ARTICLE

RHOBTB2 Mutations Expand the Phenotypic Spectrum of Alternating Hemiplegia of Childhood

Sara Zagaglia, MD,* Dora Steel, BMBCh,* S. Krithika, PhD, Laura Hernandez-Hernandez, PhD, Helena Martins Custodio, PhD, Kathleen M. Gorman, MD, Aikaterini Vezyroglou, MD, Rikke S. Møller, PhD, Mary D. King, FRCPCH, Trine Bjørg Hammer, PhD, Robert Spaull, MBBS, Walid Fazeli, MD, Tobias Bartolomaeus, MD, Diane Doummar, MD, Boris Keren, PhD, Cyril Mignot, PhD, Nathalie Bednarek, PhD, J. Helen Cross, PhD, Andrew A. Mallick, PhD, Alba Sanchis-Juan, PhD, Anna Basu, PhD, F. Lucy Raymond, FRCP, Bryan J. Lynch, FRCPI, Anirban Majumdar, FRCPCH, Hannah Stamberger, PhD, Sarah Weckhuysen, PhD, Sanjay M. Sisodiya, FRCP,* and Manju A. Kurian, PhD*

Correspondence
Prof. Kurian
manju.kurian@ucl.ac.uk
or Prof. Sisodiya
s.sisodiya@ucl.ac.uk

Neurology® 2021;96:e1539-e1550. doi:10.1212/WNL.0000000000011543

Abstract

Objective

To explore the phenotypic spectrum of *RHOBTB2*-related disorders and specifically to determine whether patients fulfill criteria for alternating hemiplegia of childhood (AHC), we report the clinical features of 11 affected individuals.

Methods

Individuals with *RHOBTB2*-related disorders were identified through a movement disorder clinic at a specialist pediatric center, with additional cases identified through collaboration with other centers internationally. Clinical data were acquired through retrospective case-note review.

Results

Eleven affected patients were identified. All had heterozygous missense variants involving exon 9 of *RHOBTB2*, confirmed as de novo in 9 cases. All had a complex motor phenotype, including at least 2 different kinds of movement disorder, e.g., ataxia and dystonia. Many patients demonstrated several features fulfilling the criteria for AHC: 10 patients had a movement disorder including paroxysmal elements, and 8 experienced hemiplegic episodes. In contrast to classic AHC, commonly caused by mutations in *ATP1A3*, these events were reported later only in *RHOBTB2* mutation–positive patients from 20 months of age. Seven patients had epilepsy, but of these, 4 patients achieved seizure freedom. All patients had intellectual disability, usually moderate to severe. Other features include episodes of marked skin color change and gastrointestinal symptoms, each in 4 patients.

Conclusion

Although heterozygous *RHOBTB2* mutations were originally described in early infantile epileptic encephalopathy type 64, our study confirms that they account for a more expansive clinical phenotype, including a complex polymorphic movement disorder with paroxysmal elements resembling AHC. *RHOBTB2* testing should therefore be considered in patients with an AHC-like phenotype, particularly those negative for *ATPA1A3* mutations.

MORE ONLINE

Videos

*These authors contributed equally to this work.

From the UCL Queen Square Institute of Neurology (S.Z., S.K., L.H.-H., H.M.C., S.M.S.), London; Chalfont Centre for Epilepsy (S.Z., S.K., H.M.C., S.M.S.), Buckinghamshire; Department of Neurology (D.S., A.V., J.H.C., M.A.K.), Great Ormond Street Hospital; Clinical Neurosciences (A.V., J.H.C.), and Neurogenetics Group (D.S., M.A.K.), Developmental Neurosciences NIHR BRC UCL Great Ormond Street Institute of Child Health, London; School of Life Sciences (S.K.), Faculty of Science and Engineering, Anglia Ruskin University, Cambridge, UK; Department of Neurology and Clinical Neurophysiology (K.M.G., M.D.K., B.J.L.), Children's Health Ireland at Temple Street, Dublin 1; School of Medicine and Medical Sciences (K.M.G., M.D.K.), University College Dublin, Dublin 4, Ireland; Danish Epilepsy Centre (R.S.M., T.B.H.), Dianalund; Department of Regional Health Research (R.S.M.), University of Southern Denmark, Odense; Department of Paediatric Neurology (R.S., A.A.M., A.M.), Bristol Royal Hospital for Children, UK; Pediatric Neurology (W.F.), Department of Pediatrics, Faculty of Medicine and University Hospital Cologne, University of Cologne; Institute of Human Genetics (T.B.), University of Leipzig Medical Center, Germany; Departement de Neuropediatrie (D.D.), Centre de Référence Neurogénetique Mouvements Anormaux, Hôpital Armand Trousseau, and Department of Genetics (B.K., C.M.), La Pitié-Salpêtrière Hospital, APHP, Sorbonne University, Paris; Centre de Référence Déficiences Intellectuelles de Causes Rares (C.M.); Departement de Pediatrie (N.B.), American Memorial Hospital, CHU Reims; CReSTIC (N.B.), University of Reims Champagne-Ardennes, France; University of Bristol (A.A.M.); Department of Haematology (A.S.-J.) and Cambridge Institute for Medical Research (F.L.R.), University of Cambridge; NIHR BioResource (A.S.-J., F.L.R.), Cambridge University Hospitals NHS Foundation Trust; Paediatric Neurology (A.B.), Great North Childrens Hospital, Newcastle upon Tyne; Population Health Sciences Institute (A.B.), Newcastle University, UK; Applied & Translational Genomics Group (H.S., S.W.), VIB-Center for Molecular Neurology, University of Antwerp; and Department of Neurology (H.S., S.W.), University Hospital Antwerp, Belgium.

Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Glossary

AHC = alternating hemiplegia of childhood; **CAPOS** = cerebellar ataxia, arreflexia, pes cavus, optic atrophy, and sensorineural hearing loss; **RRD** = *RHOBTB2*-related disorders.

