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Research paper

Mutational and phenotypic expansion of *ATP1A3*-related disorders: Report of nine cases

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ABSTRACT

Background: Mutations in the *ATP1A3* gene are known to be the cause of three distinct neurological syndromes including alternating hemiplegia of childhood (AHC), rapid-onset dystonia parkinsonism (RDP) and cerebellar ataxia, arefexia, pes cavus, optic atrophy and sensorineural hearing impairment (CAPOS). Recent studies have suggested the broader diversity of *ATP1A3*-related disorders. This study aimed to investigate the clinical spectrum in patients carrying causative mutations within the *ATP1A3* gene.
Method: The medical histories of nine unrelated patients with diverse phenotypes harboring variants in *ATP1A3* were retrospectively analyzed after they were referred to a tertiary epilepsy center in one of the two different health care systems (Germany or Thailand). Clinical features, neurophysiological data, imaging results, genetic characteristics and treatments were reviewed.
Results: Three patients harbor novel mutations in the *ATP1A3* gene. Atypical clinical features and imaging findings were observed in two cases, one with hemiplegia-hemiconvulsion-epilepsy syndrome, and the other with neurodegeneration with brain iron accumulation. All nine patients presented with intellectual impairment. Alternating hemiplegia of childhood (AHC) was the most common phenotype (67%). Flunarizine and topiramate led to symptom reduction in 83% and 25% of AHC cases administered, respectively.
Conclusion: The present case series expands the clinical and genetic spectrum of *ATP1A3*-related disorders.

Abbreviations: AED, Anti-epilepticdrug; AHC, Alternating hemiplegia of childhood; CAPOS, Cerebellar ataxia, areflexia, pes cavus, optic atrophy and sensorineural hearing loss; CBZ, Carbamazepine; EEG, Electroencephalography; GTCS, Generalized tonic clonic seizures; HHE, Hemiconvulsion-hemiplegia-epilepsysyndrome; ID, Intellectual disability; LEV, Levetiracetam; MRI, Magnetic resonance imaging; NBIA, Neurodegeneration with brain iron accumulation; NCSE, Nonconvulsive status epilepticus; PB, Phenobarbital; PHT, Phenytoin; RDP, Rapid-onset dystonia parkinsonism; SE, Status epilepticus; SD, Standard deviation; TPM, Topiramate; VPA, Valproic acid; WES, Whole exome sequencing; ZNS, Zonisamide.

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1. Introduction

The diverse consequences of mutations in the *ATP1A3* gene have become an issue of consistently growing relevance within the last couples of years. *ATP1A3* encodes the α3 subunit of Na+, K + -ATPase in neuronal cells and *ATP1A3* mutations have primarily been associated with three distinct phenotypes: alternating hemiplegia of childhood (AHC), rapid-onset dystonia-parkinsonism (RDP) and CAPOS (cerebellar ataxia, areflexia, pes cavus, optic atrophy and sensorineural hearing loss). As for RDP and CAPOS, it took several steps to link the complex phenotypes described to variants in the *ATP1A3* gene (Dobyns et al., 1993; Heinzen et al., 2014). For AHC, although being the first among them to be mentioned in 1971, it took the most years to unravel the genetic defects and pathophysiology (Rosewich et al., 2012). Recently, *ATP1A3* mutations could be identified as monogenetic causes in 74% of 98 AHC patients investigated (Heinzen et al., 2014). This triggered a series of other research studies concerning *ATP1A3* and its role in AHC and other phenotypes not breaking of until today. With the increasing publication of atypical case reports and overlapping phenotypes, it becomes clear that the phenotypic spectrum of *ATP1A3* mutations is much broader than initially thought. Rather than being fully distinct disorders only connected by their cause of pathogenic variants in the *ATP1A3* gene, the previously described clinical manifestations mark the respective end of a range of phenotypes that are often overlapping. Accordingly, the term "*ATP1A3*-related disorders" is being increasingly used. In this study, we present nine unrelated patients with proven *ATP1A3* mutations who suffer from diverse consequences. In addition to contributing to the growing phenotypic spectrum, we describe three *ATP1A3* mutations that have not been previously described.

2. Methods

2.1. Patients and families

A total of nine unrelated patients (five from Thailand, four from Germany) with *ATP1A3*-related disorders were recruited in the study. The study was approved by the institutional review board (IRB No. 054/60) of Faculty of Medicine, Chulalongkorn University, Thailand, and the ethical review committee of Faculty of Medicine, LMU Munich, Germany (amendment to No. 18–232). The study was performed according to the declaration of Helsinki. After informed consent from the patients or their legal guardians was obtained, blood samples from the patients and their parents were collected.

2.2. Mutation analysis

Genomic DNA was extracted from peripheral blood leukocytes by using Puregene blood kits (Qiagen, Hilden, Germany). In patients 1, 2, 6, 7 and 9, mutation analysis was performed by whole exome sequencing (WES). In patients 3, 4 and 5, PCR-Sanger analysis of the *ATP1A3* gene was performed using specific primers for the coding exons. In patient 8, the pathogenic variant was detected by panel-analysis using customized panels created for medically refractory epilepsies.

