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RHOBTB2 Variant p.Arg511Gln Causes Developmental and Epileptic Encephalopathy Type 64 in an Infant: A Case Report and Hotspot Variant Analysis

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ABSTRACT

Background: Developmental and epileptic encephalopathies (DEEs) are a heterogeneous group of brain disorders. Variants in the Rho-related BTB domain-containing 2 gene (*RHOBTB2*) can lead to DEE64, which is characterized by early-onset epilepsy, varying degrees of motor developmental delay and intellectual disability, microcephaly, and movement disorders. More than half of the variants are located at Arg483 and Arg511 within the BTB domain; however, the underlying mechanism of action of these hotspot variants remains unexplored.

Methods: We performed whole-exome and Sanger sequencing on the patient and his parents. We collected recurrent variant information from the literature on RHOBTB2 variants. We used Discovery Studio software to analyze the folding free energy of variant proteins, and the AlphaFold database to analyze structural alterations in mutant proteins.

Results: The patient presented with early-onset epilepsy, developmental delay, and brain structural abnormalities. Genetic analysis revealed a de novo variant in RHOBTB2, c.1532G>A, p.(Arg511Gln). To date, 60 cases of DEE patients with RHOBTB2 variants have been reported, with approximately 50% of variants located at Arg483 and Arg511. Among them, p.Arg511Gln, p.Arg483His, and p.Arg511Trp have an incidence rate exceeding 10%. The folding free energy of these high-frequency variants proteins is reduced, which may lead to increased structural stability.

Conclusion: This study highlights the importance of RHOBTB2 hotspot variants in DEE64 and provides insights into their potential mechanisms of action. We recommend RHOBTB2 gene testing for patients with relevant clinical manifestations to facilitate precise diagnosis and treatment of DEE.

1 | Introduction

Developmental and epileptic encephalopathies (DEEs) are a group of highly heterogeneous brain disorders characterized by a complex interplay of epileptic and developmental features (Scheffer et al. 2017). With the rapid advancement of genomic technologies, DEEs have been widely recognized as having a strong genetic component, with more than 400 genes being implicated in their pathogenesis (Symonds and McTague 2020). Many DEE subtypes caused by pathogenic genes exhibit similar phenotypic features, suggesting a common underlying mechanism related to specific pathways or biological functions, including voltage-gated ion channels (Bartolini et al. 2020), enzyme/enzyme regulator-mediated metabolic dysregulation (Sharma and Prasad 2017), transmembrane transport and exocytosis (Schubert et al. 2014), cell adhesion (Pederick et al. 2018), and neuronal proliferation and migration (Friocourt and Parnavelas 2010). In 2018, Straub et al. reported 10 individuals with DEE characterized by early-onset

seizures, severe intellectual disability, motor impairments, and cerebellar malformations, and they identified variants in the Rho-related BTB domain-containing 2 gene (RHOBTB2) as the underlying cause. The RHOBTB2 protein belongs to the Rho GTPase-activating protein family, which is a subfamily of the Ras superfamily of GTP-binding proteins (Ramos et al. 2002). To date, 30 RHOBTB2 gene variants have been reported in 60 individuals with neurodevelopmental disorders (NDDs) (Belal et al. 2018; Defo, Verloes, and Elenga 2022; Fonseca et al. 2021; Knijnenburg et al. 2020; Langhammer et al. 2023; Lopes et al. 2016; Maddirevula et al. 2020; Mainali et al. 2023; Niu et al. 2021; Necpál et al. 2020; Rochtus et al. 2020; Spagnoli et al. 2020; Straub et al. 2018; Valentino et al. 2021; Zagaglia et al. 2021). Heterozygous missense variants in RHOBTB2 are the primary genetic mechanism underlying DEE64 (OMIM#618004). However, a few NDD patients with biallelic RHOBTB2 variants, primarily splicing or truncating variants, have also been reported (Langhammer et al. 2023). Notably, among the reported 30 RHOBTB2 variants, those occurring at Arg511 and Arg483 (p.Arg511Gln, p.Arg511Trp, and p.Arg483His) are the most frequently observed (Belal et al. 2018; Defo, Verloes, and Elenga 2022; Fonseca et al. 2021; Knijnenburg et al. 2020; Langhammer et al. 2023; Lopes et al. 2016; Maddirevula et al. 2020; Mainali et al. 2023; Niu et al. 2021; Necpál et al. 2020; Rochtus et al. 2020; Spagnoli et al. 2020; Straub et al. 2018; Valentino et al. 2021; Zagaglia et al. 2021). However, critical knowledge regarding the full clinical spectrum and genotype–phenotype correlations is lacking.

Here, we report the case of a patient with DEE64 caused by the RHOBTB2 variant p.Arg511Gln. We reviewed the clinical characteristics of the patient and calculated the change in the folding free energy of the variant protein to elucidate the reason for its high frequency. Our aim was to improve clinical awareness of DEE through elucidating genotype–phenotype correlations, thus promoting precision diagnostics.

2 | Methods

2.1 | Subjects

The family was recruited at our hospital in March 2023. We analyzed the proband's clinical manifestations, auxiliary examinations, electroencephalography (EEG), magnetic resonance imaging (MRI), and genetic data. In addition, we analyzed previously reported cases associated with RHOBTB2 variants. Informed consent was obtained from the proband's parents, and the study was approved by the Ethics Review Committee of Hospital (2024-03).

2.2 | Whole-Exome Sequencing

To elucidate the molecular genetic cause of the patient's condition, peripheral blood samples (collected in EDTA anticoagulant) were drawn from the patient and his parents, and genomic DNA was extracted (Cowin Biotech, Beijing, China). xGen Exome Research Panel v2 capture probes (Integrated DNA Technologies, Coralville, IA, USA) were hybridized with the genomic DNA libraries in solution to enrich DNA fragments in the target region to construct exome libraries. The constructed libraries were subjected to high-throughput sequencing on the NovaSeq 6000 platform (Illumina, San Diego, CA, USA), producing 150-bp paired-end reads in FASTQ format.

2.3 | Bioinformatics Analysis

After removing reads that did not meet quality control standards, the Burrows–Wheeler aligner tool was used to align the reads to the UCSC hg19 reference genome. Variant analysis was performed using GATK v3.7, and variants with a minor allele frequency ≤ 0.005 were extracted from SNP databases (1000 Genomes, ExAC, and gnomAD). Variants were graded according to the standards and guidelines of the American College of Medical Genetics and Genomics (ACMG) (Richards et al. 2015).