Heterozygous variants in *RHOBTB2*, encoding an atypical Rho GTPase, have recently been reported in the developmental and epileptic encephalopathies.[1–3] Affected patients present with developmental delay and onset of epilepsy in the first 3 years of life.[2] Given that it is a newly identified genetic disorder, the phenotypic spectrum of *RHOBTB2*-related disorders (RRD) has not yet been fully delineated. We report a cohort of individuals with RRD whose presentation included a prominent motor phenotype with a complex polymorphic movement disorder. In several cases, the clinical features resembled those reported in patients with alternating hemiplegia of childhood (AHC).[4]

Methods

Patients were identified through the Neurogenetic Movement Disorders Clinic at Great Ormond Street Hospital and the Epilepsy Genomics service at the National Hospital for Neurology and Neurosurgery, Queen Square. Additional cases were identified through collaborating neurologists and geneticists in Ireland, Denmark, Belgium, Germany, and France. Data were obtained through a retrospective review of clinical records.

Molecular genetic diagnosis was made by whole-exome sequencing, whole-genome sequencing, or gene panel testing. All results were confirmed through accredited diagnostic laboratory services except patient 10, whose diagnosis was made through research triome whole-genome sequencing with Sanger sequencing confirmation in the proband and family (REC reference 13LO168), and patients 4, 5, and 9, who were diagnosed through research whole-exome sequencing. Genetic variants not previously reported in the literature were assessed for pathogenicity following standard American College of Medical Genetics guidelines.[5] Seizures were classified according to the 2017 International League Against Epilepsy classification and terminology.[6]

Standard Protocol Approvals, Registrations, and Patient Consents

This project was approved by the Great Ormond Street Research and Development Office (reference No. 19NM23). Because it involved retrospective collection of fully anonymized data collected during clinical care, written consent from patients' guardians was not sought but verbal assent was. When identifiable videos and photographic images are used, written informed consent for their review and publication was provided by patients or their parents or guardians.

Data Availability

The data relevant to this article are published within the text and tables.

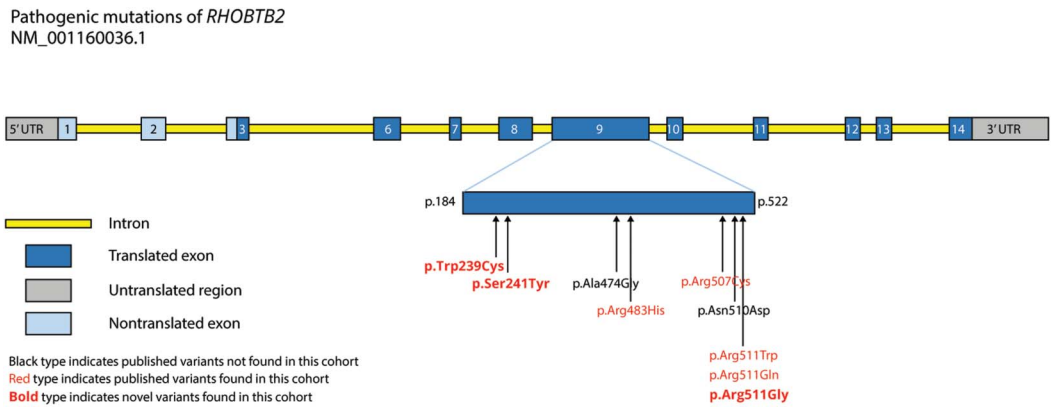
Table 1 Genetic Variants Found in RRD Cohort

Genomic DNA Variant (Chr8; GRCh37)	cDNA Change (NM_001160036.1)	Protein Change	Patients Affected	Inheritance	Previously Published as Pathogenic? (Reference)	ACMG Classification[4] if Not Previously Published (Criteria Met)
g.22865224 G>A	c.1532G>A	p.Arg511Gln	1– 3	De novo (patients 1, 2) Unconfirmed[a] (#3)	Yes[3]	NA
g.22865223C > G	c.1531C>G	p.Arg511Gly	4	De novo	No	Likely pathogenic (PS2 + PM2,5 + PP2,3)
g.22865223C > T	c.1531C>T	p.Arg511Trp	5, 6, 9	De novo (all)	Yes[2]	NA
g.22865211C > T	c.1519C>T	p.Arg507Cys	7	Unconfirmed[a]	Yes[3]	NA
g. 22865140 G>A	c.1448G>A	p.Arg483His	8	De novo	Yes[2]	NA
g.22864414C > A	c.722C>A	p.Ser241Tyr	10	De novo	No	Likely pathogenic (PS2 + PM2 + PP2,3)
g.22864409 G>C	c.717G>C	p.Trp239Cys	11	De novo	No	Likely pathogenic (PM1 + PM2 + PP2,3)

Abbreviations: ACMG = American College of Medical Genetics; CADD = Combined Annotation Dependent Depletion; gnomAD = Genome Aggregation Database; HumVar = human variation; NA = not applicable; RRD = *RHOBTB2*-related disorders; SIFT = Sorting Intolerant From Tolerant.
[a]Parental DNA not available.

Continued

Figure 1 Pathogenic Mutations Within the *RHOBTB2* Gene



Note that the longest transcript, NM_001160036.1, does not include exons 4 and 5. UTR = untranslated region.

Results

Eleven unrelated patients were identified, of whom 4 were female. Age at the latest clinical review ranged from 2 to 60 years (mean age 16.3 years, median age 9 years).

Molecular Genetic Findings

All patients had heterozygous missense variants in *RHOBTB2*. Seven of 11 involved the amino acid residue at position 511. All the other identified variants also occurred within exon 9 (figure 1). Three of the variants were novel (c.1531C>G, p.Arg511Gly; c.722C>A, p. Ser241Tyr; and c.717G>C, p. Trp239Cy) and were classified as likely pathogenic according to American College of Medical Genetics guidelines.[5] Parental testing was available for 9 of 11 patients and in all cases confirmed de novo status (table 1).