For WES in patients 1, 2 and 6, The DNA samples were sent to Macrogen Inc., Seoul, Korea. Exome libraries were captured by hybridization with the Agilent SureSelect V4 Target Enrichment Kit. WES for patients 7 and 9 was performed using a SureSelect Human All Exon 60 Mb V6 Kit (Agilent) for the enrichment and the HiSeq 4000 (Illumina) platform for sequencing. More than 98% of the exome was covered at least 20X and the average coverage was more than 126X. Sequence reads in FASTQ sequencing files were aligned to the Human Reference Genome hg19 from UCSC using Burrows-Wheeler Alignment (biobwa.sourceforge.net/) software version 0.5.8.1. Variant calling was done using GATK (www.broadinstitute.org/gatk/) version 4.0.1.1. Single nucleotide variants (SNVs) and small insertions and deletions were detected with SAMtools version 0.1.7. Copy number variations (CNVs) were detected with ExomeDepth and Pindel. We targeted the rare missense, nonsense, splice site and insertion/deletion variants in the genes related to HPO terms: seizures (HP:0001250), dystonia (HP:0001332) and hemiplegia (HP:0002301). Only variants (SNVs/small Indels) in the coding and the flanking intronic regions (±8 bp) with a minor allele frequency (MAF) < 1% were evaluated. Minor allele frequencies were taken from public databases (gnomAD, dbSNP). Subsequently, these variants were filtered out if they were present in the in-house databases comprising 1,864 unrelated Thai exomes (patients 1, 2, 6) or 16,000 exome datasets of the Munich exome server (patients 7, 9). The variants would be called novel if they were not listed in the HGMD® (http://www.hgmd.cf.ac.uk/ac/index.php) database. Classification of pathogenicity was done according to the ACMG criteria for variant classification (Richards et al., 2015).

The PCR-Sanger analysis of the *ATP1A3* gene in patients 3, 4 and 5 was performed using specific primers for the coding exons (Table 1).

The "hemiplegic migraine" panel analysis was done in patient 8. The staged sequencing for the gene *PRRT2, CACNA1A* and *ATP1A3* was performed. The coding and flanking intronic regions of the relevant genes were enriched using in solution hybridization technology (Agilent, Santa Clara, USA) and were sequenced using the Illumina HiSeq system (Illumina, San Diego, USA). Illumina bcl2fastq2 was used to demultiplex sequencing reads. Adapter removal was performed with Skewer. The trimmed reads were mapped to the human reference genome (hg19) using the Burrows Wheeler Aligner. Reads mapping to more than one location with identical mapping score were discarded. Read duplicates that likely result from PCR amplification and reads mapping to more than one genomic location were removed. The remaining high-quality sequences were used to determine sequence variants (single nucleotide changes and small insertions/deletions). Only variants (SNVs/Small Indels) in the coding and the flanking intronic regions (±8 bp) with a minor allele frequency (MAF) < 1.5% were evaluated. Known disease-causing variants (according to HGMD®)

Table 1
Primer sequences used in the PCR-Sanger sequencing in patients 3, 4 and 5.

Name	Forward primer	Reverse primer
ATP1A3-Ex1	CCAAGCCTGAGCCTGAGC	CGCACACCCAATGTCACC
ATP1A3-Ex2-Ex3	GGATAGCTGGGGCATGGAG	ACCCCAGGCCTTCACCAG
ATP1A3-Ex4	CTGGTGAAGGCCTGGGGT	GGGAAGAAGTTGGGGTGC
ATP1A3-Ex5	GGTGGAGAGTGGCTTGGG	AGACTCCCTCTGCCTGGG
ATP1A3-Ex6	TGTTAGTGTATGGGCTGGGG	AGGGCCTAAACTCCTGGGT
ATP1A3-Ex7	TGTACACAAATACCCTCCTGT	GAGGTTCTGGAGGCCTGAC
ATP1A3-Ex8.1	GTCAGGCCTCCAGAACCTC	TATCCGAGAATGAGGGAGAG
ATP1A3-Ex8.2	TGTCATGGGCCGTATCG	CTCAGGGGTCCAGGATCC
ATP1A3-Ex9	CTCGTGTCGCTCATCCAAC	GTGTGAGGGCCAGGGAC
ATP1A3-Ex10	CAGTCAGGTGAGCGCAGG	ACAGCTGAGGGGAGGACAC
ATP1A3-Ex11	CTACTGGCCTCACCTCGG	ACCTCTTTACAGGCGTCATAAG
ATP1A3-Ex12	AGGGAGCTTCCTGGTGTCTG	CTTTGGGCAGCATCACAAC
ATP1A3-Ex13	AGACATCTAGGGGCATGGG	GGTGACCATGATGACCTGC
ATP1A3-Ex14	CAAGGTGTGGCTTGGGG	AGCGGTCCCCCTGTGTCA
ATP1A3-Ex15	CTCTGTCTTTCAGGGATCAC	CCGGCCTCAGTGAGGACC
ATP1A3-Ex16	TCCTGGGCTTCTGGATCTG	CCTTGCTGGTCTCAGGCC
ATP1A3-Ex17	CAGAGGGAGTGGGGCTC	TGAGATGGCAGGGACCTAG
ATP1A3-Ex18	GCCCTGGCAGCCACCCT	GGGTCCCAAGCACCCAC
ATP1A3-Ex19	CCTCTGAGGTGCCCTGG	CGTAGGAAGTGGCCATGCA
ATP1A3-Ex20	CAAAGAGCACCGGAACGTC	CAGACACTCGGACAGGACAG
ATP1A3-Ex21	CAGGTGCAGGGTGGGTG	GGCTGAGTCTAAGGGAAGGC
ATP1A3-Ex22	TCCTGGGAGACTGCCCCT	GACCAGCTGCCTGAGACC
ATP1A3-Ex23	CCTTGCCTGTCTCTCTCCATC	GACTGACAGGGGCGGTC