2.4 | Sanger Sequencing

Sanger sequencing was performed to validate the RHOBTB2 variant in the patient and his parents. Variant-specific primers were designed using the Ensemble database (https://asia.ensembl.org/index.html), with the following sequences: F: 5′-CTGAGACAGACAGGCAATGCTAAT-3′; R: 5′-GTGGTGGTGAAGATGGACAGTT-3′. Sequencing was performed using an ABI 3500XL sequencer (Applied Biosystems, Foster City, CA, USA).

2.5 | Homology Analysis and Structural Modeling

We compared human RHOBTB2 sequences with those of other species using the ClusterW2 software to identify variable sites and their sequence conservation. WebLogo (https://weblogo.berkeley.edu/logo.cgi) was used to analyze the level of conservation at each position. Structural modeling was performed using AlphaFold v2.0 (https://alphafold.ebi.ac.uk/), and structural changes were visualized using PyMol v2.5.

2.6 | Folding Free Energy Analysis

To unveil the potential mechanisms underlying high-frequency variants, we analyzed variant protein folding free energy changes (ΔG_unfolding) (Pandey and Alexov 2024). First, protein folded-state free energy and unfolded-state free energy data were obtained using Discovery Studio software. Based on the protein structure and sequence information, the unfolded state of the protein was simulated, and thermodynamic principles were utilized to calculate free energy values. ΔG_unfolding values were determined by calculating the energy contribution terms (such as van der Waals forces, electrostatic interactions, and entropy changes).

3 | Results

3.1 | Case Presentation

The patient was a 4-month-old male infant who experienced seizures without any apparent triggers. The seizures

manifested as altered consciousness, staring, and upper limb tremors. The seizures lasted approximately 20 s and resolved spontaneously. Post-seizure fatigue was noted. The infant was the first child from a first pregnancy, with no adverse pregnancy history or family history. Physical examination on admission revealed a height of 62.4 cm (−1 SD) and weight of 7 kg (0 SD). Growth and developmental milestones were unremarkable. Auxiliary examinations, including liver/kidney function, blood ammonia, lactate, and electrolyte levels, were within normal limits. Blood and urine metabolic screening tests showed no abnormalities. EEG monitoring showed background activity of diffuse medium-to-high-amplitude 2–3-Hz delta waves and 4–6-Hz theta waves, with intermittent low-amplitude fast waves. During sleep monitoring, prolonged low-to-medium-amplitude 2–3-Hz spike-slow and polyspike-slow wave complexes were frequently observed, predominantly in the frontal pole, frontal region, and right anterior temporal region (Figure 1a). Cranial MRI revealed bilateral frontotemporal and falx cerebri-adjacent extracerebral space widening (Figure 1b). Treatment with oral sodium valproate was initiated, and the patient remained seizure-free during follow-up over 3 months.

3.2 | Genetic Testing Results

Trio-whole-exome sequencing revealed a missense variant in the RHOBTB2 gene of the patient, NM_001160036.2:c.1532G>A, p.(Arg511Gln). This variant was not detected in his parents, and Sanger sequencing confirmed the presence of this variant, suggesting it was a de novo variant (Figure 2a). The variant was not found in population databases. Several software programs predicted the variant to be potentially damaging to the protein: Polyphen2_HDIV predicted "probably damaging" (1.0), Polyphen2_HVAR predicted "probably damaging" (0.999), MutationTaster predicted "disease-causing" (0.99993), and M-CAP predicted "damaging" (0.075705). According to the ACMG guidelines, the variant was classified as "pathogenic," supported by PS2_VeryStrong + PS3_Supporting + PM1 + PM2_Supporting + PP3.

3.3 | Folding Free Energy Analysis of Hotspot Variants

We screened 10 recurrent RHOBTB2 variants, and homology analysis revealed that Arg483, Arg507, and Arg511