Clinical Features

Movement Disorder

All patients had motor features as part of their clinical phenotype. Severity varied widely, from lack of any head or truncal control (patient 11) to inability to walk (patient 7, apparently due to a combination of hypotonia, dystonia, and ataxia) to mild balance difficulties with near-normal gait (patient 10). Pyramidal signs (such as brisk reflexes or a positive Babinski sign) were evident in 5 of 11 patients, although clear velocity-dependent spasticity was present in only 2 of them. In addition to gait difficulties, 5 of 11 patients had a generalized hyperkinetic choreiform movement disorder (videos 1–3). Ten of 11 patients had additional paroxysmal movements: this included hemiplegic episodes in 8, episodic focal dystonia in 7 (videos 4–6), episodic ataxia in 4, episodic

Table 1 *(continued)*

CADD Score	Mutation Taster	PolyPhen-2 (HumVar)	SIFT	gnomAD Minor Allele Frequency
32	Disease causing (1)	Probably damaging (0.999)	Tolerated (0.11)	Absent
27.6	Disease causing (1)	Probably damaging (0.999)	Deleterious (0.05)	Absent
33	Disease causing (1)	Probably damaging (1.000)	Deleterious (0.01)	Absent
32	Disease causing (1)	Probably damaging (0.931)	Deleterious (0)	Absent
31	Disease causing (1)	Probably damaging (1.000)	Deleterious (0.03)	Absent
26	Disease causing (1)	Probably damaging (0.999)	Deleterious (0)	Absent
27.9	Disease causing (1)	Probably damaging (0.999)	Deleterious (0)	Absent

Table 2 Patients With RRD: Epilepsy, EEG Features, Medications, and Other Neurologic/Systemic Features

Patient	Age at Last Review, y	Epilepsy Confirmed?	EEG Findings	Relevant Medication	MRI Findings
1	8	No: only confirmed seizures were febrile convulsions	Initially mild slowing only. From 7 y, multifocal discharges appeared. Multiple episodes had no EEG correlate.	Topiramate, gabapentin (stopped); levetiracetam Betashot; flunarizine; carbamazepine, phenobarbitone; clonidine	Thin corpus callosum
2	2	Yes: onset at 5 mo with SE. Seizure types: focal with and without impairment of awareness and evolution to bilateral tonic-clonic; atypical absences. Seizure-free >1 y.	Initially slowing only; subsequently focal seizures captured. Multiple episodes had no EEG correlate.	Sodium valproate (stopped); carbamazepine	Normal
3	37	Yes: onset at 18 mo. Seizure types: focal onset with impaired awareness.	Slowing and epileptiform activity (NB first EEG at 10 y old).	Vigabatrin (stopped); carbamazepine; clobazam	None available
4	20	No: but had electrographic seizures during hemiplegic episode at 3 y of age.	Focal electrographic seizures during hemiplegic episode; subsequent EEGs slowing only.	Carbamazepine; acetazolamide	Mild static cerebellar atrophy
5	14	Yes: onset 3 mo. Seizure types: focal onset with and without impairment of awareness and evolution to bilateral tonic-clonic; SE ×2. Seizure-free >5 y.	Initially slowing with focal discharges. Subsequently slowing and asymmetry; no further epileptiform activity. Multiple episodes had no EEG correlate.	Phenobarbitone; phenytoin; pyridoxine (all stopped); carbamazepine	Normal
6	5	Yes: onset at 3 mos. Seizure types: focal onset (some of myoclonic subtype) with and without impairment of awareness and evolution to bilateral tonic-clonic; with secondary generalization; atypical absences. Seizure-free ≈1 y.	Initially normal; from 6 months frontal sharp waves noted.	Sodium valproate; oxcarbazepine; pyridoxine; clobazam; lamotrigine (all stopped); acetazolamide	Nonspecific left occipital changes
7	60	Yes: onset at 1 y. Seizure types: focal onset with impairment of awareness and evolution to bilateral tonic-clonic	Initially normal; subsequently abnormal background; epileptiform activity captured occasionally	Sodium valproate; lamotrigine; fosphenytoin; levetiracetam (all stopped); topiramate; zonisamide; clobazam; carbamazepine	None available
8	3	No	No epileptiform activity; poor organisation	Flunarizine; topiramate	Mild widening of temporal horns
9	3	Yes: onset at 11 wk. Seizure types: left focal onset with and without impairment of awareness and evolution to bilateral tonic-clonic; tonic. Seizure-free 2 y.	Slow background activity; left frontocentral spike and slow wave discharges during sleep	Levetiracetam; clobazam; sodium valproate (all stopped)	Meningeal enhancement at time of SE. Thin corpus callosum; delayed myelination
10	18	No	Spikes and slow waves during encephalopathic episode at 3 y of age. Subsequently normal. Multiple episodes had no EEG correlate.	Nil	Normal
11	9	Yes: onset at 4 y 6 mo at time of encephalopathic episode. Generalized-onset tonic and tonic-clonic seizures.	Generalized spike and slow wave during SE. Otherwise, slowing only.	Topiramate; levetiracetam; carbamazepine; clobazam; ketogenic diet	Normal at 4 y of age; cereberocerebellar atrophy at 5 y of age

Abbreviations: RRD = *RHOBTB2*-related disorders; SE = status epilepticus.

Continued

exacerbation of dyskinesia in 4, and nonepileptic myoclonus in 2 patients. Three patients also exhibited stereotypies. The pattern and nature of paroxysmal movements often varied for each individual over time. For example, in patient 1, dyskinetic episodes were prominent when he was a toddler and then settled with age before re-emerging at 8 years of age. Five

Table 2 *(continued)*

Intellectual Disability	Neurodevelopmental Regression	Behavioral/ Psychiatric Features	Encephalopathic Episode?	Other
Severe (uses single words)	At times of increased abnormal movement (13 mo, 28 mo, 7 y)	Irritability (correlates with increased abnormal movements)	Possibly: episodes of regression with increased abnormal movements	Episodes of gastrointestinal discomfort; episodes of pallor; acquired microcephaly
Moderate to severe global developmental delay (no speech yet)	No	Emotional lability	Possibly: but sudden onset of epilepsy with SE	Gastroesophageal reflux disease; episodes of redness/ pallor; acquired microcephaly
Severe (nonverbal)	No	Challenging behavior (aggression)	No	Nil
Severe (nonverbal; some understanding of speech)	No	Nil	Possibly: prolonged hemiplegic episode with abnormal contralateral EEG at 3 y of age	Nil
Severe (nonverbal)	No, but developmental slowing concurrent with epilepsy onset	Behavioral and sensory issues	Possibly: sudden onset of epilepsy with SE	Episodes of redness/ pallor
Severe (nonverbal, some understanding of speech)	No, but developmental stagnation at 3 y concurrent with increased seizures/ movements	Nil (sociable and happy)	Yes: at 3 mo, fever, rash, cluster of seizures (negative bacterial cultures)	Constipation
Moderate (speaks in 2-word phrases)	Unknown	Nil	No	Constipation
Moderate (speaks a few words; understands instructions)	Yes, motor regression at 30 mo of age, after prolonged hemiplegic episode	Challenging behavior	No	Nil
Severe developmental delay	Developmental stagnation at 11 wk (onset of epilepsy)	Nil	Possibly: but sudden onset of epilepsy with SE	Nil
Mild	No	Emotional lability	Yes: drowsiness, vomiting, and abnormal EEG at 3 y of age	Nil
Profound (nonverbal) after regression	Yes: severe lasting regression after encephalopathic episode at 4 y 6 mo	Nil	Yes: 3-mo comatose illness with SE and hypothermia at 4 y 6 mo	Episodes of redness/ pallor

