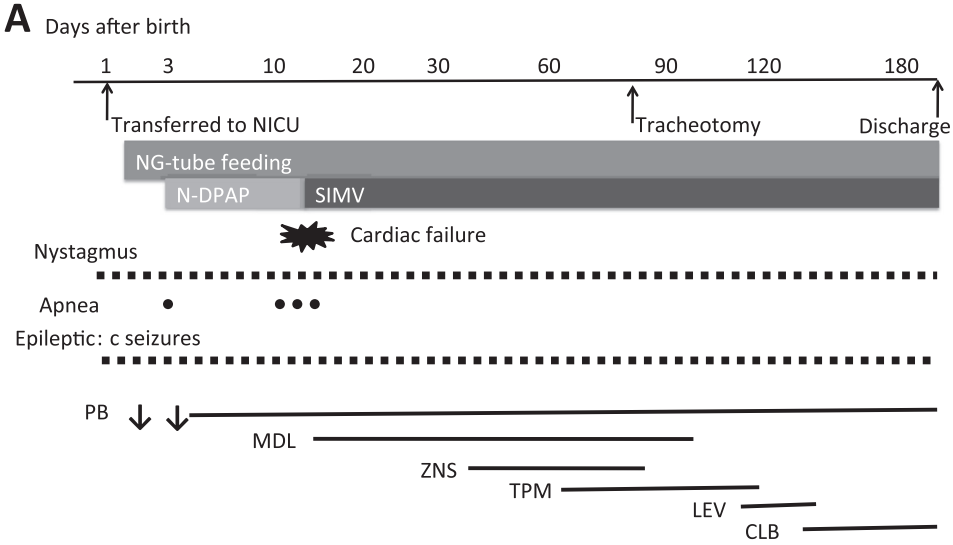
acknowledged that *ATP1A3* mutations also cause cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS) [5], relapsing encephalopathy with cerebellar ataxia (RECA) [6], and early infantile epileptic encephalopathy [7].

ATP1A3–related disorders also manifest with various onsets. RDP usually occurs at between 10 and 30 years of age, whereas AHC usually starts prior to the first 18 months of life [7]. We here report a neonatal case of catastrophic early life epilepsy, respiratory failure, postnatal microcephaly, and severe developmental disability, associated with a novel heterozygous mutation in the *ATP1A3* gene.

2. Case report

A boy was born to nonconsanguineous parents with a normal delivery at 39 weeks and 3 days. His Apgar scores were 7 and 8 at 1 and 5 min, respectively. His body length was 45.1 cm (−1.9 SD), body weight was 2785 g (−0.7 SD), and head circumference was 33.8 cm (+0.4 SD). He was transferred to a neonatal intensive care unit (NICU) due to respiratory failure at 2 days postnatally. He also showed extreme hypotonia, episodic oculomotor abnormality and tachycardia, and frequent epileptic seizures including non-epileptic tonic or dystonic fits. His clinical course during the NICU period is shown in Fig. 1A. Nasogastric-tube feeding was required because of poor oral intake, and gastrostomy was performed at 8 months. A nasal-DPAP was required for the respiratory failure. On day 17, he had severe attacks of cardiac failure and apnea, and intravenous inotropic drugs and mechanical ventilation were administrated. No specific findings were observed in electrocardiogram or ultrasound of the heart. Nystagmus was evident with or without epileptic seizures. Phenobarbital was administrated intravenously to ameliorate the seizures but they remained intractable to multiple anti-epileptic drugs. Neurological characteristics or severity of the symptom did not change before and after



B

		Day17	3 months	1y3mo	1y5mo	
Plasma	Lactate	62.2	26.1	8.7	7.9	mg/dL
	Pyruvate	2.46	1.51	0.59	0.59	mg/dL
CSF	Lactate	94.8	n.d.	n.d.	13.7	mg/dL
	Pyruvate	4.34	n.d.	n.d.	1.09	mg/dL

n.d.: no data

Fig. 1. Clinical course in the NICU and longitudinal lactate and pyruvate data of the study patient. (A) Clinical course of the study patient in the NICU. Nasogastric-tube feeding was required due to a poor oral intake, and gastrostomy was performed at 8 months. Nasal-DPAP was required for respiratory failure. On day 17, the patient had severe attacks of cardiac failure and apnea, and intravenous inotropic drugs and mechanical ventilation were administrated. Nystagmus was seen with or without epileptic seizures. Phenobarbital was administrated intravenously for sedating the epileptic seizures bit these remained intractable to multiple anti-epileptic drugs. (B) Longitudinal data for lactate and pyruvate. On day 17 when the patient had severe attacks of cardiac failure and apnea, the lactate/pyruvate ratio was extremely elevated in both the plasma and cerebrospinal fluid. At 3 months however, this level had decreased and had returned to within a normal range at 1 year.