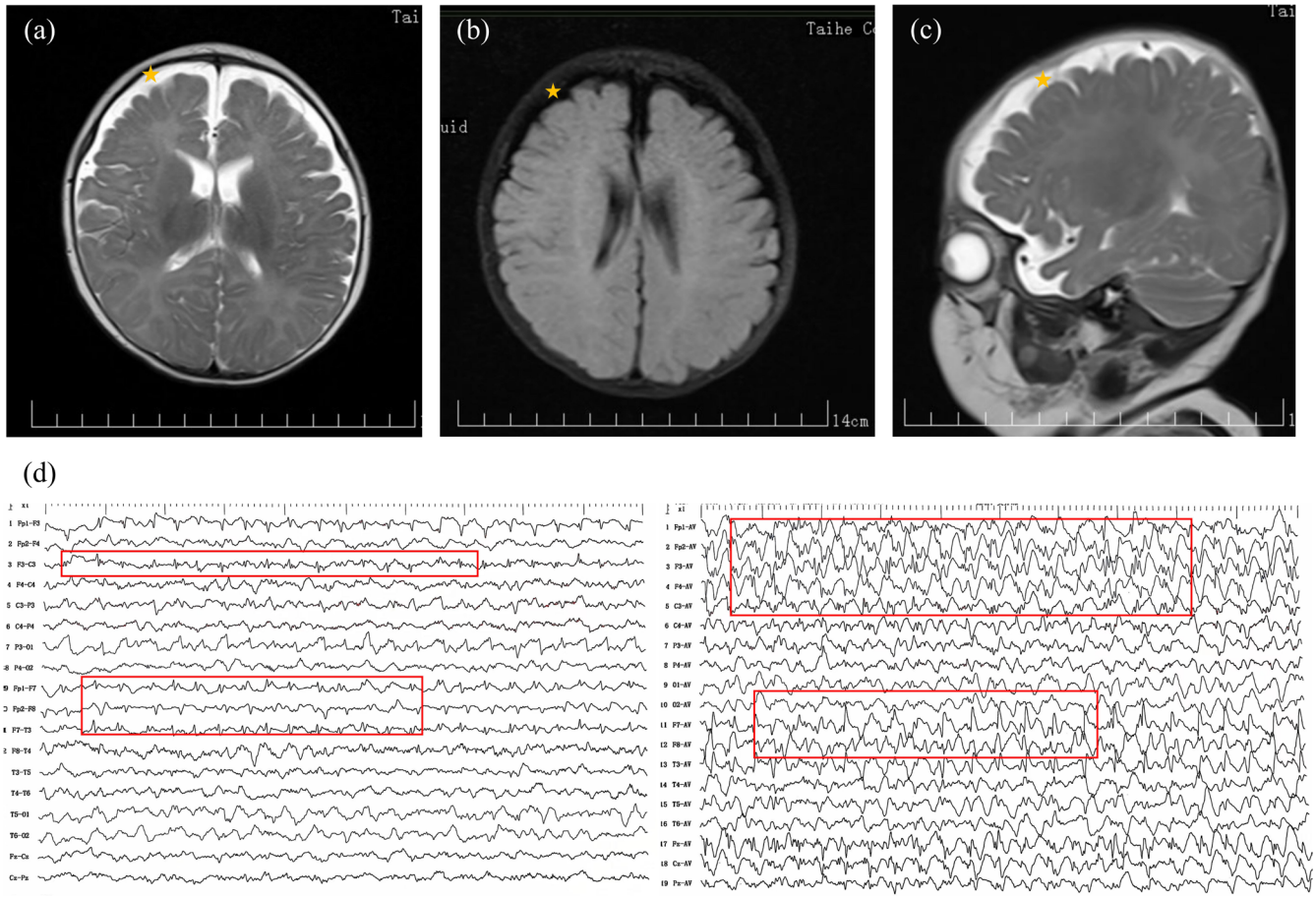

FIGURE 1 | Clinical data of the patient. (a) Axial T2WI scan showing widened extracranial space in the bilateral frontal regions, with high signal changes in the cerebrospinal fluid (CSF) at the widened areas (yellow star). (b) Axial T1WI scan indicating widened extracranial space in the bilateral frontal regions, with low signal changes in the CSF at the widened areas (yellow star). (c) Sagittal T2WI scan revealing widened extracranial space in the bilateral frontal regions, with high signal changes in the CSF at the widened areas (yellow star). (d) Video electroencephalogram monitoring during sleep period showing frequent discharge of long-duration low-to-medium amplitude 2–3-Hz spike-slow and polyspike-slow wave complexes, with significant activity in the frontal poles, frontal regions, and right anterior temporal area (red boxes show exceptional areas).

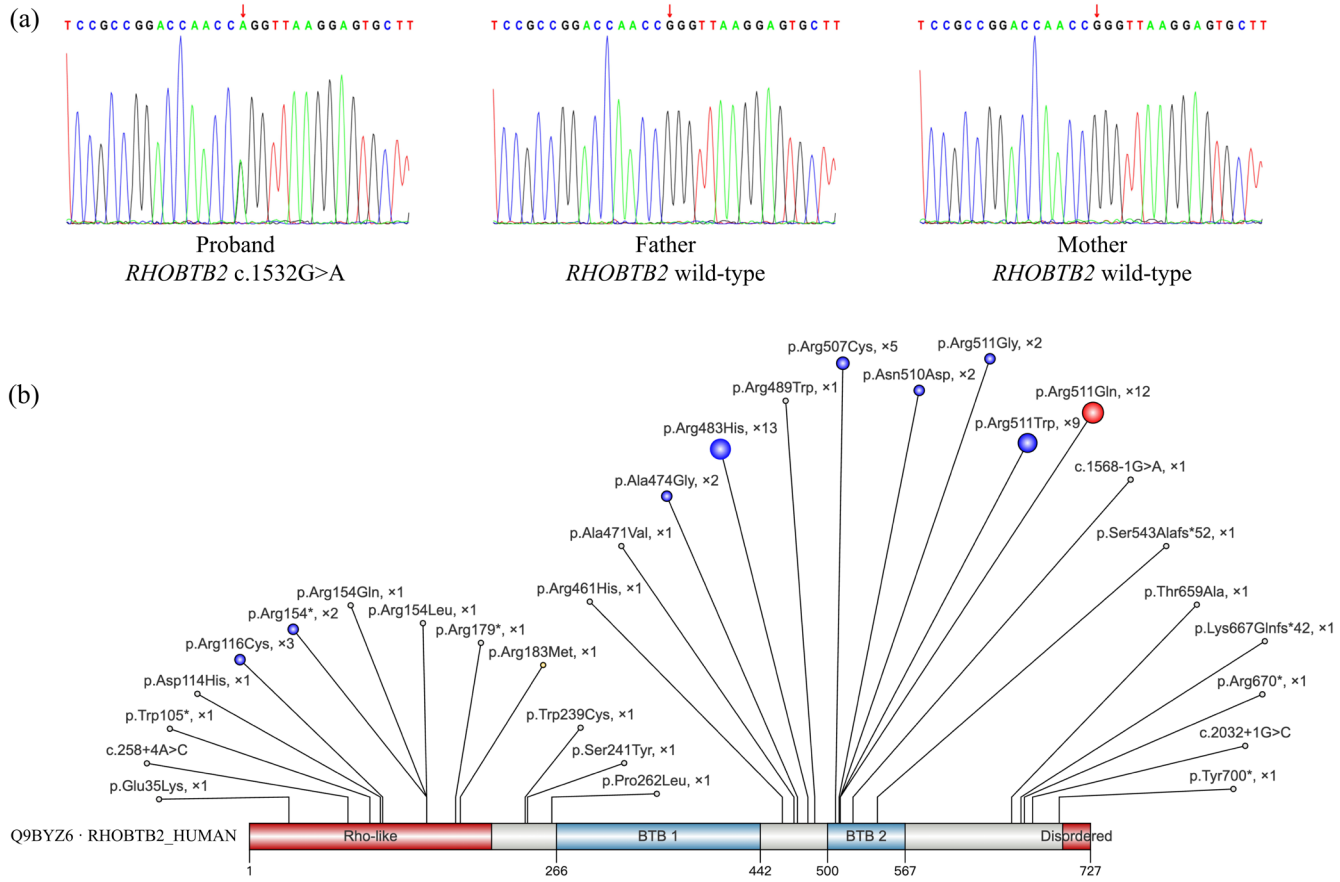

FIGURE 2 | Variant information. (a) Sanger validation suggested that the proband had *RHOBTB2* variant c.1532G>A, p.(Arg511Gln). (b) Schematic diagram of reported *RHOBTBT2* variant distribution. Blue circles represent recurrent variants, the red circle represents the variant in the present study, and circle size represents the variant frequency.