were evaluated in up to ± 30 bp of flanking regions and up to 5% MAF. Minor allele frequencies were taken from public databases (gnomAD, dbSNP) and an in-house database.

2.3. Statistical analyses

Pseudonymized patient data were collected from medical records. Descriptive statistics were obtained using SPSS version 25 (IBM Corporation, Armonk, NY).

3. Results

3.1. Demographic and clinical data

Among the nine *ATP1A3* cases, the age of onset ranged from six weeks to twelve years. The median age at onset of first symptoms was at five months (interquartile range; IQR = 5 months). The median time difference between onset of symptoms and genetic testing was 6 years.

All patients were intellectually impaired. While two patients (22%) (patients 6 and 8) were only mildly disabled, the remaining seven cases (78%) had severe cognitive deficits. The family history regarding similar symptoms was unremarkable in all individuals. Six out of nine patients (67%) presented with hemiplegic attacks (Table 2). The attacks lasted from one hour to 72 h. The frequency ranged from two episodes a year to two episodes every week. Reported triggers were exercise, fever and changes in temperature or weather. Five out of six AHC patients (83%) had a history of epileptic seizures. While four of them (80%) suffered from generalized tonic-clonic seizures without clinically lateralizing features, the remaining patient (20%) experienced focal motor seizures occasionally evolving into non-convulsive status epilepticus. For two of the non-lateralizing epileptic patients, attacks transitioned in status epilepticus. Three patients (33%) did not show signs of AHC features. One of them (patient 9) experienced seizures during infancy that were first triggered by fever. The other two patients without AHC showed hemidystonia or muscular hypotonia, respectively (patients 6 and 7).

Six AHC patients were treated with flunarizine, which resulted in a significant reduction in frequency and duration of hemiplegic attacks in five patients (83%). Topiramate was administered in four cases, but only proved effective in one patient. Seizures, if present, were commonly refractory to standard antiepileptic drugs. The clinical manifestations, genetic findings and treatment information are summarized in Table 2.

3.2. Neurophysiological data and neuroimaging studies

Neurophysiological data (electroencephalography, EEG) were available in eight out of nine patients (89%). Of these eight patients, one (12.5%) revealed lateralized interictal epileptiform discharges (patient 2) and three patients (37.5%) showed signs of encephalopathy, which showed either bilateral focal (patient 7) or generalized patterns (patients 3 and 8) (Table 2).

Magnetic resonance imaging (MRI) of the brain results were obtained in eight patients (89%). Two of them (25%) revealed abnormal images shown in Fig. 1. The brain MRI of patient 2 revealed diffused atrophy with white matter gliosis of right cerebral hemisphere with Wallerian degeneration of the right brainstem consistent with hemiplegia-hemiconvulsion syndrome. In patient 6, T2*W gradient echo images revealed signal loss involving bilateral globi pallidi indicating deposition of paramagnetic substances.

3.3. ATP1A3 gene mutations

Pathogenic and likely pathogenic heterozygous missense variants in the *ATP1A3* gene with distinct changes of the polypeptide chain of the protein as depicted in Fig. 2A were identified in all nine unrelated patients. Three alterations (44%) have not been previously described. These novel mutations, c.2479A > T (p.Arg827Trp), c.2600G > T (p.Gly867Val) and c.2425G > C (p.Ala809Pro) were found in patients 2, 6 and 7, respectively (Table 3). They were located in the transmembrane domain VI (patient 7), the intracellular loop between domains VI and VII (patient 2) and the extracellular loop between domains VII and VIII (patient 6).Fig. 3.

The variants in patients 1, 2, 4, 5 and 7 occurred *de novo*. For patient 6, the variant was not identified in the mother but was not tested in the father due to unavailable paternal DNA. The parental sequencing was not performed in patients 3, 8 and 9. Several lines of evidence suggest that the previously undescribed variants are disease causing. The variants were neither identified in GnomAD nor in our in-house exome databases. *In silico* analysis predicted the amino acid changes to be possibly pathogenic (Table 3). The amino acid residues altered by the variants are highly conserved among N+/K + ATPase isoforms across different species (Fig. 2B). The variants were classified as "pathogenic" in eight cases and "likely pathogenic" in one (patient 6) according to the ACMG standard guidelines (Table 3). There was no correlation of the *ATP1A3* mutations with the clinical phenotype including severity of mental retardation, occurrence and length of hemiplegic disorders or seizure severity.

