Pediatric Neurology 139 (2023) 59–64

Contents lists available at ScienceDirect

Pediatric Neurology

journal homepage: www.elsevier.com/locate/pnu

Research Paper

PEBAT, an Intriguing Neurodegenerative Tubulinopathy Caused by a Novel Homozygous Variant in *TBCD*: A Case Series and Literature Review

Claudia Ocampo-Chih, MD [a, †], Hailey Dennis, MMSc, CGC [b, †], Neil Lall, MD [c, d], Nga Pham, MD [c, e], Bo Liang, PhD [f], Sumit Verma, MD [c, g], Juanita Neira Fresneda, MD [b, *]

[a] *Monroe Carell Jr. Children's Hospital, Vanderbilt University, Nashville, Tennessee*
[b] *Department of Medical Genetics, Emory University School of Medicine, Atlanta, Georgia*
[c] *Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia*
[d] *Department of Radiology, Emory University School of Medicine, Atlanta, Georgia*
[e] *Department of Pediatric Critical Care Medicine, Emory University School of Medicine, Atlanta, Georgia*
[f] *Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia*
[g] *Department of Neurology, Emory University School of Medicine, Atlanta, Georgia*

ARTICLE INFO

Article history:
Received 29 May 2022
Accepted 8 November 2022
Available online 23 November 2022

Keywords:
PEBAT
Hypotonia
TBCD
WES
Neurodegeneration

ABSTRACT

Progressive encephalopathy with brain atrophy and thin corpus callosum (PEBAT) is a severe and rare progressive neurodegenerative disease (OMIM 617913). This condition has been described in individuals with pathogenic variants affecting tubulin-specific chaperone protein D (TBCD), which is responsible for proper folding and assembly of tubulin subunits. Here we describe two unrelated infants from Central America presenting with worsening neuromuscular weakness, respiratory failure, polyneuropathy, and neuroimaging findings of severe cerebral volume loss with thin corpus callosum. These individuals harbored the same homozygous variant of uncertain significance in the *TBCD* gene on whole exome sequencing (WES). Predicted protein modeling of this variant confirmed disruption of the protein helix at the surface of TBCD. The goal of this report is to emphasize the importance of rapid WES, careful interpretation of uncertain variants, prognostication, and family counseling especially when faced with a neurodegenerative clinical course.

Introduction

Microtubules are dynamic structures that are fundamental for neuronal differentiation, polarization, synapse formation, axonal growth, and signal conduction.[1] Microtubules are composed of α and β tubulin subunits, whose polymerization and depolymerization are carried out with the help of five tubulin-specific chaperone (TBC) proteins (TBCA, TBCB, TBCC, TBCD, and TBCE).[2,3]

Biallelic pathogenic variants in the *TBCD* gene have been shown to cause progressive encephalopathy with brain atrophy and thin corpus callosum (PEBAT) (OMIM 617913). Published literature review of this rare and progressive recessive genetic disorder, revealed 41 molecularly confirmed cases (before April 2022) with phenotypes ranging from mild to severe neurological dysfunction.[4-14]

In this case series and literature review, we highlight two unrelated patients with neurodegenerative disease courses, who were identified through WES to harbor a homozygous variant of uncertain significance (VUS) in the *TBCD* gene. Parental and local institutional consents were obtained for this report.

Case studies

Patient 1

Clinical presentation

This patient was born at 40 weeks, and his delivery was complicated by tight nuchal chord, late decelerations, and

Conflicts of interest statement: The authors of this manuscript have no financial or personal conflict of interest to disclose.
Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
* Communications should be addressed to: Dr. Fresneda; Department of Medical Genetics, Emory University School of Medicine, 1365 Clifton Road, Building B Suite 2200; Atlanta, GA 30322.
E-mail address: jneiraf@emory.edu (J. Neira Fresneda).
† Indicates shared first authorship.

https://doi.org/10.1016/j.pediatrneurol.2022.11.006
0887-8994/

meconium aspiration. He had noticeable hypotonia since birth and had a two-week neonatal intensive care unit admission for feeding difficulties. Brain magnetic resonance imaging (MRI) (Fig 1D) at birth was interpreted as normal for age. He was readmitted at 11 weeks of life with worsening hypotonia, muscle weakness, and respiratory failure necessitating ventilatory support. His examination was remarkable for a head circumference of 38 cm (18th percentile, - 0.88 S.D.) and plagiocephaly with no appreciable dysmorphic features. Ophthalmologic examination revealed optic nerve atrophy, nystagmus, absent tracking, and bilateral ptosis. He had paucity of movements in the upper and lower extremities and lacked antigravity strength. Tendon reflexes were absent with no palmar or plantar grasp and only subtle withdrawal to noxious stimulus in the lower extremities. Electrodiagnostics showed sensory motor axonal polyneuropathy and active denervation of the muscles.