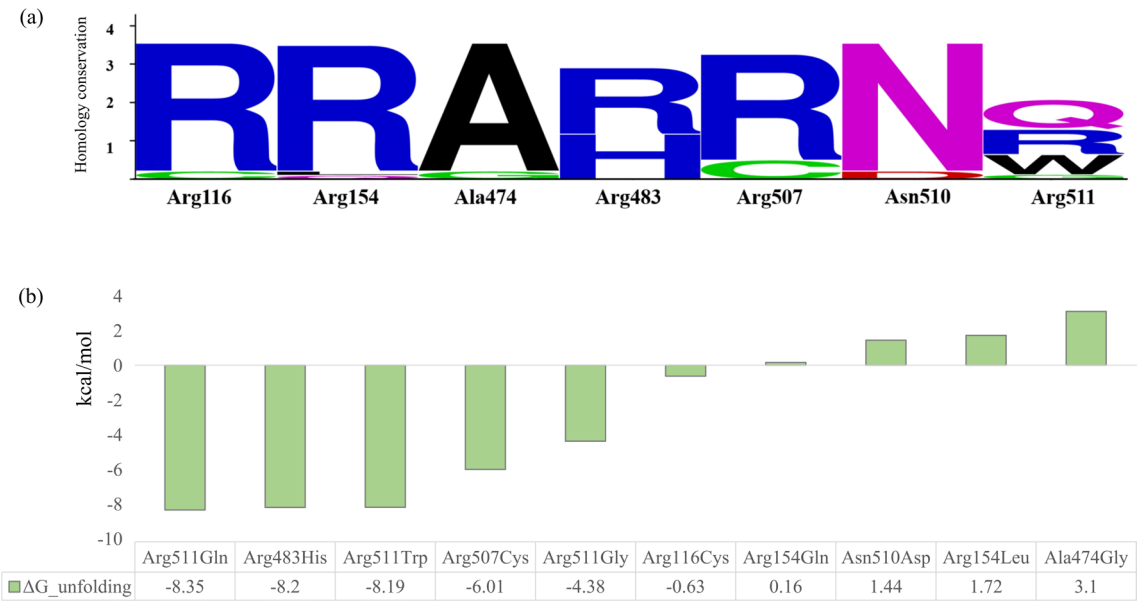

FIGURE 3 | Hot spot variant analysis. (a) WebLogo analysis of the degree of conservation of recurrent variant sites in the RHOBTB2 protein. (b) Changes in the protein folding free energy of 10 recurrent variant proteins. Hotspot variants p.Arg511Gln, p.Arg483His, and p.Arg511Trp lead to a large decrease in folding free energy, indicating a stabilization of the protein structure.

are non-conserved residues (Figure 3a). We calculated the changes in folding free energy induced by each variant. The results showed that variants p.Arg511Gln, p.Arg483His, p.Arg511Trp, p.Arg507Cys, p.Arg511Gly, and p.Arg116Cys led to a decrease in folding free energy, suggesting a more stable protein structure (Figure 3b). Notably, p.Arg511Gln,

p.Arg483His, p.Arg511Trp, p.Arg507Cys, and p.Arg511Gly exhibited the most significant differences in folding free energy (Table 1). Combined with the non-conserved nature of these residues, this finding may explain the reason for the high frequency of these variants.

3.4 | Structural Simulation Results

Analysis of the structural characteristics of the RHOBTB2 protein with p.Arg511Gln, p.Arg483His, p.Arg511Trp, p.Arg507Cys, and p.Arg511Gly variants revealed structural alterations. Arg511 does not interact with surrounding residues via non-polar interactions; however, p.Arg511Gln forms a new hydrogen bond with Glu514, whereas p.Arg511Gly exhibits a smaller entropy change in α-helix formation. p.Arg511Trp forms hydrophobicity owing to its indole functional group. Another high-frequency variant, p.Arg483His, forms a new hydrogen bond with Glu475. Similarly, p.Arg507Cys forms a new hydrogen bond with Ala503. These newly formed hydrogen bonds may contribute to a more stable protein structure (Figure 4).

4 | Discussion

We report the case of a patient with DEE carrying a hotspot variant p.Arg511Gln in RHOBTB2. The patient presented with recurrent seizures accompanied by brain structural and EEG abnormalities, consistent with previously reported phenotypes (Straub et al. 2018). To date, 30 RHOBTB2 variants have been reported (Figure 2b), with variants at Arg511 and Arg483 accounting for approximately 50% of patients. Of these, approximately 32% are located at Arg511 (17% being p.Arg511Gln, 13% being p.Arg511Trp, and 2% being p.Arg511Gly), and approximately 18% are p.Arg483His (Belal et al. 2018; Defo, Verloes, and Elenga 2022; Fonseca et al. 2021; Knijnenburg et al. 2020; Langhammer et al. 2023; Lopes et al. 2016; Maddirevula et al. 2020; Mainali et al. 2023; Niu et al. 2021; Necpál et al. 2020; Rochtus et al. 2020; Spagnoli et al. 2020; Straub et al. 2018; Valentino et al. 2021; Zagaglia et al. 2021). Homology analysis suggested that both Arg483 and Arg511 are non-conserved sites. By analyzing the protein folding free energy of the hotspot variants, we found that the intramolecular van der Waals force and the energy at the high-frequency variant sites Arg483 and Arg511 were increased, resulting in a lower folding free energy, suggesting a more stable structure. In addition, structural simulation results revealed that p.Arg483His and p.Arg511Gln form new hydrogen bonds with surrounding residues, indicating that variants at these sites may lead to a more stable structure. Changes in protein folding free energy serve as a thermodynamic indicator for detecting the effects of variants on protein stability and binding. Therefore, we attempted to utilize this indicator to unveil the underlying mechanisms of action of RHOBTB2 hotspot variants (Joerger and Fersht 2007).

RHOBTB2, located on chromosome 8p, encodes Rho-related BTB domain-containing 2, a small Rho GTPase that acts as a potential tumor suppressor. It was first reported in association with breast cancer and was previously named "deleted in breast cancer 2 gene (Hamaguchi et al. 2002)." This protein contains a GTPase domain, two tandem BTB domains, and a conserved C-terminal region (Straub et al. 2018). RHOBTB2 is highly expressed in neural tissues and interacts with the ubiquitin ligase scaffold protein CUL3, recruiting target proteins for degradation via its BTB domain, thus participating in regulating the cell cycle, apoptosis, cytoskeleton, and membrane trafficking (Belal et al. 2018). Cancer-related studies have revealed that RHOBTB2 variants can affect the protein interaction with CUL3, disrupting CUL3-dependent ubiquitination (Wilkins, Ping, and Carpenter 2004). However, Straub et al., demonstrated that variants in the BTB domain could impair proteasomal degradation, leading to increased protein abundance without affecting its interaction with CUL3, contradicting the expected mechanism of cancer development. In Drosophila, overexpression of Rhobtb2 increased the susceptibility to epilepsy and disrupted dendritic cell development. Nevertheless, the detailed mechanism of RHOBTB2 remains largely unexplored.