4. Discussion

We characterized nine unrelated patients with a broad spectrum of clinical manifestations of *ATP1A3*-related disorders. Genetic testing revealed eight different variants out of which three have not yet been described. All of these novel variants were located in the C-terminal domain of the ATPase cation transporter (Fig. 2A) (Ogawa et al., 2009; Heinzen et al., 2012; Heinzen et al., 2014). Previous studies suggest that amino acid alterations in the C-terminal domain cause structural changes and dysfunctions of the ATP1α3 protein (Hunanyan et al., 2015). As pointed out above, all our results obtained from analysis and prediction tools supported the pathogenicity of the newly discovered alterations. Still, further functional studies will be required to determine the pathogenicity of the novel variants.

Six patients were clinically categorized as AHC. Overlapping features with hemiconvulsion-hemiplegia-epilepsy (HHE) syndrome and neurodegeneration with brain iron accumulation (NBIA) were observed in patients 2 and 6, respectively. These findings expand the phenotypic spectrum of *ATP1A3*-related disorders. Although neuroimaging findings in patients with *ATP1A3*-related disorders are typically normal (Ishii et al., 2013), diffuse bilateral atrophy and cerebellar atrophy have been reported in a proportion of the cases in the subsequent clinical course of the disease (Ishii et al., 2013). However, severe unilateral brain atrophy as described in patient 2 carrying the novel p.Arg827Trp variant has not been reported in genetically proven *ATP1A3*-related disorders. One might speculate that recurrent status epilepticus could result in unilateral neuronal damage in this patient. This causal relationship was supported as an initial brain MRI at the age of two years was normal and the subsequent MRI at the age of six years (after the occurrence of several status epilepticus) revealed the extended atrophy of the right hemisphere. The patient also showed permanent hemiparesis of the left side. Thus, the clinical course of this patient is compatible with hemiconvulsion-hemiplegia-epilepsy (HHE) syndrome (Gastaut et al., 1960). The HHE syndrome is a rare condition caused by various etiologies (i.e. prolonged febrile seizures, viral infections, brain anomalies, cortical dysplasia) resulting in prolonged seizures. However, the causes of HHE could not be established in the majority of cases (Auvin et al., 2012). Several factors including excitotoxic cell injury, impairment of neuronal energy metabolism, inflammation and blood brain barrier permeability as well as genetic factors have been proposed to contribute to the pathogenesis of HHE (Auvin et al., 2012). Muta-

Table 2
Summary of the genetic and clinical features of all nine patients.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Current age/sex	7 years/Male	16 years/Male	21 years/Male	16 years/Female	7 years/Female	21 years/Female	0.9 year/Male	7 years/Male	19 years/Female
Phenotype	AHC	AHC/HHE	AHC	AHC	AHC	Right hemidystonia/ NBIA	RDP	AHC	Generalized hyperkinesia
Presenting symptom (s)	Seizures	Nystagmus(age 8 months)	Developmental regression(age 6 weeks)	Opsoclonus(age 5 months)	Disconjugate eye movements(age 5 months)	Right hemidystonia(age 12 years)	Developmental regression(age 2 months)	Reduced muscle tone and loss of head control(age 1 year and 7 months)	Choreoathetosis (age 2 months)
Seizure onset	5 months	11 months	13 years	5 years(single episode)	7 months	NA	NA	NA	2 months
Seizure semiologies	Tonic, focal clonic seizures, NCSE	Focal clonic, tonic seizures, GTC, SE	GTC, hypomotor seizures, SE	GTC	GTC	NA	NA	NA	Febrile seizures
Hemi/ quadriplegia onset	10 months	11 months	6 months	1 year and 3 months	7 months	NA	NA	6 months	NA
Frequency	1–2 per month	3–4 per month	2–3 per year	2–3 per month	2 per week	NA	NA	2–3 per month	NA
Duration	48–72 h	1–2 h	2–4 h	1–2 h	48–72 h	NA	NA	4–8 h	NA
Triggers	Not reported	Exercise	2–4 h	Fever, exercise, hot water	Cold bath	Not reported	Not reported	Weather change	NA
Other symptoms	NA	Gait ataxia,Left hemiparesis	NA	Gait ataxia	NA	NA	Muscular hypotonia, dystonia	Dystonia, eye rolling, opisthotonos	NA
EEG	Age 1 year and 8 months: normal	Age 2 years: epileptiform discharge over the left paracentral areas	Age 13 years: mild generalized encephalopathy. Intermittent slow bifrontal regions	Age 1.5 years: normal	Age 2 years: normal	Not performed	Moderate encephalopathy biposterior slowing	Generalized intermittent slow	Normal
Brain magnetic resonance imaging	Age 1 year and 6 months: normal	Age 2 years: normalAge 6 years: right cerebral atrophy	Not available	Age 5 years: normal	Age 2 years: normal	Age 19 years: Paramagnetic substances deposit bilateral globi pallidi	Age 3.5 months: normal	Normal	Normal
Development	Severe ID	Severe ID	Severe ID	Severe ID (IQ 23)	Severe ID	Mild ID	Severe ID	Mild ID	Severe ID
Medications	CBZ, LTG, TPM, flunarizine, LEV PB, PHT, Clonazepam, acetazolamide	PHT, PB, LEV, VPA, flunarizine	PHT, VPA, TPM, flunarizine, CLB, PB, chloral hydrate, ZNS	CBZ, TPM, flunarizine, propanolol	VPA, propanolol, flunarizine, PHT	Trihexyphenidyl, clonazepam, carbidopa-levodopa, baclofen	NA	TPM, flunarizine	VPA, PHB
Effective treatment(s)	None	flunarizine	flunarizine	flunarizine	flunarizine	None	NA	TPM, flunarizine	None
Genetic testing	WES trio	WES trio	Targeted sequencing	PCR-Sanger	PCR-Sanger	WES singleton	WES trio	Panel	WES singleton
ATP1A3 variants	c.2552A > Cp.Gln851Pro	c.2479A > T p.Arg827Trp	c.410C > A, p.Ser137Tyr	C.2429 T > Gp.Ile810Ser	C.2401G > Ap.Asp801Asn	c.2600G > T p.Gly867Val	c.2425G > C p.Ala809Pro	c.2263G > T p.Gly755Cys	C.2401G > Ap.(Asp801Asn)
	De novo	*De novo*	Not confirmed	*De novo*	*De novo*	Not confirmed	*De novo*	Not confirmed	Not confirmed

