RHOBTB2 mutations expand the phenotypic spectrum of alternating hemiplegia of childhood

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Abstract

Objective: To explore the phenotypic spectrum of *RHOBTB2*-related disorders, and specifically to determine whether patients fulfil 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 paediatric centre, with additional cases identified through collaboration with other centres internationally. Clinical data was acquired through retrospective case-note review.

Results: 11 affected patients were identified. All had heterozygous missense variants involving exon 9 of *RHOBTB2*, confirmed as *de novo* in nine cases. All had a complex motor phenotype, including at least two 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 eight experienced hemiplegic episodes. In contrast to classical AHC, commonly caused by mutations in *ATP1A3*, these events were only reported later in *RHOBTB2*-mutation-positive patients, from twenty months of age. Seven patients had epilepsy, but of these, four achieved seizure-freedom. All patients had intellectual disability, usually moderate to severe. Other features include episodes of marked skin colour change and gastrointestinal symptoms, each in four patients.

Conclusion: Although heterozygous *RHOBTB2* mutations were originally described in early infantile epileptic encephalopathy (EIEE64), 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.

Introduction

Heterozygous variants in *RHOBTB2*, encoding an atypical Rho GTPase, have recently been reported in the developmental and epileptic encephalopathies.[1,2,3] Affected patients present with developmental delay and onset of epilepsy in the first three 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 on 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 (WES), whole-genome sequencing (WGS) or gene panel testing. All results were confirmed through accredited diagnostic laboratory services except #10, whose diagnosis was made through research triome WGS with Sanger sequencing confirmation in the proband and family (REC reference 13LO168), and #4, #5 and #9 who were diagnosed through research WES. Genetic variants not previously reported in the literature were assessed for pathogenicity followed standard American College of Medical Genetics (ACMG) guidelines.[5] Seizures were classified according to the 2017 International League Against Epilepsy (ILAE) classification and terminology.[6]

Standard Protocols, Approvals, Registrations and Patient Consents

This project was approved by the Great Ormond Street Research and Development Office (Reference Number 19NM23). As it involved retrospective collection of fully-anonymised data collected during clinical care, written consent from patients' guardians was not sought but verbal assent was. Where identifiable videos and photographic images are used, written informed consent for their review and publication was provided by patients and/or their parents or guardians.

Data Availability Statement: The data relevant to this article is published within the text and tables.

Results

11 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 (Table 1)

All patients had heterozygous missense variants in *RHOBTB2.* 7/11 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 ACMG guidelines.[5] Parental testing was available for 9/11 patients and in all cases confirmed *de novo* status (**Table 1**).

Clinical Features (Table 2, Table 3)

Movement disorder: All patients had motor features as part of their clinical phenotype. Severity varied widely, from lack of any head or truncal control (#11) through inability to walk (#7, apparently due to a combination of hypotonia, dystonia and ataxia) to mild balance difficulties with near-normal gait (#10). Pyramidal signs (such as brisk reflexes or a positive Babinski) were evident in 5/11 patients although clear velocity-dependent spasticity was only present in two of these. In addition to gait difficulties, 5/11 patients had a generalised hyperkinetic choreiform movement disorder (**Video 1, 2, 3**). 10/11 patients had additional paroxysmal movements: this included hemiplegic episodes in eight, episodic focal dystonia in seven (**Video 4, 5, 6**), episodic ataxia in four, episodic exacerbation of dyskinesia in four, and non-epileptic myoclonus in two 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, then settled with age before re-emerging at eight years old. Five 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 (#1, #2), possibly associated with changes in position, movement or temperature. Two other families (#4, #5) reported a diurnal variation in episodes of unsteadiness, with increased occurrence in the mornings.

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

Epilepsy: 7/11 patients had a diagnosis of epilepsy (mean age of onset 14 months, range 11 weeks to 4 years 6 months), and two others had experienced at least one seizure (electrographic seizures in #4 detected on EEG aged three years, and febrile convulsions in #1 aged eight months). All of the patients with epilepsy had at least one EEG, either ictal or interictal, which showed focal or multifocal epileptiform discharges. Most interictal recordings were either 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 6/7 patients.