families described reliable sensory triggers such as temperature change, which elicited episodes of abnormal movement. This appeared to relate to a sudden change in temperature, for example, coming out of the bath, rather than to extremes of environmental temperature per se. Car journeys also triggered events in some families (of patients 1 and 2), possibly associated with changes in position, movement, or temperature. Two other families (of patients 4 and 5) reported a diurnal variation in episodes of unsteadiness, with increased occurrence in the mornings (tables 2 and 3).

Table 3 RRD Patients: Baseline and Paroxysmal Movement Disorders

Patient	Sex	Age at Last Review, y	Motor Abilities	Baseline Movement Disorder				
				Any?	Dyskinesia/Chorea	Dystonia	Ataxia/ Unsteadiness	Pyramidal Signs
1	M	8	Walks a few steps with support	Y	Y (generalized, improved with age)	N	Stiff-legged, arrhythmic gait	Brisk reflexes only
2	M	2	Sat at 20 mo, not yet walking	Y	Y	N	Balance poor	Brisk reflexes only
3	M	37	Walked from 3 y	Y	Y (orofacial)	Y (trunk, neck, hands)	Broad-based gait	Brisk reflexes, unilateral upgoing plantar
4	M	20	Walked from 3 y	Y	N	N	Broad-based (and crouched) gait	N
5	M	14	Gait broad-based	Y	N	N	Broad-based gait	N
6	F	5	Walked from 2 y, gait ataxic	Y	N	Y	Y	N
7	F	60	Unable to walk	Y	?	?	?	?
8	M	3	Walked at 27 mo	N	N	N	N	N
9	F	3	Walks with support	Y	N	N	N	Spasticity, brisk reflexes
10	F	18	Walked at 1 years, gait normal	Y	Y (upper limb and mild orofacial)	N	Mildly poor balance	N
11	M	9	Bedridden, no head control	Y	Y	Y	NA: no truncal control	Spasticity, brisk reflexes

Abbreviation: NA = not applicable; RRD = *RHOBTB2*-related disorders.

Continued

Hemiplegic episodes were not reported in infancy. The earliest such episode was seen at 20 months of age (patient 8), although for the adult patients exact age at onset was not recorded. These episodes consisted of unilateral or asymmetrical weakness accompanied by what parents described as a vacant appearance (although without loss of responsiveness to external stimuli), associated with drooling and reduced activity (videos 7 and 8). Hemidystonia was reported in 1 patient (patient 8). Duration varied from minutes to 2 weeks (patients 6 and 7). In patient 6, an EEG undertaken during the longest hemiparetic episode showed contralateral voltage reduction but no epileptiform discharges, rendering nonconvulsive status epilepticus unlikely. In some but by no means all cases, hemiplegic episodes followed an identifiable trigger such as a seizure (patient 7) or a minor head injury (patient 6).

Epilepsy

Seven of 11 patients had a diagnosis of epilepsy (mean age at onset 14 months, range 11 week–4 years 6 months), and 2 others had experienced at least 1 seizure (electrographic seizures in patient 4 detected on EEG at 3 years of age, and febrile convulsions in patient 1 at 8 months of age). All of the patients with epilepsy had at least 1 EEG, either ictal or interictal, which showed focal or multifocal epileptiform discharges. Most interictal recordings either were normal or showed only nonspecific slowing. The commonest seizure types were focal seizures with or without impaired awareness and/or evolution to bilateral tonic-clonic seizures, seen in 6 of 7 patients.

Five patients (patients 2, 5, 6, 9, and 11) experienced status epilepticus. For 4 of them (patients 2, 5, 6, and 9), this occurred at between 2 and 5 months of age. For patient 6, but not the others, status epilepticus occurred in the context of a

Table 3 *(continued)*

Paroxysmal Movement Disorder							
Any?	**Dyskinesia/ Chorea**	**Dystonia**	**Ataxia/ Unsteadiness**	**Generalized Weakness**	**Hemiplegia**	**Other Episodes**	**Triggers**
Y	Y (variable frequency; general, minutes)	Y (focal or generalized; seconds to minutes)	N	Y (daily, up to 30 mins)	Y (asymmetry more evident with age)	Tongue protrusion, head and eye deviation	Temperature changes; car travel; excitement
Y	Y (many per day; general, minutes)	Y (general, 30 s)	N	Y (daily, a few minutes)	Y	Head and eye deviation (seconds, frequent)	Car travel
N	N	N	N	N	N	Stereotypies	N
Y	N	N	Y (increased unsteadiness 1–2 h, mornings)	N	Y (infrequent, hours–days, from 3–8 y of age only)	Stereotypies	N
Y	Y	Y (limb posturing)	Y	Y	N	Tongue protrusion, head and eye deviation	N
Y	N	Y (focal: L ankle)	Y (brief episodes most days)	N	Y (3 episodes lasting hours to 2 wk; first episode at 2 y)	Tremor myoclonus (epileptic or movement?)	2 Hemiplegic episodes triggered by minor head injuries
Y	N	Episodes of arm stiffening (dystonia?)	N	N	Y (infrequent; last hours–2 wk)	N	Some hemiplegic episodes follow seizures
Y	N	Y	N	N	Y (from 20 mo; flaccid paresis or hemidystonia lasting hours–3 d)	Episodes of head and eye deviation	Fatigue; fever
Y	Y (3 times a day, 1–3 min, arms and trunk)	N	Y (2–4 times a day, very brief)	N	N	N	Movement; fever; excitement
Y	N	Y (focal, brief, infrequent)	N	N	Y (up to 1 h, infrequent)	Stereotypies; myoclonus (mild)	No
Y	Y	Y	N	N	Y (age 4 y, single episode lasting 10 d)	Nystagmus and mouth opening	Illness; fatigue; bathing

febrile illness. Viral encephalitis was suspected, but no infective agent was found in blood or CSF. She had clustered seizures requiring intubation and ventilation for a period of 6 days (including 2 extubation attempts that failed due to recurrence of seizures). The duration of the episode is not known for patient 5 but was ≈40 minutes in patient 2 and 2 hours in patient 9. Only 1 of these patients, patient 5, went on to have a second episode of status epilepticus, which occurred at 8 years of age. Patient 11 had a single prolonged episode of status epilepticus at 4 years 6 months of age in the context of a severe encephalitis-like episode during which he was comatose for 3 months and experienced hypothermia and autonomic disturbances. After this, he experienced severe and permanent neurodevelopmental impairment.