Table 2 (*Continued*)

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
	Reported (Yang et al., 2019)	Novel	Reported (Heinzen et al., 2012)	Reported (Hunanyan et al., 2015)	Reported (Heinzen et al., 2012; Rosewich et al., 2012; Ishii et al., 2013; Hoei-Hansen et al., 2014; Sasaki et al., 2014; Yang et al., 2014; Viollet et al., 2015)	Novel	Novel	Reported (Rosewich et al., 2012; Yang et al., 2014)	Reported (Heinzen et al., 2012; Rosewich et al., 2012; Ishii et al., 2013; Hoei-Hansen et al., 2014; Sasaki et al., 2014; Yang et al., 2014; Viollet et al., 2015)

CBZ; carbamazepine, GTC; generalized tonic clonic seizures, HHE; hemiconvulsion hemiplegia epilepsy syndrome, ID; intellectual disability, LEV; levetiracetam, NCSE; nonconvulsive status epilepticus, PB; phenobarbital, PHT; phenytoin, RDP; rapid onset dystonia-parkinsonism, SE; status epilepticus, TPM; topiramate, VPA; valproic acid, WES; whole exome sequencing, ZNS; Zonisamide, NA; not applicable

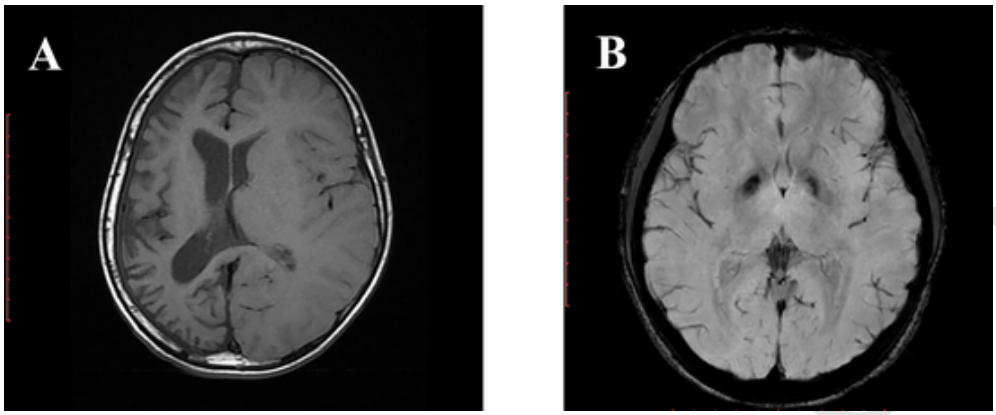

Fig. 1. A, A follow-up brain MRI at age 6 years in patient 2 showed diffuse atrophy of the right cerebral hemisphere. The prolonged seizures followed by atrophy of one hemisphere and hemiplegia ipsilateral to the side of convulsion is compatible with hemiconvulsion-hemiplegia-epilepsy (HHE) syndrome. B, Brain MRI at age 16 years in patient 6 revealed signal loss (dark signal) at bilateral globi pallidi seen on GRE T2*WI (gradient recalled echo T2-weighted imaging) and SWI (susceptibility weighted imaging) indicating deposition of paramagnetic substances.