Five patients (#2, #5, #6, #9, #11) experienced status epilepticus. For four of these (#2, #5, #6, #9) this occurred at between two and five months old. For #6, but not the others, status epilepticus occurred in the context of a 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 six days (including two extubation attempts which failed due to recurrence of seizures). The duration of the episode is not known for #5 but was approximately 40 minutes in #2 and two hours in #9. Only one of these patients, #5, went on to have a second episode of status epilepticus, which occurred at eight years old. #11 had a single prolonged episode of status epilepticus at four years six months old, in the context of a severe encephalitis-like episode during which he was comatose for three 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 behavioural changes were captured on EEG in some patients (#1, #2, #5, #8, #10 and #11) and proved to have no electrographic correlate. For #4, #6 and #7, however, ictal EEG was not captured for the majority of event types.

All patients except #9 and #10 were taking anti-seizure 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 7/11 patients, which was felt to be clearly beneficial in at least two cases (#1, #5), resulting in reduced frequency of episodes, possibly including reduced paroxysmal movements as well as epileptic seizures. Topiramate improved seizure control in at least one patient (#7), whose seizures reduced from 20 per week to one per week, and another patient (#8, who did not have epilepsy) experienced reduced frequency of abnormal movements on a combination of topiramate and flunarizine. (In another patient, #1, however, neither topiramate nor flunarizine showed any benefit for non-epileptic paroxysmal movements.) Two patients (#6, #4) also experienced some improvement with acetazolamide, which was prescribed primarily to treat episodic ataxia. One family (#1) also reported a benefit from a medium-chain triglyceride dietary supplement.[7] The impact of other medications was less clear in this patient cohort.

Other paroxysmal events:

Eight patients (#1, #2, #4, #5, #6, #9, #10, #11) had a history of an acute encephalitis-like illness but the forms this took were variable. In five cases (#2, #5, #6, #9, #11) the episode included convulsive status epilepticus, as described above. Two of these episodes (#6 and #11) also included thermoregulatory changes – hypothermia in #11, hyperthermia in #6 – and skin changes, described as flushing in #11 but simply as "rash" in #6. #6 additionally went on to have three further episodes of loss of consciousness aged between three and four years old, two of which immediately followed minor head trauma. Vomiting occurred on at least one occasion. Unconsciousness lasted for around 24 hours on the first occasion and a few hours on the subsequent times, but was followed by hemiplegia which took up to two weeks to resolve. EEG was captured during unconsciousness on two 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.

#4, aged three years, had an episode of hemiplegia lasting several hours associated with contralateral electrographic seizure activity in the mid-temporal region. MRI brain in the acute phase showed changes consistent with acute ischaemia in the affected hemisphere but subsequently normalised. #10, also at age three, 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. #1 had three separate episodes of behavioural and neurodevelopmental regression associated with increased frequency of paroxysmal abnormal movements and increased vasomotor skin changes aged 13 months, 28 months and seven years. His EEG

initially showed only mild slowing but epileptiform discharges – without clinical correlate – appeared only from the age of seven years onwards.

Paroxysmal abnormal eye movements were reported in patients #1, #2, #5, #8 and #11. In #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. #11 had episodes of nystagmus.

One patient, #1, experienced recurrent episodes of respiratory disturbance, starting from the age of seven years. These consisted of shallow or irregular breathing or sometimes complete apnoea requiring bag-valve-mask ventilation, accompanied by tachycardia and pupillary dilatation, lasting for up to 20 minutes. Ventilation was never technically challenging and there was no suggestion of 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 paralysed and flaccid. Episodes could occur from sleep or waking and there were no obvious triggers. There was no post-event 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 four individuals (#1, #2, #5, #11; **Figure 2**).

Other co-morbidities: All patients had intellectual disability (ID): formal IQ testing was not available for most patients but for 10/11, ID was clinically assessed as moderate or severe, with either absent or significantly impaired verbal communication and comprehension. Patient #10, however, had a milder impairment when compared to the rest of the cohort. Only two patients experienced developmental regression. In #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 #1, regression occurred on three occasions, each time coinciding with exacerbation of the movement disorder. In three other patients (#5, #6, #9), developmental slowing was noted after the onset of epilepsy, which in all these cases was very early (3 months of age, or less): milestones (such as social smiling, visual attentiveness and partial head control) prior to this age had been apparently normal, but were delayed afterwards. Patient #6 also experienced a further period of developmental stagnation aged three years, at a time of increased seizure frequency.