A number of events clinically suspected to be seizures such as myoclonus, paroxysmal lateral deviation of the head and eyes, and behavioral changes were captured on EEG in some patients (patients 1, 2, 5, 8, 10, and 11) and proved to have no electrographic correlate. For patients 4, 6, and 7, however, ictal EEG was not captured for the majority of event types.

All patients except patients 9 and 10 were taking antiseizure medications at their last review, although in several instances, the main indication was for treatment of abnormal movements rather than epilepsy. The most widely used drug was carbamazepine in 7 of 11 patients, which was felt to be clearly beneficial in at least 2 cases (patient 1 and 5), resulting in reduced frequency of episodes, possibly including reduced paroxysmal movements and epileptic seizures. Topiramate improved seizure control in at least 1 patient (patient 7), whose seizures reduced from 20 per week to 1 per week, and another patient (patient 8, who did not have epilepsy) experienced reduced frequency of abnormal movements on a combination of topiramate and flunarizine. (In another patient [patient 1], however, neither topiramate nor flunarizine showed any benefit

for nonepileptic paroxysmal movements.) Two patients (patients 6 and 4) also experienced some improvement with acetazolamide, which was prescribed primarily to treat episodic ataxia. One family (of patient 1) also reported a benefit from a medium-chain triglyceride dietary supplement.[7] The effect of other medications was less clear in this patient cohort.

Other Paroxysmal Events

Eight patients (patients 1, 2, 4–6, and 9–11) had a history of an acute encephalitis-like illness, but the forms this took were variable. In 5 cases (patients 2, 5, 6, 9, and 11), the episode included convulsive status epilepticus, as described above. Two of these episodes (patients 6 and 11) also included thermoregulatory changes—hypothermia in patient 11 and hyperthermia in patient 6—and skin changes, described as flushing in patient 11 but simply as rash in patient 6. Patient 6 in addition went on to have 3 further episodes of loss of consciousness between 3 and 4 years of age, 2 of which immediately followed minor head trauma. Vomiting occurred on at least 1 occasion. Unconsciousness lasted for ≈24 hours on the first occasion and a few hours on the subsequent times but was followed by hemiplegia, which took up to 2 weeks to resolve. EEG was captured during unconsciousness on 2 of these occasions and did not show any epileptiform activity, although on the first occasion voltage was reduced in the hemisphere contralateral to the hemiplegia.

At the age of 3 years, patient 4 had an episode of hemiplegia lasting several hours associated with contralateral electrographic seizure activity in the midtemporal region. MRI of the brain in the acute phase showed changes consistent with acute ischemia in the affected hemisphere but subsequently normalized. Patient 10, also at the age of 3 years, experienced an episode of vomiting and drowsiness severe enough to require brief mechanical ventilation. EEG during the episode was encephalopathic but did not show seizure activity. Patient 1 had 3 separate episodes of behavioral and neurodevelopmental regression associated with increased frequency of paroxysmal abnormal movements and increased vasomotor skin changes at 13 months, 28 months, and 7 years of age. His EEG initially showed only mild slowing, but epileptiform discharges—without clinical correlate—appeared only from the age of 7 years on.

Paroxysmal abnormal eye movements were reported in patients 1, 2, 5, 8, and 11. In patients 1, 2, 5, and 8, these took the form of brief lateral deviation of both eyes, sometimes accompanied by turn of the head in the ipsilateral direction and/or by tongue protrusion. These events were captured on EEG and had no electrographic ictal correlate. Events could occur multiple times per day but were only seconds in duration. Patient 11 had episodes of nystagmus.

One patient (patient 1) experienced recurrent episodes of respiratory disturbance starting at the age of 7 years. These consisted of shallow or irregular breathing or sometimes complete apnea requiring bag-valve-mask ventilation, accompanied by tachycardia and pupillary dilation, lasting for up to 20 minutes. Ventilation was not technically challenging, and there was usually no laryngeal dystonia. The child often remained able to move his head and appeared to be aware of people around him, but his limbs and trunk were paralyzed and flaccid. Episodes could occur from sleep or waking, and there were no obvious triggers. There was no postevent drowsiness. The nature of these episodes remains unclear.

Other reported episodic phenomena included striking episodes of flushing or pallor, sometimes associated with increased abnormal movements, in 4 individuals (patients 1, 2, 5, and 11; figure 2).

Other Comorbid Conditions

All patients had intellectual disability. Formal IQ testing was not available for most patients, but for 10 of 11, intellectual disability was clinically assessed as moderate or severe, with either absent or significantly impaired verbal communication and comprehension. Patient 10, however, had a milder impairment compared to the rest of the cohort. Only 2 patients experienced developmental regression. In patient 11, there was a severe and lasting regression at the age of 54 months, following a prolonged encephalopathic episode accompanied by status epilepticus and resulting in near-total and permanent loss of developmental abilities. In patient 1, regression occurred on 3 occasions, each time coinciding with exacerbation of the movement disorder. In 3 other patients (patients 5, 6, and 9), developmental slowing was noted after the onset of epilepsy, which in all these cases was very early (≤3 months of age). Milestones (such as social smiling, visual attentiveness, and partial head control) before this age had been apparently normal but were delayed afterward. Patient 6 also experienced a further period of developmental stagnation at 3 years of age at a time of increased seizure frequency.

Marked emotional lability was reported in 2 patients (patients 2 and 10), irritability in 1 patient (patient 1), and challenging behavior in 3 more individuals (patients 3, 5, and 8). Gastrointestinal symptoms, including gastroesophageal reflux, constipation, and episodes of abdominal discomfort, were described in 4 patients (patients 1, 2, 6, and 7).