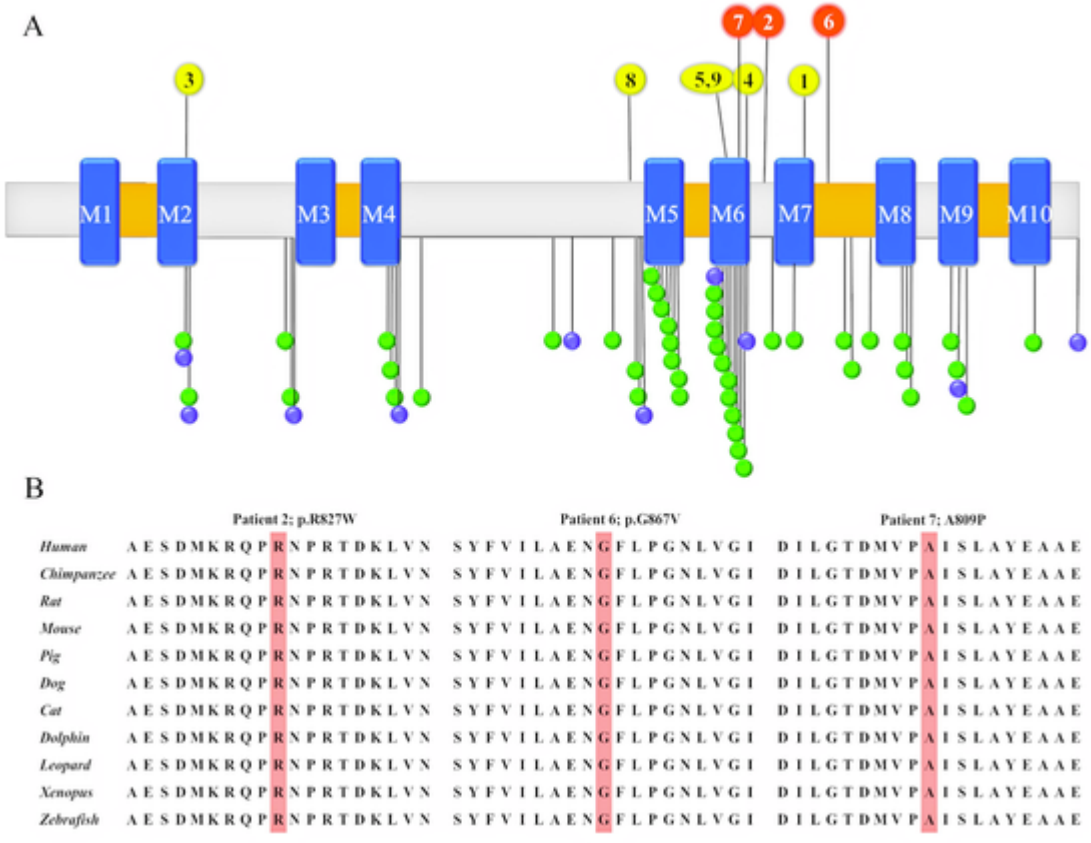

Fig. 2. A, The predicted location of amino-acid alterations in the ATP1A3 protein. red circles; novel variants identified in this study (2; p.Arg827Trp, 6; p.Gly867Val, 7; p.Ala809Pro,), yellow circles; previously reported variants identified in this study (1; p.Gln851Pro, 3; p.Ser137Tyr, 4; p.Ile810Ser, 5./9; p.Asp801Asn, 8; p.Gly755Cys), green dots; variants previously reported in patients with AHC, blue dots; variants previously reported in patients with RDP, gray bar; cytoplasmic domain, orange bar; extracellular domain. M1-10; transmembrane domains 1–10. B, Sequence alignment of partial amino acid sequence of ATP1A3 from various species. The arginine, glygine and alanine residues at codon 827, 867 and 809, respectively, are indicated by red bars.

tions within *SCN1A* and *CACNA1A1* were detected in some HHE cases (Sakakibara et al., 2009; Yamazaki et al., 2011). A case of a 15-year-old girl with clinical characteristics of AHC with status epilepticus and predominantly left-hemispheric cortical necrosis on brain MRI was reported but genetic testing was not mentioned in the article (Algahtani et al., 2017). Thus, the occurrence of HHE syndrome in patients with AHC should be acknowledged and would support timely and consequent treatment of any occurring status epilepticus in these patients.

Patient 6 harboring the novel p.Gly867Val variant had right hemidystonia started at age 12 years without parkinsonian features. For *ATP1A3*-related rapid onset dystonia-parkinsonism (RDP), the abrupt onset of generalized dystonia typically occurs during the second or third decade of life and accompanies with parkinsonian features (Heinzen et al., 2014). Notably, asymmetric dystonia is unusual but has been reported in a 24-year-old Chinese female with a p.Glu277Lys mutation in *ATP1A3* (Liu et al., 2016). The mechanism of laterality