Marked emotional lability was reported in two patients (#2, #10), irritability in one (#1), and challenging behaviour in three more individuals (#3, #5, #8). Gastrointestinal symptoms including gastro-oesophageal reflux, constipation and episodes of abdominal discomfort were described in four patients (#1, #2, #6, #7).

Brain MRI findings

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

Discussion

RHOBTB2 is a relatively newly recognised disease-causing gene. It encodes a Rho GTPase, one of a family of proteins involved in regulating actin dynamics, and is believed to play a key role in cell morphology and, more specifically, dendritic arborisation. It is highly expressed in brain, especially the cortex and basal ganglia.[8]

Previously, pathogenic mutations in *RHOBTB2* have 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, where seven of the nine mutations are previously described. Moreover, all known pathogenic variants are found clustered in Exon 9, which encodes the BTB1 protein domain as well as part of BTB2. In our cohort, seven out of 11 patients carried a pathogenic variant affecting residue Arg511. This residue was also identified as the most frequent mutational hot-spot 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 (#10) has a mutation slightly outside the main cluster of reported pathogenic variants, so too does #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 four years of age, and 11 patients presenting before one year of age. As such, this

disorder was designated early infantile epileptic encephalopathy type 64 (EIEE64, MIM 618004), though 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 co-morbidities, including intellectual disability, neurobehavioural features, acute encephalopathic episodes and, as demonstrated by our data, a prominent movement disorder.

11/13 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. Our cohort of 11 patients all display a complex, polymorphic movement disorder, usually with both a paroxysmal and non-paroxysmal 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 two 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, from 3/11 patients who were non-ambulant, 2/11 who could walk only with support, and one who 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 classical AHC caused by mutations in *ATP1A3,*[9] in that hemiplegic episodes first appeared later in childhood: from 20 months onwards rather than by 18 months as in the accepted criteria of AHC[10] (**Table 4**). Interestingly, the hemiplegic spells appeared to share many features with episodes which were characterised 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 (#1, #2 and #10) these symmetrical episodes appeared some time before the bilateral asymmetrical episodes. As described in classical 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 #1, #2, #5 and #8, but these did not particularly resemble the specific eye movement disorders such as monocular nystagmus which 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. 4/7 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, as 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 non-epileptic paroxysmal events. Frequently, episodes which clinicians had believed to be clearly epileptic (such as the head deviation/eye deviation/tongue thrusting episodes seen in #1, #2, #5 and #8) proved to have no electrographic ictal correlate. It remains possible however, that paroxysmal abnormal movements are mediated by electrical 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 non-epileptic – often changed over time, with the frequency of particular episode-types waxing and waning. A fuller understanding of the co-existence 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 alternating hemiplegia of childhood due to mutations in *ATP1A3*.

In all but two 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 physiological role for *RHOBTB2* in neurodevelopment. Two patients (#1 and #11) experienced developmental regression, either in association with exacerbation of the movement disorder (#1) or following an encephalopathic illness including status epilepticus (#11). Developmental stagnation was seen in three patients, again either during a period of increased abnormal movements (#6) or coinciding with epilepsy onset and/or worsening (#5, #6, #9). Control of both seizures and paroxysmal movements may therefore be required in order to optimise neurodevelopmental outcome, but in view of the different therapeutic approaches required it remains important – though 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 anti-epileptic activity – also showed some benefit for ataxic episodes in two patients (#4

and #6) in whom good control of epilepsy was already achieved. However, 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 neurological and systemic co-morbidities. Many patients had behavioural 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 one patient (#1).

Based on the findings in our cohort, there is no clear pattern of progression over time. Other than #11, who had a single episode of severe regression with lasting consequences, and #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.

In conclusion, our findings suggest that the phenotype of RRD extends beyond EIEE and encompasses a complex neurological disorder characterised 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 CAPOS 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.

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Video Legends

Video 1: #2 as a toddler is sitting in his highchair. Whilst laughing, choreiform dyskinesia and rhythmic tremulous movements involving all four limbs, together with some dystonic posturing of the feet, is seen.

Video 2: #1 as a baby with severe generalised hyperkinesia and chorea involving the trunk (leading to truncal instability) as well as all limbs. He is seen to arch to the right side and also backwards.