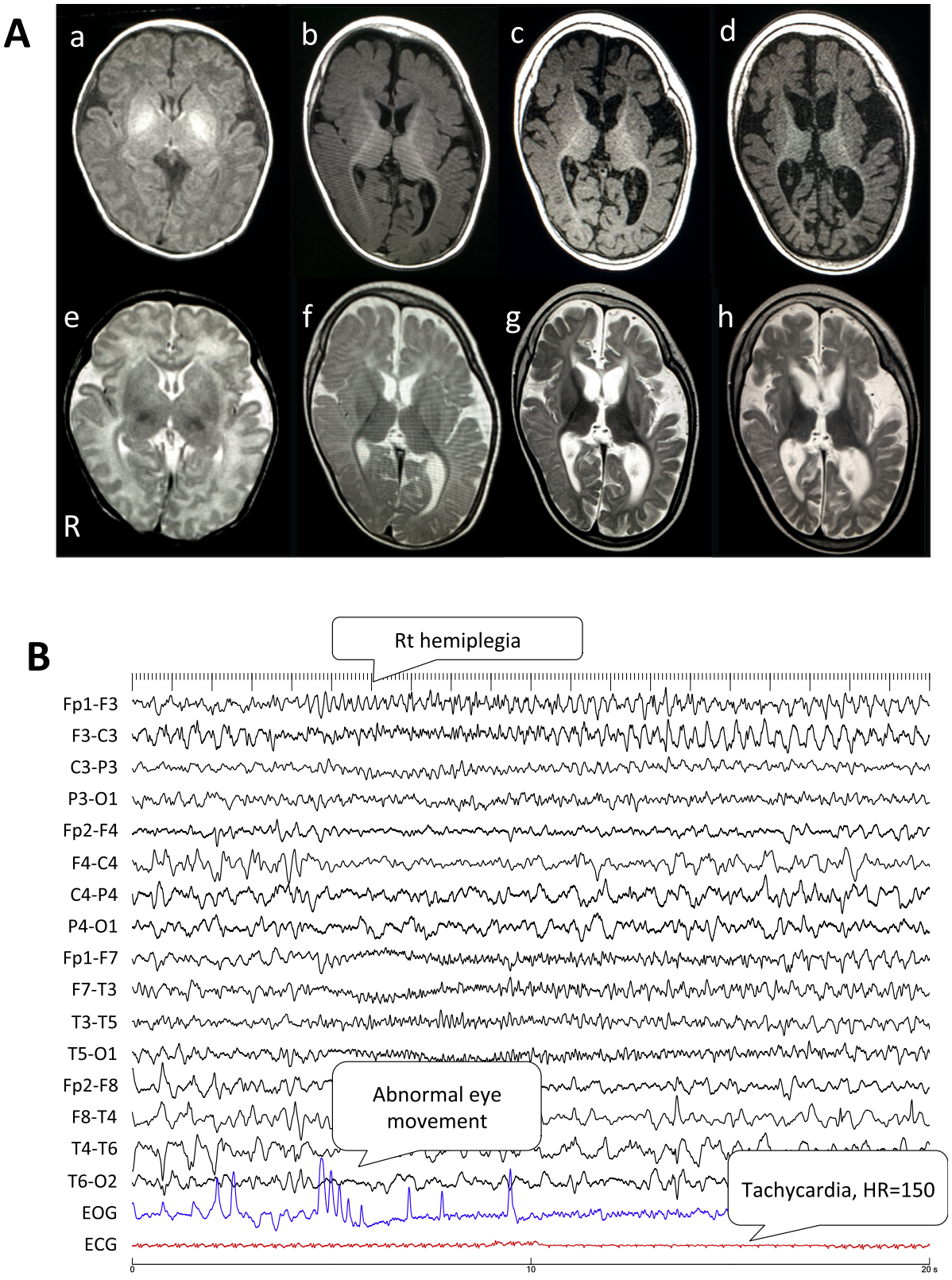


Fig. 2. Brain MRI and ictal EEG of the study patient. (A) Brain MRI of the study patient. T1-weighted images (a–d) and T2-weighted images (e–h) are shown at different postnatal timepoints: 6 days (a, e), 10 months (b, f), 2 years (c, g), and 4 years (d, h). (B) Ictal EEG of the study patient. The seizure shown in the figure commenced from the left frontal area and these were first noticed with tachycardia. Abnormal eye movements were then observed, followed by hemiplegia. However, the pattern in ictal EEG was the typical findings in focal seizure, not for the plegic episode of the AHC. Rhythmic ictal activity arose at different frequency in different areas, but the migration of the epileptic foci was not observed.