Table 3
Interpretation of the identified variants in the *ATP1A3* gene.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Variant†	c.2552A > C(p.Gln851Pro)	c.2479A > T (p.Arg827Trp)	c.410C > A (p.Ser137Tyr)	C.2429 T > G(p.Ile810Ser)	C.2401G > A(p.Asp801Asn)	c.2600G > T (p.Gly867Val)	c.2425G > C (p.Ala809Pro)	c.2263G > T (p.Gly755Cys)	c.2401G > A (p.Asp801Asn)
Inheritance	*De novo*	*De novo*	Not confirmed	*De novo*	*De novo*	Not confirmed	*De novo*	Not confirmed	Not confirmed
SIFT	D	D	D	D	D	D	D	D	D
Polyphen-2	P.D.	P.D.	P.D.	P.D.	P.D.	P.D.	P.D.	P.D.	P.D.
M–CAP	P.P.	P.P.	P.P.	P.P.	P.P.	P.P.	P.P.	P.P.	P.P.
CADD	27.4	34	26.6	26.5	27.4	33	27.4	33	27.4
gnomAD	–	–	–	–	–	–	–	–	–
dbSNP	–	–	rs542652468	rs536681257	rs80356537	–	–	rs557052809	rs80356537
Classification‡	Pathogenic	Pathogenic	Pathogenic	Pathogenic	Pathogenic	Likely pathogenic	Pathogenic	Pathogenic	Pathogenic

D, deleterious; P.D., probably damaging; P.P., possibly pathogenic
SIFT, sorting intolerant from tolerant (http://sift.jcvi.org/); Polyphen-2, prediction of functional effects of human SNPs (http://genetics.bwh.harvard.edu/pph2/); M–CAP, Mendelian clinically applicable pathogenicity score (http://bejerano.stanford.edu/mcap/); CADD, combined annotation dependent depletion (https://cadd.gs.washington.edu/; recommended pathogenicity threshold greater than 20); dbSNP (https://www.ncbi.nlm.nih.gov/projects/SNP/); gnomAD, https://gnomad.broadinstitute.org/
† NCBI (National Center of Biotechnology Information) reference sequence: NM_152296.5
‡ According to the American College of Medical Genetics and Genomics interpretations guidelines (PMID 25741868).

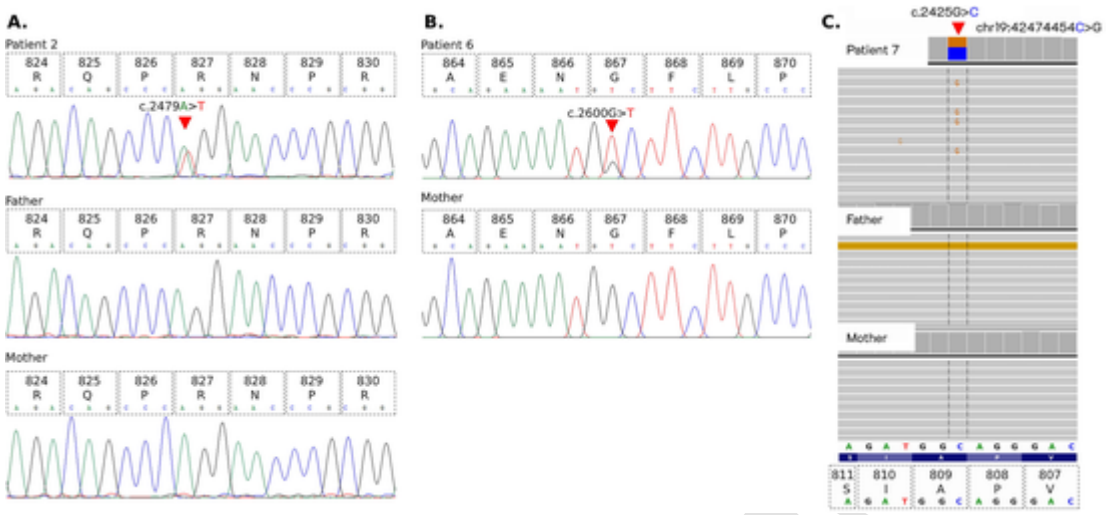

Fig. 3. A, Electropherograms showing the c.2479A > T (p.Arg827Trp) variant found in patient 2. B, Electropherograms showing the c.2600G > T (p.Gly867Val) variant found in patient 6. C, Sequence alignment from integrative genomics viewer (IGV) for c.2425G > C (p.Ala809Pro). The picture shows the genomic location chr19:42474454C > G (a reverse strand).

is unclear in both patient 6 and the previously reported case. Of note, alteration of the same amino acid residue p.Gly867Asp in the *ATP1A3* gene has been previously reported in a 15-year-old female with overlapping phenotypes between AHC and RDP (Rosewich et al., 2014). The neuroimaging findings in patient 6 are also unique. The brain MRI at age 16 years revealed signal loss on T2*-weighted gradient echo sequences involving bilateral globi pallidi. This resembles the neuroimaging findings associated with NBIA (neurodegeneration with brain iron accumulation) (Kruer et al., 2012). NBIA encompasses a group of heritable disorders characterized by deposition of iron or paramagnetic substances in the brain predominantly in the basal ganglia. The patients may present with dystonia, parkinsonism and/or neuropsychiatric abnormality. The mechanism of iron accumulation in the basal ganglia is secondary consequence of different primary mechanisms (Meyer et al., 2015). Several genes associated with NBIA play pivotal roles in neuronal health including regulation of membrane lipid homeostasis, maintaining the membrane potential, mitochondrial function and autophagy (Meyer et al., 2015). The *ATP1A3* gene encodes the transport proteins that maintain electrochemical gradient of Na + and K + across the plasma membrane and is highly expressed in GABAergic neurons of the basal ganglia. We speculate that *ATP1A3* dysfunction could cause disturbance in cellular biology of the basal ganglia nuclei and consequently lead to iron accumulation.