Video 3: #10 aged 18 years. Subtle dysmetria (double-tapping), hyperkinesia (possibly including low-amplitude myoclonic jerks) of the extremities and orofacial dyskinesia are evident.

Video 4: #1, aged 1 year, is seen playing on the floor. Note dystonic posturing of the feet, as well as intermittent eye deviation.

Video 5: #2 as a baby is seen after a bath: parents report that changes of temperature such as bathing often trigger these episodes. Asymmetrical posturing of all four limbs, together with dystonic tremor, are present.

Video 6: #3, age 37 years, is seen walking, with asymmetrical dystonic posturing of the upper limbs. Note also his broad-based and slightly asymmetrical and unsteady gait pattern.

Video 7: #1 is seen as a toddler experiencing an episode of generalised weakness. He is quiet, still and drooling (with tenting of the mouth), but still alert and responsive to touch. Two brief episodes of upward eye deviation are seen.

Video 8: #2 as a baby experiences an episode of generalised weakness. He is noticeably pale as well as floppy and weak, with tenting of the mouth, but remains alert. At the end of the video, as he begins to recover some movement, his colour also starts to improve.

Figure Legends

Figure 1: Pathogenic mutations within the *RHOBTB2* gene (NB the longest transcript, NM_001160036.1, does not include exons 4 and 5)

Figure 2: Patient #2, age one year, demonstrating an episode of patchy erythema and pallor during a bath.

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)	CADD score	Mutation Taster	PolyPhen-2 (HumVar)	SIFT	Gnomad minor allele frequency
g.22865224 G>A	c.1532G>A	p.Arg511Gln	#1, #2, #3	De novo (#1, #2) Unconfirmed* (#3)	Yes[3]	N/A	32	Disease causing (1)	Probably damaging (0.999)	Tolerated (0.11)	Absent
g.22865223 C>G	c.1531C>G	p.Arg511Gly	#4	De novo	No	Likely pathogenic (PS2 + PM2,5 + PP2,3)	27.6	Disease causing (1)	Probably damaging (0.999)	Deleterious (0.05)	Absent
g.22865223 C>T	c.1531C>T	p.Arg511Trp	#5, #6, #9	De novo (all)	Yes[2]	N/A	33	Disease causing (1)	Probably damaging (1.000)	Deleterious (0.01)	Absent
g.22865211 C>T	c.1519C>T	p.Arg507Cys	#7	Unconfirmed*	Yes[3]	N/A	32	Disease causing (1)	Probably damaging (0.931)	Deleterious (0)	Absent
g. 22865140 G>A	c.1448G>A	p.Arg483His	#8	De novo	Yes[2]	N/A	31	Disease causing (1)	Probably damaging (1.000)	Deleterious (0.03)	Absent
g.22864414 C>A	c.722C>A	p.Ser241Tyr	#10	De novo	No	Likely pathogenic (PS2 + PM2 + PP2,3)	26	Disease causing (1)	Probably damaging (0.999)	Deleterious (0)	Absent
g.22864409 G>C	c.717G>C	p.Trp239Cys	#11	De novo	No	Likely pathogenic (PM1 + PM2 + PP2,3)	27.9	Disease causing (1)	Probably damaging (0.999)	Deleterious (0)	Absent

Table 1: Genetic variants found in RRD cohort

*Parental DNA not available

Table 2: RRD patients: epilepsy, EEG features, medications and other neurological/systemic features