TABLE 1 | Folding free energy and contribution data of recurrent *RHOBTB2* variants (some of which are at the same locus).

No.	Mutation	Frequency	Van der Waals term	Electrostatic term	Entropy term	Calculated mutation energy	ΔG_ unfolding
1	p.Arg511Gln	20% (12/60)	−4.45	0.79	−0.49	−4.2	−8.35
2	p.Arg483His	22% (13/60)	−4.46	0.04	0.32	−4.1	−8.2
3	p.Arg511Trp	15% (9/60)	−3.58	−0.82	0.31	−4.1	−8.19
4	p.Arg507Cys	8% (5/60)	−4.5	0.59	0.9	−3	−6.01
5	p.Arg511Gly	3% (2/60)	−1.12	0.12	−1.19	−2.19	−4.38
6	p.Arg116Cys	5% (3/60)	0.46	−0.56	−0.23	−0.3	−0.63
7	p.Arg154Gln	2% (1/60)	−0.18	−0.07	0.33	0.08	0.16
8	p.Asn510Asp	3% (2/60)	2.17	−1.08	−0.37	0.72	1.44
9	p.Arg154Leu	2% (1/60)	3.12	−1.38	−0.88	0.86	1.72
10	p.Ala474Gly	3% (2/60)	3.84	−0.51	−1.78	1.55	3.1

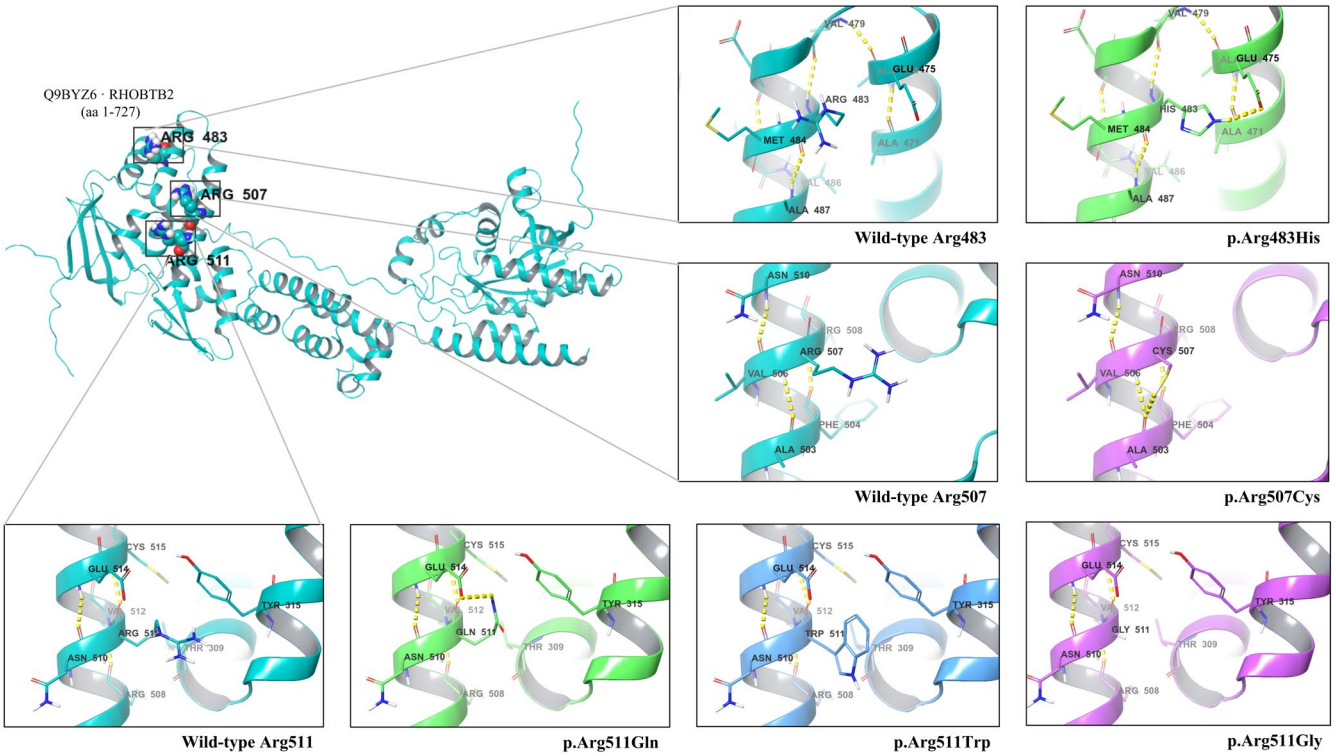

FIGURE 4 | Structural analysis of the five variants with the largest decreases in folding free energy, revealing that high-frequency variants p.Arg511Gln, p.Arg483His, and p.Arg507Cys form new hydrogen bonds. Secondarily, the indole functional group in p.Arg511Trp forms a hydrophobic interaction. The p.Arg511Gly exhibits a reduced entropy change in the α-helix, promoting structural stability.

Twelve variants, including the three hotspot variants, are located within or between the BTB domains, with a majority concentrated in amino acid residues 483 to 511 (Belal et al. 2018; Defo, Verloes, and Elenga 2022; Fonseca et al. 2021; Knijnenburg et al. 2020; Langhammer et al. 2023; Lopes et al. 2016; Maddirevula et al. 2020; Mainali et al. 2023; Niu et al. 2021; Necpál et al. 2020; Rochtus et al. 2020; Spagnoli et al. 2020; Straub et al. 2018; Valentino et al. 2021; Zagaglia et al. 2021). Analyzing changes in the folding free energy of mutated proteins can provide a novel perspective on the mechanisms underlying RHOBTB2 hotspot variants. Folding free energy analysis involves a comprehensive analysis of the free energy of the folded and unfolded states. The former reflects the folding stability of the protein in its native state, whereas the latter indicates the free energy of the protein when fully unfolded and devoid of biological activity (Pandey and Alexov 2024). Our findings revealed that the folding free energy of RHOBTB2 with hotspot variants was consistently reduced, implying increased structural stability. This decrease was most pronounced for the three hotspot variants. According to Straub et al., variants in the BTB domain lead to an increase in protein abundance. In addition to impaired protease function, the stability of the mutated protein may be a reason for the increase in protein abundance.