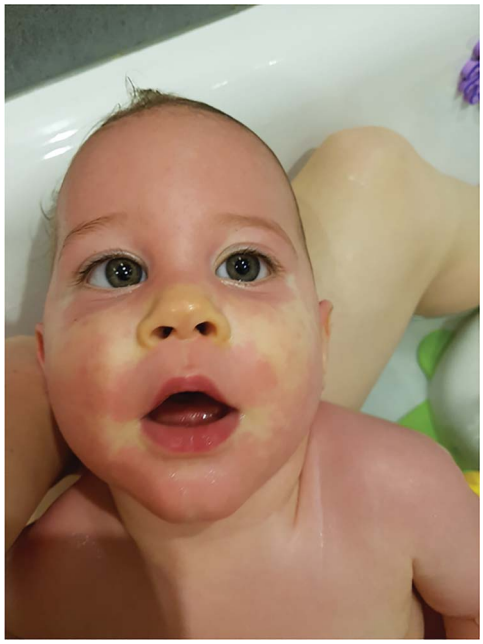
Brain MRI Findings

MRI brain results were available for 9 of 11 patients. Three patients had normal scans, and 1 other patient (patient 11) had a normal scan at 4 years of age with cerebrocerebellar atrophy apparent on a scan a year later, after a period of significant developmental regression. Two patients (patients 1 and 9) had a thin corpus callosum. Other findings found in 1 patient each included mild static cerebellar atrophy (patient 4), mild dilation of the temporal horns (patient 10), nonspecific occipital lobe changes (patient 6), delayed myelination (patient 9), and meningeal enhancement seen on a scan performed at the time of an episode of status epilepticus (patient 9).

Discussion

RHOBTB2 is a relatively newly recognized disease-causing gene. It encodes a Rho GTPase, one of a family of proteins

Figure 2 Patient 2, 1 Year of Age, Demonstrating an Episode of Patchy Erythema and Pallor During a Bath



involved in regulating actin dynamics, and is believed to play a key role in cell morphology and, more specifically, dendritic arborization. It is highly expressed in brain, especially the cortex and basal ganglia.[8]

Pathogenic mutations in *RHOBTB2* have previously been reported in 13 patients.[2,3] All reported variants are de novo missense changes, suggesting either complete or very high penetrance. Several of the reported variants are recurrent mutations, an observation further confirmed in our study, in which 7 of the 9 mutations are previously described. Moreover, all known pathogenic variants are found clustered in exon 9, which encodes the BTB1 protein domain and part of BTB2. In our cohort, 7 of 11 patients carried a pathogenic variant affecting residue Arg511. This residue was also identified as the most frequent mutational hotspot in previously reported cohorts.[2,3] We did not, however, identify a significant difference in phenotype between patients who had a mutation involving this residue and those with mutations affecting other sites. Exploration of any genotype-phenotype correlation would require analysis of a larger cohort, which may be expected to become available as more patients are diagnosed with RRD. Although our most mildly affected patient (patient 10) has a mutation slightly outside the main cluster of reported pathogenic variants, so too does patient 11, who has an exceptionally severe phenotype (figure 1).

Developmental and epileptic encephalopathy was a universal feature in previously reported cases, with all having onset of seizures by 4 years of age and 11 patients presenting before 1 year of age. Therefore, this disorder was designated early infantile epileptic encephalopathy type 64 (Mendelian Inheritance in Man 618004), although it is clear that the clinical phenotype is more extensive. Epilepsy is not always present, and besides epilepsy, RRD can also present with a number of other comorbid conditions, including intellectual disability, neurobehavioral features, acute encephalopathic episodes, and, as demonstrated by our data, a prominent movement disorder.

Eleven of 13 of the previously reported cases were deemed to have a movement disorder of some description, including dystonia, dyskinesia, paroxysmal disorders, and stereotypies, but this has not been described in detail. All 11 patients in our cohort display a complex, polymorphic movement disorder, usually with both a paroxysmal and nonparoxysmal element. This was, in most cases, a more salient and disabling feature than their epilepsy.

All patients within our cohort had a complex motor phenotype, with at least 2 types of movement disorder. Most patients experienced a hyperkinetic movement disorder with dystonia and/or dyskinesia, but other abnormal movements such as myoclonus were also seen. Severity of functional impact varied widely: 3 of 11 patients were nonambulant; 2 could walk only with support; and 1 had only mild gait disturbance. Gait difficulties in RRD may arise as from a combination of delayed motor development and an underlying movement disorder.

The paroxysmal movement disorders were also variable but notably in the majority included paroxysmal hemiplegia. This differed somewhat from classic AHC caused by mutations in *ATP1A3*[9] in that hemiplegic episodes first appeared later in childhood, from 20 months on rather than by 18 months as in the accepted criteria of AHC[4] (table 4). The hemiplegic spells appeared to share many features with episodes that were characterized by symmetrical weakness, affecting both sides of the body, often accompanied by pallor, quietness, and a vacant appearance but continued responsiveness to external stimuli. In several individuals (patients 1, 2, and 10), these symmetrical episodes appeared some time before the bilateral asymmetrical episodes. As described in classic AHC, other paroxysmal phenomena were also evident in this cohort, including exacerbations of dyskinesia, episodic ataxia, and focal dystonia, often associated with reproducible sensory triggers. Unlike in *ATP1A3*-related AHC, episodes did not reliably terminate with sleep. Some disordered eye movements were observed, including frequent lateral deviation of both eyes in patients 1, 2, 5, and 8, but these did not particularly resemble the specific eye movement disorders such as monocular nystagmus that are reported in *ATP1A3*-related disease.