Patient	Age at last review	Epilepsy confirmed?	EEG findings	Relevant medication	MRI findings	Intellectual disability	Neurodevelopmental regression	Behavioural/psychiatric features	Encephalopathic episode?	Other
1	8y	No – only confirmed seizures were febrile convulsions	Initially mild slowing only. From 7y, multifocal discharges appeared. Multiple episodes had no EEG correlate.	Topiramate (stopped); Levetiracetam Betashot; Flunarizine; Carbamazepine	Thin corpus callosum	Severe (uses single words)	At times of increased abnormal movement (13m, 28m, 7y)	Irritability (correlates with increased abnormal movements)	Possibly: episodes of regression with increased abnormal movements	Episodes of gastrointestinal discomfort; Episodes of pallor Acquired microcephaly
2	2y	Yes: onset at 5m with SE. Seizure types: focal with and without impairment of awareness and evolution to bilateral tonic-clonic~~,~~ atypical absences. Seizure-free >1y.	Initially slowing only; subsequently focal seizures captured. Multiple episodes had no EEG correlate.	Sodium valproate (stopped); Carbamazepine	Normal	Moderate-severe global developmental delay (no speech yet)	No	Emotional lability	Possibly: but sudden onset of epilepsy with SE	Gastro-oesophageal reflux disease; Episodes of redness/pallor; Acquired microcephaly
3	37y	Yes: onset 18m. Seizure types: focal onset with impaired awareness.	Slowing and epileptiform activity (NB first EEG at 10y old).	Vigabatrin (stopped); Carbamazepine; Clobazam	None available	Severe (non-verbal)	No	Challenging behaviour (aggression)	No	Nil
4	20y	No – but had electrographic seizures during hemiplegic episode aged 3y.	Focal electrographic seizures during hemiplegic episode; subsequent EEGs slowing only.	Carbamazepine; Acetazolamide	Mild static cerebellar atrophy	Severe (non-verbal; some understanding of speech)	No	Nil	Possibly: prolonged hemiplegic episode with abnormal contralateral EEG aged 3y	Nil
5	14y	Yes: onset 3m. Seizure types: focal onset with and without impairment of awareness and evolution to bilateral tonic-clonic~~,~~; SE X2. Seizure free >5y.	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	Severe (non-verbal)	No – but developmental slowing concurrent with epilepsy onset	Behavioural and sensory issues	Possibly: sudden onset of epilepsy with SE	Episodes of redness/pallor
6	5y	Yes: onset 3m. Seizure types: focal onset (some of myoclonic subtype) with and without impairment of awareness and evolution to bilateral tonic-clonic~~,~~; atypical absences; Seizure free approx. 1y.	Initially normal; from 6m frontal sharp waves noted.	Sodium valproate; Oxcarbazepine; Pyridoxine; Clobazam; Lamotrigine (all stopped); Acetazolamide	Non-specific left occipital changes	Severe (non-verbal, some understanding of speech)	No – but developmental stagnation at 3y concurrent with increased seizures/movements	Nil (sociable and happy)	Yes: at 3m, fever, rash, cluster of seizures (negative bacterial cultures)	Constipation
7	60y	Yes: onset 1y. Seizure types: focal onset with impairment of awareness and evolution	Initially normal; subsequently abnormal background; epileptiform activity	Sodium valproate; Lamotrigine; Fosphenytoin; Levetiracetam (all stopped); Topiramate;	None available	Moderate (speaks in two-word phrases)	Unknown	Nil	No	Constipation

		to bilateral tonic-clonic.	captured occasionally	Zonisamide; Clobazam; Carbamazepine						
8	3y	No	No epileptiform activity; poor organisation	Flunarizine; Topiramate	Mild widening of temporal horns	Moderate (speaks a few words; understands instructions)	Yes – motor regression aged 30m, following prolonged hemiplegic episode	Challenging behaviour	No	Nil
9	3y	Yes: onset 11w. Seizure types: left focal onset with and without impairment of awareness and evolution to bilateral tonic-clonic tonic. Seizure free 2 years.	Slow background activity; left fronto-central spike and slow wave discharges during sleep	Levetiracetam; Clobazam; Sodium valproate (all stopped)	Meningeal enhancement at time of SE. Thin corpus callosum; delayed myelination.	Severe developmental delay	Developmental stagnation at 11w (onset of epilepsy).	Nil	Possibly: but sudden onset of epilepsy with SE	Nil
10	18y	No	Spikes and slow waves during encephalopathic episode aged 3y. Subsequently normal. Multiple episodes had no EEG correlate.	Nil	Normal	Mild	No	Emotional lability	Yes: drowsiness, vomiting and abnormal EEG aged 3y	Nil
11	9y	Yes: onset 4y6m at time of encephalopathic episode. Generalised onset tonic and tonic-clonic seizures.	Generalised spike and slow wave during SE. Otherwise, slowing only.	Topiramate; Levetiracetam; Carbamazepine; Clobazam; Ketogenic diet	Normal aged 4y; cerebero-cerebellar atrophy aged 5y	Profound (non-verbal) following regression	Yes: severe lasting regression following encephalopathic episode at 4y 6m	Nil	Yes: 3m comatose illness with SE and hypothermia at 4y 6m	Episodes of redness/pallor

Abbreviations: SE: status epilepticus.