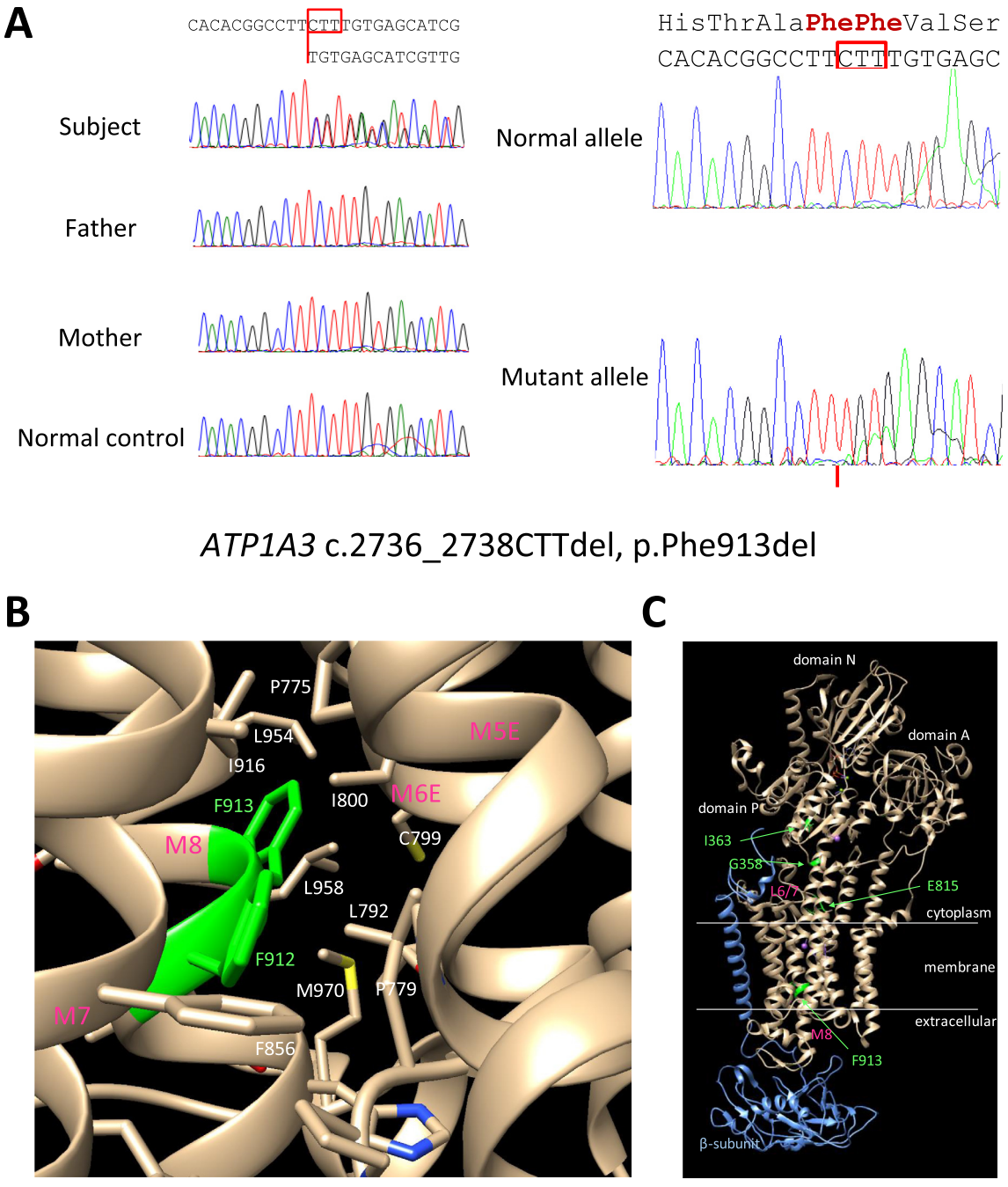


Fig. 3. Genetic analysis of the study patient. (A) Sequencing of the *ATP1A3* gene in the study patient and his parents. A heterozygous mutation in *ATP1A3* (c.2736_2738CTTdel) was detected in the case. (B) Structural models of the ATP1α3 protein. Phe913 (green) is located in the 8th transmembrane domain (M8) and is surrounded by hydrophobic amino acids contained in the other transmembrane domains, suggesting that the Phe916del mutation may affect the protein structure. (C) Structural positions of other amino acids in ATP1α3 found to be mutated in early onset epilepsy cases. Glu815 is located in the loop between M6 and M7 (L6/7), whereas 358Val and Ile363 are located in the P domain. However, the positions of these previously described amino acids in the ATP1α3 protein structure seem to be unrelated to the Phe913 position.

the apneic episode. The patient was discharged from the NICU at 6 months but continued to show daily seizure clusters and require 24-h mechanical ventilation.

Brain MRIs of our current case are shown in Fig. 2A. There were no particular abnormalities found on these scans at day 6 except for a high intensity at the bilateral pallidum on a T1WI. At 10 months, the patient showed delayed myelination and an abnormally high intensity in the white matter, particularly in the area surrounding the anterior horn of the lateral ventricle on a T2WI. At 2 and 4 years of age, progressive brain atrophy and an abnormal intensity of white matter were seen.

Table 1
Clinical data of our case and literature review of early-onset cases.

Case ID	ATP1A3 mutation	Age/Sex	Age at onset	Initial symptoms	Motor status	Intellec-tual status	Head MRI	Ictal EEG	Respiratory status	Postnatal micro-cephaly	Reference Ref.