To date, 60 patients with RHOBTB2-associated NDD have been reported, with the vast majority of patients exhibiting early-onset epilepsy, varying degrees of motor developmental delay and intellectual disability, microcephaly, and movement disorders. EEG typically reveals focal epileptic discharges during seizures, whereas MRI shows brain structural abnormalities such as brain atrophy, corpus callosum hypoplasia, or delayed myelination (Belal et al. 2018; Defo, Verloes, and Elenga 2022; Fonseca et al. 2021; Knijnenburg et al. 2020; Langhammer et al. 2023; Lopes et al. 2016; Maddirevula et al. 2020; Mainali et al. 2023; Niu et al. 2021; Necpál et al. 2020; Rochtus et al. 2020; Spagnoli et al. 2020; Straub et al. 2018; Valentino et al. 2021; Zagaglia et al. 2021). Regarding the seizure characteristics of this syndrome, we reviewed the literature on patients with hotspot variants, including the present case. Thirteen patients have been reported with p.Arg511Gln, nine with p.Arg511Trp, and 13 with p.Arg483His (Table 2). Among these 35 patients, the seizure characteristics are diverse, including status epilepticus, febrile seizures, focal epileptic seizures, and tonic–clonic seizures, with some patients also experiencing myoclonic and atypical absence seizures. The seizure characteristics of these patients reveal a high phenotypic heterogeneity, with no clear genotype–phenotype correlations.

The treatment of epilepsy-related encephalopathy associated with RHOBTB2 variants primarily relies on symptomatic treatment, with antiepileptic drugs (AEDs) being the mainstay. Drug responses vary significantly among patients. Treatments range from monotherapies to combination therapies depending on age, developmental milestones, and treatment efficacy (Fonseca et al. 2021; Langhammer et al. 2023). Some patients respond well to valproate, carbamazepine, and pyridoxine (Belal et al. 2018; Langhammer et al. 2023; Straub et al. 2018). However, some patients still experience partial or non-continuous seizures during combined therapy, and others, despite multiple changes in AEDs, continue to have epileptic seizures or status epilepticus (Fonseca et al. 2021; Langhammer et al. 2023). Additionally, a few patients have seizures

TABLE 2 | Summary of epilepsy onset and treatment in 13 reported patients with p.Arg511Gln, including the present case, and 22 patients with other hotspot variants.

	Sex	Age	Variant	Inheritance	Seizure onset	Seizure type	AEDs	Seizure treatment response	Ref.
1	F	14 years	Arg511Gln	De novo	0.08 months	SE	VPA, PNX	Yes, responds to PNX	Straub et al. 2018
2	F	17 years	Arg511Gln	De novo	4 months	/	LTG, TPM, PB, CBZ	Yes, responds to CBZ	Straub et al. 2018
3	M	20 years	Arg511Gln	De novo	3 months	TS, febrile SE	PHT	Controlled with PHT from infancy	Belal et al. 2018
4	F	9 years	Arg511Gln	De novo	3 months	Febrile focal SE	VPA, TPM, LEV	Responds to VPA, refractory to LEV	Spagnoli et al. 2020
5	F	/	Arg511Gln	De novo	/	SE	/	/	Knijnenburg et al. 2020
6	M	8 years	Arg511Gln	De novo	/	Febrile seizures	TPM, GBP, LEV, CBZ, PB, clonidine, flunarizine	/	Zagaglia et al. 2021
7	M	2 years	Arg511Gln	De novo	5 months	SE with focal seizures	VPA, CBZ	Controlled with CBZ	Zagaglia et al. 2021
8	M	37 years	Arg511Gln	Unconfirmed	18 months	Focal seizures with impaired awareness	VGB, CBZ, CLB	/	Zagaglia et al. 2021
9	M	36 years	Arg511Gln	Unconfirmed	/	/	/	/	Mainali et al. 2023
10	M	6 years	Arg511Gln	Unconfirmed	1 months	Focal to bilateral TCS	CBZ, LEV, PB, VPA, CZP, TPM, LTG, OXC, ACZ	Responds to ACE for dystonia	Langhammer et al. 2023
11	M	12 years	Arg511Gln	De novo	4 months	Febrile, complex partial, SE	LEV	Yes, responds to CBZ	Langhammer et al. 2023
12	M	7 years	Arg511Gln	De novo	6 months	Multiple seizures	LEV, ACZ, OXC, PNX	Experiences partial, non-sustained seizures	Langhammer et al. 2023
13	M	7 years	Arg511Trp	De novo	4 months	Complex partial, GTC	LEV	Yes	Straub et al. 2018
14	M	3 years	Arg511Trp	De novo	3 months	Focal status	LEV	Yes	Straub et al. 2018
15	M	14 years	Arg511Trp	De novo	3 months	Focal onset with and without impairment of awareness and evolution to bilateral TCS, 2× SE	PB, PHT, CBZ, pyridoxine	Controlled with CBZ	Zagaglia et al. 2021
16	F	5 years	Arg511Trp	De novo	3 months	Focal onset with myoclonic subtype, generalization, atypical absences	VPA, OXC, CLB, LTG, pyridoxine, ACZ	Controlled with ACZ	Zagaglia et al. 2021

(Continues)

TABLE 2 | (Continued)

	Sex	Age	Variant	Inheritance	Seizure onset	Seizure type	AEDs	Seizure treatment response	Ref.
17	F	3 years	Arg511Trp	De novo	0.21 months	Left focal onset with evolution to bilateral TCS	LEV, CLB, VPA	Controlled without treatment	Zagaglia et al. 2021
18	M	8 years	Arg511Trp	De novo	6 months	SE	CBZ, PHT	Yes, two seizures per year	Langhammer et al. 2023
19	F	7 years	Arg511Trp	De novo	5 months	Acute encephalopathy with hemiparesis, breakthrough focal seizures, SE	CBD, cyproheptadine, VPA, DZP, felbamate, OXC	Intractable seizures; multiple changes to AEDs	Langhammer et al. 2023
20	M	4 years	Arg511Trp	De novo	6 months	Right eye deviation with recurrent TCS	Initially, LEV, TPM, OXC, VPA; currently LEV+OXC, with recent addition of CBZ for dystonia	Partially controlled	Langhammer et al. 2023
21	M	7 years	Arg511Trp	De novo	5 months	5 months: focal motor SE, 15 months: Focal seizures, 5–7 years: Focal onset seizures evolving into bilateral convulsive SE	6 months, VPA; 2 years, CBZ; 5 years, PHT + LEV	VPA at 5 months, switched to CBZ at 15 months because of developmental delay, and added PHT at 5–7 years when LEV proved ineffective for seizure control	Fonseca et al. 2021
22	F	8 years	Arg483His	De novo	2 months	/	CBZ	Yes	Straub et al. 2018
23	M	3.5 years	Arg483His	De novo	0.01 months	Generalized	PB, CBZ	Yes	Straub et al. 2018
24	F	8 years	Arg483His	De novo	6 months	SE	VPA, TPM, ZNS, LCS, CLB, PB, LEV, ketogenic diet	Drug resistance	Straub et al. 2018
25	F	11 years	Arg483His	De novo	0.19 months	Focal, secondarily generalized, SE	PB, LEV, CZP, memantine	No seizures after age 4 years, later refractory epilepsy including SE	Straub et al. 2018
26	F	7 years	Arg483His	De novo	3 months	TS, febrile SE	PB, KBr, OXC, CZP	Refractory to PB, KBr, OXC, CZP	Belal et al. 2018
27	F	3 years	Arg483His	De novo	/	/	/	/	Necpál et al. 2020