Here, flunarizine has been established as the first line drug for preventive therapy. The calcium entry blocker showed to reduce the severity and duration of AHC attacks in 50% and the frequency in 25% of 230 patients reviewed (Neville and Ninan, 2007). Several independent case studies supported the efficacy of flunarizine and the numbers were confirmed throughout (Mikati et al., 2000; Sasaki et al., 2001; Pisciotta et al., 2017). Five out of six patients in our cohort had reduced hemiplegic attacks after flunarizine. Nevertheless, flunarizine rarely leads to complete and long-lasting remission of AHC attacks. The second choice often is topiramate – an AED with promising effects in AHC patients. When administered in patients 1, 3 and 4, it was ineffective. There were trials of ketogenic diet in AHC patients (Ulate-Campos et al., 2014; Roubergue et al., 2015). Although it has not been administered in the patients in our cohort it is considered as another therapeutic approach.

As for RDP (patient 7), therapeutic options are sparse. Classic drugs in anti-Parkinson therapy such as levodopa fail to provide sufficient effects in improving Parkinsonian symptoms (Dobyns et al., 1993; Pittock et al., 2000; Brashear et al., 2007; McKeon et al., 2007; Brashear et al., 2012). Benzodiazepines, anticholinergics and tetrabenazine were reported to alleviate dystonia in a few patients (Brashear et al., 2007; Heinzen et al., 2014). There are rare case reports concerning the usage of deep brain stimulation in RDP patients. While some of them reported the absence of benefits (Deutschlander et al., 2005), some showed minimal benefits (Kamm et al., 2008). It was not performed in our RDP patients. Since patient 7 is still at a very young age, he has not yet received any treatment.

The limited sample size of our study might partly explain our inability to establish a clear genotype-phenotype correlation. In previous studies, the mutations associated with AHC phenotypes were clustered in the C-terminal cation ATPase domain (transmembrane domain 6) whereas the mutations associated with the RDP phenotype distributed across the *ATP1A3* gene and were more evenly distributed (Rosewich et al., 2012; Heinzen et al., 2014; Rosewich et al., 2014). Patients 5 and 9 demonstrate that genotype-phenotype correlation seems to be a general difficulty in *ATP1A3*. Although these two unrelated patients harbored the same mutation, p.Asp801Asn, their phenotypes differed widely. While patient 5 suffered from typical symptoms of AHC, patient 9 presented with severe developmental delay and hyperkinetic movements. This strongly supports the hypothesis that there are other factors influencing the severity of genetic diseases. This is consistent with findings of other recent studies concerning *ATP1A3*.

In conclusion, our study reports nine unrelated patients harboring *ATP1A3* causal variants displaying a broad range of neurological phenotypes. The use of comprehensive genomic approaches such as whole-exome sequencing unraveled not only novel genetic variants but also the linkage of unusual clinical manifestations to *ATP1A3*. Our findings support that mutations in the *ATP1A3* gene need to be considered in a broader context than previously expected. This extension beyond the currently defined phenotypes (AHC, RDP and CAPOS) implies HHE syndrome and NBIA and implies the application of next-generation sequencing early in the disease course in patients with early-onset seizures, movement disorders and developmental delay.

CRediT authorship contribution statement

Ponghatai Boonsimma: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Marius Michael Gasser:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Wiracha Netbaramee:** Investigation, Resources, Writing - review & editing. **Thanin Wechapinan:** Investigation, Resources, Writing - review & editing. **Chalurmpon Srichomthomg:** Methodology, Investigation, Writing - review & editing.

Chupong Ittiwut: Investigation, Data curation, Formal analysis, Writing - review & editing. **Matias Wagner:** Methodology, Investigation, Data curation, Formal analysis, Writing - review & editing. **Martin Krenn:** Investigation, Data curation, Writing - review & editing. **Fritz Zimprich:** Investigation, Data curation, Writing - review & editing. **Angela Abicht:** Investigation, Supervision, Writing - review & editing. **Saskia Biskup:** Investigation, Data curation, Writing - review & editing. **Timo Roser:** Resources, Writing - review & editing. **Ingo Borggraefe:** Conceptualization, Methodology, Supervision, Formal analysis, Resources, Writing - review & editing, Funding acquisition. **Kanya Suphapeetiporn:** Conceptualization, Supervision, Writing - original draft, Writing - review & editing, Project administration, Funding acquisition. **Vorasuk Shotelersuk:** Supervision, Resources, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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