II-1	E815K	13y/Male	17d	Tonic fits	Stands with support	Only words	Cerebellar atrophy	N/A	Normal	N/A	[11]
III-1	E815K	32y/Female	2d	Tonic fits	Walks with support	Only words	Cerebellar atrophy	N/A	On ventilator	N/A	[11]
IV-1	E815K	6y/Male	1d	Upward gaze, tonic fits	Sit alone	Only words	Normal	N/A	Apnea	N/A	[11]
IX-1	E815K	9y/Male	Infant	Apnea	Unable to sit	No words	N/A	N/A	Apnea	N/A	[11]
X-1	E815K	1y/Male	Neo-natal	Nystagmus, downward gaze, tonic fits	Rolling over	Delay	Normal	N/A	Apnea	N/A	[11]
LR11-328	G358V	Died 16 m/ Female	4hr	Epileptic seizures	N/A	N/A	Progressive atrophy	Yes	Failure	N/A	[7]
LR11-147	I363N	4y/Male	6w	Apnea	Quadri-plegia	Occasinal vocalization	Normal	No EEG correlation	On ventilator	Yes	[7]
7	D756del	4y/Male	3m	Clonic seizures	(Improved with KD)	Moderately delay	Normal	Yes	Apnea	N/A	[12]
#1	D742Y	16y/Female	6w	Tonic spells, myoclonic jerks	Walks with support	Only words	Short corpus callosum,atrophy	Yes	Normal	No	[13]
#2	C346R	6y/Male	12d	Apnea	Able to crawl, unable to sit	Only words	Normal	Yes	Normal	No	[13]
#3	D609Y	4y/Female	1m	Spells	Able to crawl and sit	No words	Unremark-able	Yes	Normal	No	[13]
	G371S	9m/Male	1d	Eyelid colonies	No head control	N/A	Lt hippocampal screrosis	N/A	Apnea	Yes	[14]
Our case	F913del	4y/Male	1d	Apnea	Bed ridden, no head control	No vocalization,	Progressive atrophy	Yes	On ventilator	Yes	

Ictal electroencephalograms (EEG) were also taken during the seizures (Fig. 2B) which started from the left frontal area, were clustered, and lasted for 2 h. Because of extreme hypotonia, we first noticed these seizures with tachycardia. Abnormal eye movements were then also seen followed by hemiplegia. Electric seizure activities were also observed on the left side and in the right hemisphere.