(Continues)

TABLE 2 | (Continued)

	Sex	Age	Variant	Inheritance	Seizure onset	Seizure type	AEDs	Seizure treatment response	Ref.
28	M	8 years	Arg483His	De novo	0.01 months	Opisthotonus, head rotation	PB, VPA, LEV	Yes	Knijnenburg et al. 2020
29	F	11 years	Arg483His	De novo	0.19 months	/	/	/	Rochtus et al. 2020
30	M	3 years	Arg483His	De novo	/	/	Flunarizine, TPM	/	Zagaglia et al. 2021
31	F	11 years	Arg483His	De novo	5 months	Generalized TCS	TPM, PB	Yes	Langhammer et al. 2023
32	M	7 years 10 months	Arg483His	De novo	9 h after birth	TCS	VPA, sultiam, CBZ	Yes	Langhammer et al. 2023
33	M	11 years	Arg483His	De novo	3 months	Eye deviation, limp, reddened face, vomiting	TPM, CZP, lacosamide, baclofen	3 years seizure-free, recent seizure due to fever	Langhammer et al. 2023
34	F	11 years	Arg483His	De novo	/	Refractory seizures	/	/	Langhammer et al. 2023
35	M	4 months	Arg511Gln	De novo	4 months	Focal to bilateral TCS	VPA	Yes, responds to VPA	Our patient

Abbreviations: /, no data; ACZ, acetazolamide; AEDs, antiepileptic drugs; CBZ, carbamazepine; CLB, clobazam; CZP, clonazepam; F, female; GBP, gabapentin; KBr, potassium bromide; LEV, levetiracetam; LTG, lamotrigine; M, male; OXC, oxcarbazepine; PB, phenobarbital; PHT, phenytoin; PNX, pyridoxine; SE, status epilepticus; TCS, tonic–clonic seizures; TPM, topiramate; VGB, vigabatrin; VPA, valproate.

related to triggering factors such as fever (Langhammer et al. 2023), highlighting the complexity and variability in managing epilepsy in patients with RHOBTB2 variants. In the present case, seizure were controlled with valproate, similar to the case reported by Fonseca et al.; however, vigilance is warranted for possible recurrences as the patient grows, which may require a change or combination with other AEDs, such as carbamazepine or phenytoin (Fonseca et al. 2021). As research on the role of RHOBTB2 in neurological disorders expands, the potential for precise and effective therapeutic targets and personalized care strategies increases.

In summary, we described the case of a Chinese infant patient with epilepsy caused by the hotspot variant p.Arg511Gln in RHOBTB2. Approximately 50% of the 60 reported cases carry variants at positions Arg483 and Arg511. Protein folding free energy analysis suggested that hotspot variants (p.Arg511Gln, p.Arg511Trp, and p.Arg483His) may lead to increased structural stability, potentially contributing to their occurrence. In conclusion, expanding reports on RHOBTB2 variants will enrich our understanding of genotype–phenotype correlation and may foster greater research interest in this class of diseases.

Author Contributions

Conceptualization, Q.L. and F.L.; methodology, Q.L., Q.R., and N.W.; software, Q.R., N.W., and Z.F.; investigation, Q.L., F.L., and N.W.; data curation, Q.L., F.L., Q.R., N.W., and Z.F.; writing – original draft preparation, Q.L.; writing – review and editing, Q.L. and F.L. All authors have read and agreed to the published version of the manuscript.

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Ethics Statement

This study adhered to the principles outlined in the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Taihe County People's Hospital (approval number 03, granted March 2024).

Consent

Informed consent to publish the study was obtained from the proband's parent.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon request.

References

Bartolini, E., R. Campostrini, L. Kiferle, et al. 2020. "Epilepsy and Brain Channelopathies From Infancy to Adulthood." *Neurological Sciences: Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology* 41, no. 4: 749–761. https://doi.org/10.1007/s10072-019-04190-x.

Belal, H., M. Nakashima, H. Matsumoto, et al. 2018. "De Novo Variants in RHOBTB2, an Atypical Rho GTPase Gene, Cause Epileptic

Encephalopathy." *Human Mutation* 39, no. 8: 1070–1075. https://doi.org/10.1002/humu.23550.

Defo, A., A. Verloes, and N. Elenga. 2022. "Developmental and Epileptic Encephalopathy Related to a Heterozygous Variant of the RHOBTB2 Gene: A Case Report From French Guiana." *Molecular Genetics & Genomic Medicine* 10, no. 6: e1929. https://doi.org/10.1002/mgg3.1929.

Fonseca, J., C. Melo, C. Ferreira, M. Sampaio, R. Sousa, and M. Leão. 2021. "RHOBTB2 p.Arg511Trp Mutation in Early Infantile Epileptic Encephalopathy-64: Review and Case Report." *Journal of Pediatric Genetics* 12, no. 2: 155–158. https://doi.org/10.1055/s-0040-1722288.

Friocourt, G., and J. G. Parnavelas. 2010. "Mutations in ARX Result in Several Defects Involving GABAergic Neurons." *Frontiers in Cellular Neuroscience* 4: 4. https://doi.org/10.3389/fncel.2010.00004.

Hamaguchi, M., J. L. Meth, C. von Klitzing, et al. 2002. "DBC2, a Candidate for a Tumor Suppressor Gene Involved in Breast Cancer." *Proceedings of the National Academy of Sciences of the United States of America* 99, no. 21: 13647–13652. https://doi.org/10.1073/pnas.212516099.