The majority of our cohort did have epilepsy, with focal seizures being the commonest. Epilepsy was not in general the most debilitating feature of the disorder. Four of 7 patients with epilepsy were seizure-free (on or off medication) at the time of their last review. It is plausible that some of the seizures described, including myoclonic, gelastic, and atypical absence episodes, were not always truly epileptic events,

Table 4 Comparison Between Classic AHC and Alternating Hemiplegia Occurring in RRD

Diagnostic Criterion for AHC [4]	Present in AHC	Present in *RRD*
A. Recurrent attacks of hemiplegia alternating between the 2 sides of the body and fulfilling criterion B	✓	✓
B. Onset before the age of 18 mo	✓	–
C. At least 1 other paroxysmal phenomenon associated with the bouts of hemiplegia or occurring independently	✓	✓
D. Evidence of mental and/or neurologic deficit(s)	✓	✓
E. Not attributed to another disorder	✓	✓
Indicator of Possible *ATP1A3*-Related Disorder[10]	**Present in *ATP1A3*-Related Disorders**	**Present in RRD**
Infancy/early childhood		
Alternating hemiparesis, hemiplegia, or dystonia	✓	✓
Paroxysmal episodes of monocular nystagmus with or without other motor signs or symptoms	✓	–
Paroxysmal conjugate or dysconjugate ocular movement abnormalities	✓	✓
Acute flaccid quadriparesis persisting for hours to days	✓	✓
Recurrent paroxysmal tonic or dystonic seizure-like episodes	✓	✓
Child or adult		
Paroxysmal onset of ataxia, which becomes fixed or remains episodic	✓	✓
Paroxysmal dystonia or hemidystonia	✓	✓
Acute, fluctuating motor function deficits persisting for hours to days, which may include ataxia, chorea, hemiplegia, or paresis	✓	✓
Paroxysmal episodes of motor signs with EEG monitoring documenting the absence of epileptiform activity	✓	✓
Episodes clinically consistent with generalized or focal epilepsy (with or without ictal EEG)	✓	✓
Any age		
Asymmetric paroxysmal onset of hemiplegia or paresis, quadriplegia or paresis, spasticity, dystonia, and dyskinesia, with or without the subsequent appearance of fixed neurologic deficits	✓	✓
Rostrocaudal gradient (topographic, not temporal) of fixed or fluctuating motor involvement	✓	–
Multiple environmental triggers, including physical exertion, extremes of temperature, emotional stimuli, and chemicals	✓	✓
Seizure-like paroxysmal tremor affecting ≥1 limbs or inclusive of whole-body tremors	✓	✓
Paroxysmal bulbar symptoms with or without resolution over hours to days	✓	–
Suspected epileptic event with normal EEG recording during a typical spell, especially if associated with tonic or dystonic posturing or migratory paresis	✓	✓

Abbreviations: AHC = alternating hemiplegia of childhood; RRD = *RHOBTB2*-related disorders; ✓ = feature usually present in condition; – = feature not usually present in condition.

because very similar events in other patients were found to have no EEG correlate. In most cases, it was often hard or impossible to distinguish clinically between epileptic and nonepileptic paroxysmal events. Frequently, episodes that clinicians had believed to be clearly epileptic (such as the head deviation/eye deviation/tongue thrusting episodes seen in patients 1, 2, 5, and 8) proved to have no electrographic ictal correlate. It remains possible, however, that paroxysmal abnormal movements are mediated by electric discharges analogous to epilepsy but involving deeper foci that may be inaccessible by standard EEG.

Moreover, although ictal EEG was recorded for most patients, for some individuals who experienced a wide range of different event types, not every type was captured. Evaluation is further complicated by the fact that the types and frequencies of events, both epileptic and nonepileptic, often changed over time, with the frequency of particular episode types waxing

and waning. A fuller understanding of the coexistence of epilepsy and paroxysmal movement disorders in patients with RRD could be achieved in the future by a prospective longitudinal study, ideally including both serial EEGs and repeated re-evaluations of the semiology of clinical events. We note that similar issues affect evaluation of paroxysmal episodes in individuals with AHC due to mutations in *ATP1A3*.

In all but 2 patients (patients 5 and 9, whose epilepsy started in the first months of life), developmental delay was already evident before the first seizure, suggesting a key physiologic role for *RHOBTB2* in neurodevelopment. Two patients (patients 1 and 11) experienced developmental regression, either in association with exacerbation of the movement disorder (patient 1) or after an encephalopathic illness including status epilepticus (patient 11). Developmental stagnation was seen in 3 patients, again either during a period of increased abnormal movements (patient 6) or coinciding with epilepsy onset and/or worsening (patients 5, 6, and 9). Control of both seizures and paroxysmal movements may therefore be required to optimize neurodevelopmental outcome, but in view of the different therapeutic approaches required, it remains important, although challenging, to distinguish between paroxysmal movements and epileptic seizures. On the whole, control of epileptic seizures was achieved more successfully in our cohort than control of paroxysmal movements. Some agents such as carbamazepine may have been efficacious for both. Acetazolamide, used primarily for movement disorders although it does have antiepileptic activity, also showed some benefit for ataxic episodes in 2 patients (patients 4 and 6) in whom good control of epilepsy was already achieved. However, the numbers in our cohort are certainly too small to draw firm conclusions as to the most effective therapeutic approaches, and this should be a priority for future research.

RRD are complex, and our study confirms association with a number of neurologic and systemic comorbid conditions. Many patients had behavioral and/or emotional difficulties as well as gastrointestinal features and, interestingly, vasomotor symptoms (excessive flushing and/or pallor), often observed during periods of increased paroxysmal movements. These could potentially represent unexplored dysautonomic phenomena. The same may be true of the paroxysmal respiratory disturbances seen in 1 patient (patient 1).

On the basis of the findings in our cohort, there is no clear pattern of progression over time. Other than patient 11, who had a single episode of severe regression with lasting consequences, and patient 1, who experienced several episodes of regression, most patients achieved some neurodevelopmental gains over time, and neither epilepsy nor movement disorders were reported to worsen with age. However, many patients in our cohort are still very young, and an accurate longitudinal assessment of natural history would clearly be valuable to determine whether RRD are associated with progressive disease features in the longer term.

Our findings suggest that the phenotype of RRD extends beyond early infantile epileptic encephalopathy and encompasses a complex neurologic disorder characterized by prominent motor features, a wide variety of episodic phenomena, a broad spectrum of intellectual and motor disability, and, in some cases, distinctive dysautonomic or vasomotor phenomena. There seems to be a significant degree of overlap with the *ATP1A3*-related disease spectrum, including AHC, albeit with hemiplegic episodes emerging later in childhood, as seen in CAPOS (cerebellar ataxia, arreflexia, pes cavus, optic atrophy, and sensorineural hearing loss) and intermediate phenotypes. A registry of patients with *RHOBTB2* mutations and a prospective study of their natural history would facilitate a better understanding of this disorder and identify targets for therapeutic intervention.