We performed laboratory examinations for an inborn error of metabolism in this case and found an increased ratio of lactate/pyruvate in the plasma and cerebrospinal fluid (Fig. 1B). On day 17, when he had severe attacks including cardiac failure and apnea, the lactate/pyruvate ratio was extremely elevated in both the plasma and cerebrospinal fluid. However, this ratio had decreased 3 months later, and was within a normal range at 1 year. Screening for mitochondrial disease including sequencing of the mitochondrial DNA and histochemical and enzymatic analyses using muscle biopsy sample was negative.

After obtaining informed consents, whole exome sequencing was performed for this index case and his parents as previously described [8]. The study protocol was approved by the Ethical Review Board for Human Genome Studies at the Fujita Health University. We thereby identified a *de novo* heterozygous mutation, c.2736_2738CTTdel, p.Phe913del in the *ATP1A3* gene, which was validated by Sanger sequencing (Fig. 3A). This mutation has not been reported previously in the dbSNP147 (URL: http://www.ncbi.nlm.nih.gov/projects/SNP/index.html), ExAC (http:// http://exac.broadinstitute.org/), Integrative Japanese Genome Variation Database (https://ijgvd.megabank.tohoku.ac.jp/), nor in the Japanese database HGVD (URL: http://www.hgvd.genome.med.kyoto-u.ac.jp/). The deleted Phe913 residue in our present patient is highly conserved among vertebrates. Notably, a p.Val919del mutation, which is located in a same transmembrane domain as Phe913, reduces the activity of ATP1α3 [9], suggesting that p.Phe913del is pathogenic.

At age 4, our patient's body length was 85.3 cm (−3.8SD), body weight was 8.94 kg (−4.2SD), and head circumference was 43.8 cm (−4.0 SD). He underwent a tracheotomy and required mechanical ventilation due to apnea at night and during a seizure. He also received a gastrostomy due to hypotonia and poor sucking. The seizures persisted for a few minutes or occasionally for a few hours, and did not always accompany the hemiplegic symptom. Duration of ocular symptoms are a few seconds or minutes, while that for tachycardia was a few minutes or hours. The patient had conjugate deviation and horizontal nystagmus at 0.5–5c/s, which might be the part of ictal symptom of epileptic seizure or non-epileptic ocular movements in AHC. Since a ketogenic diet was started at the age of 1 year and 3 months, the frequency of seizure was reduced to approximately 50%. However, the epileptic seizures continued to occur daily, and he thus required extremely high doses of multiple anti-epileptic drugs. Flunarizine dihydrochloride was used as a specific treatment with approval of the Institutional Review Board of Fujita Health University.

3. Discussion

Our present case study broadens the clinical spectrum of *ATP1A3*-related disorders by adding a new phenotype consisting of epileptic encephalopathy in the newborn period with respiratory distress and cardiac failure. Intractable neonatal seizures, early life epilepsy, and status epilepticus have been reported in children with clinically-defined AHC [4], and an association has been made with p.Glu815Lys mutations in *ATP1A3* and neonatal-onset seizures [10]. Recently, two cases of EIEE with heterozygous mutations in *ATP1A3* were reported (p.Gly358Val and p.Ile363Asn) who were not meeting the diagnostic criteria for AHC, one of whom also had catastrophic early life epilepsy and another who manifested epilepsy and life-threatening recurrent apnea leading to severely impaired neurodevelopment [7]. The observed mutations in these previous cases resulted in a significant reduction of ATP1α3 activity *in vitro*, which indicates that they underlie the most severe phenotypes of the *ATP1A3*-related disorder spectrum. More recently, additional three EIEE cases with the *ATP1A3* mutation were reported. The clinical characteristics of these cases are summarized in Table 1.

The mutation found in our present case results in a single amino acid deletion in the 8th transmembrane domain of ATP1α3 (F913del, Fig. 3B) that likely affected the protein structure. Previously reported phenotypes for mutations in the 8th transmembrane domain of this protein include classical AHC and RDP [6]. p.Glu815Lys, which occurs in the 6th transmembrane domain, has been reported as a genotype of EIEE [6] and a severe form of AHC [9,10]. On the other hand, although the p.Gly358Val and p.Ile363Asn mutations in the P domain of ATP1α3 are also classified as causative for EIEE [6], they are reported as a different phenotype from AHC [7]. Indeed, the positions of these mutated amino acids in the protein structure seem unrelated (Fig. 3C). Further functional studies are thus required to clarify the relationship between these *ATP1A3* mutations and such distinct phenotypes.

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