Joerger, A. C., and A. R. Fersht. 2007. "Structural Biology of the Tumor Suppressor p53 and Cancer-Associated Mutants." *Advances in Cancer Research* 97: 1–23. https://doi.org/10.1016/S0065-230X(06)97001-8.

Knijnenburg, A. C. S., J. Nicolai, L. A. Bok, et al. 2020. "Acute Encephalopathy After Head Trauma in a Patient With a RHOBTB2 Mutation." *Neurology. Genetics* 6, no. 3: e418. https://doi.org/10.1212/NXG.0000000000000418.

Langhammer, F., R. Maroofian, R. Badar, et al. 2023. "Genotype-Phenotype Correlations in RHOBTB2-Associated Neurodevelopmental Disorders." *Genetics in Medicine: Official Journal of the American College of Medical Genetics* 25, no. 8: 100885. https://doi.org/10.1016/j.gim.2023.100885.

Lopes, F., M. Barbosa, A. Ameur, et al. 2016. "Identification of Novel Genetic Causes of Rett Syndrome-Like Phenotypes." *Journal of Medical Genetics* 53, no. 3: 190–199. https://doi.org/10.1136/jmedgenet-2015-103568.

Maddirevula, S., H. E. Shamseldin, A. Sirr, et al. 2020. "Exploiting the Autozygome to Support Previously Published Mendelian Gene-Disease Associations: An Update." *Frontiers in Genetics* 11: 580484. https://doi.org/10.3389/fgene.2020.580484.

Mainali, A., T. Athey, S. Bahl, et al. 2023. "Diagnostic Yield of Clinical Exome Sequencing in Adulthood in Medical Genetics Clinics." *American Journal of Medical Genetics. Part A* 191, no. 2: 510–517. https://doi.org/10.1002/ajmg.a.63053.

Necpál, J., M. Zech, A. Valachová, et al. 2020. "Severe Paroxysmal Dyskinesias Without Epilepsy in a RHOBTB2 Mutation Carrier." *Parkinsonism & Related Disorders* 77: 87–88. https://doi.org/10.1016/j.parkreldis.2020.06.028.

Niu, X. Y., Y. Sun, Y. Yang, et al. 2021. "RHOBTB2 Gene Associated Epilepsy and Paroxysmal Movement Disorder: Two Cases Report and Literature Review." *Acta Epileptologica* 3: 20. https://doi.org/10.1186/s42494-021-00056-y.

Pandey, P., and E. Alexov. 2024. "Most Monogenic Disorders Are Caused by Mutations Altering Protein Folding Free Energy." *International Journal of Molecular Sciences* 25, no. 4: 1963. https://doi.org/10.3390/ijms25041963.

Pederick, D. T., K. L. Richards, S. G. Piltz, et al. 2018. "Abnormal Cell Sorting Underlies the Unique X-Linked Inheritance of PCDH19 Epilepsy." *Neuron* 97, no. 1: 59–66.e5. https://doi.org/10.1016/j.neuron.2017.12.005.

Ramos, S., F. Khademi, B. P. Somesh, and F. Rivero. 2002. "Genomic Organization and Expression Profile of the Small GTPases of the RhoBTB Family in Human and Mouse." *Gene* 298, no. 2: 147–157. https://doi.org/10.1016/s0378-1119(02)00980-0.

Richards, S., N. Aziz, S. Bale, et al. 2015. "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." *Genetics in Medicine: Official Journal of the American College of Medical Genetics* 17, no. 5: 405–424. https://doi.org/10.1038/gim.2015.30.

Rochtus, A., H. E. Olson, L. Smith, et al. 2020. "Genetic Diagnoses in Epilepsy: The Impact of Dynamic Exome Analysis in a Pediatric Cohort." *Epilepsia* 61, no. 2: 249–258. https://doi.org/10.1111/epi.16427.

Scheffer, I. E., S. Berkovic, G. Capovilla, et al. 2017. "ILAE Classification of the Epilepsies: Position Paper of the ILAE Commission for Classification and Terminology." *Epilepsia* 58, no. 4: 512–521. https://doi.org/10.1111/epi.13709.

Schubert, J., A. Siekierska, M. Langlois, et al. 2014. "Mutations in STX1B, Encoding a Presynaptic Protein, Cause Fever-Associated Epilepsy Syndromes." *Nature Genetics* 46, no. 12: 1327–1332. https://doi.org/10.1038/ng.3130.

Sharma, S., and A. N. Prasad. 2017. "Inborn Errors of Metabolism and Epilepsy: Current Understanding, Diagnosis, and Treatment Approaches." *International Journal of Molecular Sciences* 18, no. 7: 1384. https://doi.org/10.3390/ijms18071384.

Spagnoli, C., L. Soliani, S. G. Caraffi, et al. 2020. "Paroxysmal Movement Disorder With Response to Carbamazepine in a Patient With RHOBTB2 Developmental and Epileptic Encephalopathy." *Parkinsonism & Related Disorders* 76: 54–55. https://doi.org/10.1016/j.parkreldis.2020.05.031.

Straub, J., E. D. H. Konrad, J. Grüner, et al. 2018. "Missense Variants in RHOBTB2 Cause a Developmental and Epileptic Encephalopathy in Humans, and Altered Levels Cause Neurological Defects in Drosophila." *American Journal of Human Genetics* 102, no. 1: 44–57. https://doi.org/10.1016/j.ajhg.2017.11.008.

Symonds, J. D., and A. McTague. 2020. "Epilepsy and Developmental Disorders: Next Generation Sequencing in the Clinic." *European Journal of Paediatric Neurology: EJPN* 24: 15–23. https://doi.org/10.1016/j.ejpn.2019.12.008.

Valentino, F., L. P. Bruno, G. Doddato, et al. 2021. "Exome Sequencing in 200 Intellectual Disability/Autistic Patients: New Candidates and Atypical Presentations." *Brain Sciences* 11, no. 7: 936. https://doi.org/10.3390/brainsci11070936.

Wilkins, A., Q. Ping, and C. L. Carpenter. 2004. "RhoBTB2 Is a Substrate of the Mammalian Cul3 Ubiquitin Ligase Complex." *Genes & Development* 18, no. 8: 856–861. https://doi.org/10.1101/gad.1177904.

Zagaglia, S., D. Steel, S. Krithika, et al. 2021. "RHOBTB2 Mutations Expand the Phenotypic Spectrum of Alternating Hemiplegia of Childhood." *Neurology* 96, no. 11: e1539–e1550. https://doi.org/10.1212/WNL.0000000000011543.