Acknowledgment

The authors thank the participants and their families for their involvement. M.A.K.'s research group (including D.S.) is funded by sources including the National Institute of Health Research, Rosetrees Trust, and the Jules Thorn Trust. They thank Dr. Victoria Nesbitt for her involvement in diagnosis of a patient. They also thank the Epilepsy Society for support. Part of this work was undertaken at University College London Hospitals, which received a proportion of funding from the National Institute of Health Research Biomedical Research Centres funding scheme.

Study Funding

No specific funding of sponsorship was received for this work. M.A. Kurian and D. Steel are funded by a National Institute of Health Research Professorship, and M.A.K.'s research group also benefits from funding from the Sir Jules Thorn Trust and Rosetrees Trust. All research at UCL Great Ormond Street Institute of Child Health is supported by the NIHR Great Ormond Street Hospital Biomedical Research Centre.

Disclosure

The authors report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

Publication History

Received by *Neurology* May 5, 2020. Accepted in final form December 9, 2020.

Appendix Authors

Author	Location	Contribution
Sara Zagaglia, MD	UCL Queen Square Institute of Neurology, London, UK	Compiled and analyzed the data; drafted the manuscript
Dora Steel, BMBCh	Institute of Child Health, London, UK	Compiled and analyzed the data; drafted the manuscript

Continued

Appendix *(continued)*

Author	Location	Contribution
Krithika S., PhD	UCL Queen Square Institute of Neurology, London, UK	Identified, diagnosed, and phenotyped cases
Laura Hernandez-Hernandez, PhD	UCL Queen Square Institute of Neurology, London, UK	Identified, diagnosed, and phenotyped cases
Helena Martins Custodio, PhD	UCL Queen Square Institute of Neurology, London, UK	Identified, diagnosed, and phenotyped cases
Kathleen Gorman, MD	Children's Health Ireland at Temple Street, Dublin	Identified, diagnosed, and phenotyped cases
Aikaterini Verzyroglou, MD	Institute of Child Health, London, UK	Phenotyped cases; advised on correlations with AHC/*ATP1A3*
Rikke S. Møller, PhD	Danish Epilepsy Centre, Dianalund, Denmark	Identified, diagnosed, and phenotyped cases
Mary D. King, FRCPCH	Children's Health Ireland at Temple Street, Dublin	Identified, diagnosed, and phenotyped cases
Trine Bjørg Hammer, PhD	Danish Epilepsy Centre, Dianalund, Denmark	Identified, diagnosed, and phenotyped cases
Robert Spaull, MBBS	Bristol Royal Hospital for Children, UK	Identified, diagnosed, and phenotyped cases
Walid Fazeli, MD	University Hospital Cologne, Germany	Identified, diagnosed, and phenotyped cases
Tobias Bartolomaeus, MD	Institute of Human Genetics, University of Leipzig, Germany	Identified, diagnosed, and phenotyped cases
Diane Doummar, MD	Centre de Référence Neurogénetique Mouvements Anormaux, Paris, France	Identified, diagnosed, and phenotyped cases
Boris Keren, PhD	Department of Genetics, La Pitié-Salpêtrière Hospital, Paris, France	Identified, diagnosed, and phenotyped cases
Cyril Mignot, PhD	Department of Genetics, La Pitié-Salpêtrière Hospital, Paris, France	Identified, diagnosed, and phenotyped cases
Nathalie Bednarek, PhD	American Memorial Hospital, Reims, France	Identified, diagnosed, and phenotyped cases
J. Helen Cross, PhD	Institute of Child Health, London, UK	Phenotyped cases; advised on correlations with AHC/*ATP1A3*
Andrew A. Mallick, PhD	Bristol Royal Hospital for Children, UK	Identified, diagnosed, and phenotyped cases
Alba Sanchis-Juan, PhD	NIHR BioResource, Cambridge University Hospitals, UK	Conducted whole-genome sequencing

Appendix *(continued)*

Author	Location	Contribution
Anna Basu, PhD	Great North Children's Hospital, Newcastle, UK	Provided phenotypic data
F. Lucy Raymond, PhD	NIHR BioResource, Cambridge University Hospitals, UK	Conducted whole-genome sequencing
Brian J. Lynch, FRCPI	Children's Health Ireland at Temple Street, Dublin	Identified, diagnosed, and phenotyped cases
Anirban Majumdar, FRCPCH	Bristol Royal Hospital for Children, UK	Identified, diagnosed, and phenotyped cases
Hannah Stamberger, PhD	University Hospital Antwerp, Belgium	Identified, diagnosed, and phenotyped cases
Sarah Weckhuysen, PhD	University Hospital Antwerp, Belgium	Identified, diagnosed, and phenotyped cases
Sanjay M. Sisodiya, FRCP	UCL Queen Square Institute of Neurology, London, UK	Conceptualized the project; revised the manuscript for intellectual content
Manju A. Kurian, PhD	Institute of Child Health, London, UK	Conceptualized the project; revised the manuscript for intellectual content

References

1. Lopes F, Barbosa M, Ameur A, et al. Identification of novel genetic causes of Rett syndrome-like phenotypes. J Med Genet 2016;53:190–199.
2. Straub J, Konrad EDH, Grüner J, et al. Missense variants in RHOBTB2 cause a developmental and epileptic encephalopathy in humans, and altered levels cause neurological defects in drosophila. Am J Hum Genet 2018;102: 44–57.
3. Belal H, Nakashima M, Matsumoto H. De novo variants in RHOBTB2, an atypical Rho GTPase gene, cause epileptic encephalopathy. Hum Mutat 2018; 39:1070–1075.
4. IHS Classification ICHD-3. London: International Headache Society; 2019. Available at: ichd-3.org/appendix/a1-migraine/a1-6-episodic-syndromes-that-may-be-associated-with-migraine/a1-6-5-alternating-hemiplegia-of-childhood/. Accessed September 2019.
5. 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.
6. 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.
7. ClinicalTrials.gov. Use of Betashot in Children and Adults With Epilepsy (study in progress). Available at: clinicaltrials.gov/ct2/show/NCT02825745. Accessed October 2019.
8. UK Brain Expression Consortium (UKBEC). Available at:braineac.org. Accessed November 2019.
9. Heinzen EL, Swoboda J, Hitomi Y, et al. De novo mutations in ATP1A3 cause alternating hemiplegia of childhood. Nat Genet 2012;44:1030–1034.
10. Rosewich H, Sweney MT, DeBrosse S, et al. Research conference summary from the 2014 International Task Force on ATP1A3-related disorders. Neurol Genet 2017;3: e139.