Table 3: RRD patients: baseline and paroxysmal movement disorders

Patient	Sex	Age at last review	Motor abilities	Baseline movement disorder					Paroxysmal movement disorder							
				Any?	Dyskinesia/ chorea	Dystonia	Ataxia/ unsteadiness	Pyramidal signs	Any?	Dyskinesia/ chorea	Dystonia	Ataxia/ unsteadiness	Generalised weakness	Hemiplegia	Other episodes	Triggers

1	M	8y	Walks a few steps with support	Y	Y (generalised, improved with age)	N	Stiff-legged, arrhythmic gait	Brisk reflexes only	Y	Y (variable frequency; general, minutes)	Y (focal or generalised; 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
2	M	2y	Sat at 20m, not yet walking	Y	Y	N	Balance poor	Brisk reflexes only	Y	Y (many per day; general, minutes)	Y (general, 30s)	N	Y (daily, a few mins)	Y	Head and eye deviation (seconds, frequent)	Car travel
3	M	37y	Walked from 3y	Y	Y (orofacial)	Y (trunk, neck, hands)	Broad-based gait	Brisk reflexes, unilateral upgoing plantar	N	N	N	N	N	N	Stereotypies	N
4	M	20y	Walked from 3y	Y	N	N	Broad-based (and crouched) gait	N	Y	N	N	Y (increased unsteadiness 1-2hrs, mornings)	N	Y (infrequent, hrs-days, from 3y to 8y only)	Stereotypies	N
5	M	14y	Gait broad-based	Y	N	N	Broad-based gait	N	Y	Y	Y (limb posturing)	Y	Y	N	Tongue protrusion, head and eye deviation	N
6	F	5y	Walked from 2y, gait ataxic	Y	N	Y	Y	N	Y	N	Y (focal: L ankle)	Y (brief episodes most days)	N	Y (3 episodes lasting hours to 2w; first episode at 2y)	Tremor Myoclonus (?epileptic or movement)	2 hemiplegic episodes triggered by minor head injuries
7	F	60y	Unable to walk	Y	?	?	?	?	Y	N	Episodes of arm stiffening ?dystonia	N	N	Y (infrequent; last hours-2w)	N	Some hemiplegic episodes follow seizures
8	M	3y	Walked at 27m	N	N	N	N	N	Y	N	Y	N	N	Y (from 20m; flaccid paresis or hemidystonia lasting hours to 3d)	Episodes of head and eye deviation	Fatigue; fever
9	F	3y	Walks with support	Y	N	N	N	Spasticity, brisk reflexes	Y	Y (3X/day, 1-3 mins, arms and trunk)	N	Y (2-4X/day, very brief)	N	N	N	Movement; fever; excitement
10	F	18y	Walked at 1y, gait normal	Y	Y (upper limb and mild orofacial)	N	Mildly poor balance	N	Y	N	Y (focal, brief, infrequent)	N	N	Y (up to 1hr, infrequent)	Stereotypies; Myoclonus (mild)	No
11	M	9y	Bedridden, no head control	Y	Y	Y	N/A: no truncal control	Spasticity, brisk reflexes	Y	Y	Y	N	N	Y (age 4y, single episode lasting 10d)	Nystagmus and mouth opening	Illness; fatigue; bathing

Table 4: Comparison between classical AHC and alternating hemiplegia occurring in RRD

Diagnostic criterion for Alternating Hemiplegia of Childhood[10]	**Present in AHC**	**Present in *RHOBTB2*-related disorders**

A Recurrent attacks of hemiplegia alternating between the two sides of the body and fulfilling criterion B	✓	✓
B Onset before the age of 18 months	✓	–
C At least one other paroxysmal phenomenon associated with the bouts of hemiplegia or occurring independently	✓	✓
D Evidence of mental and/or neurological deficit(s)	✓	✓
E Not attributed to another disorder	✓	✓
Indicator of possible *ATP1A3*-related disorder[11]	**Present in *ATP1A3*-related disorders**	**Present in *RHOBTB2*-related disorders**
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 one or more 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	✓	✓

✓ : feature usually present in condition; –: feature not usually present in condition