Imaging

Repeat brain MRI at 11 weeks showed cerebral volume loss, lack of progression of myelination, and a thin corpus callosum (Fig 1C and D). A small area of posterior insular/perisylvian polymicrogyria was present bilaterally, and the optic nerves appeared small for age.

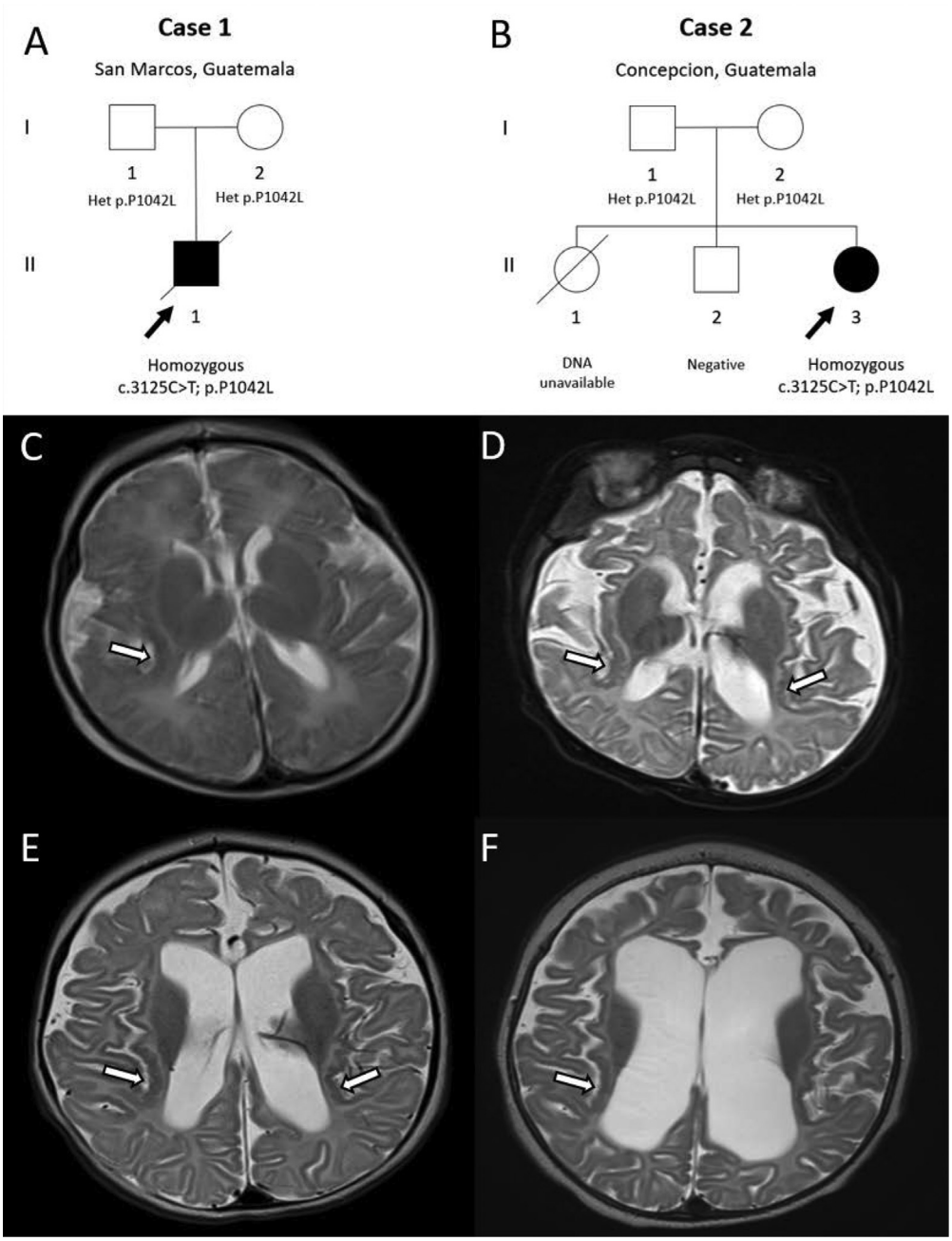

FIGURE 1. Pedigrees and magnetic resonance imaging (MRI) findings of cases 1 and 2. (A) Pedigree of case 1. (B) Pedigree of case 2. (C and D) Axial T2 MRIs of case 1 at age three days and 11 weeks, respectively. (E and F) Case 2 at age 10 weeks (three weeks corrected gestational age) and 19 weeks (12 weeks corrected gestational age), respectively. These images show progressive volume loss with increasing *ex vacuo* dilation of the lateral ventricles. Focal polymicrogyria is present in the right greater than left posterior insular/perisylvian cortex (arrows) in both patients. The color version of this figure is available in the online edition.

Genetic investigation

Family history was noncontributory (Fig 1A). His parents are from Guatemala and denied consanguinity. A chromosomal microarray revealed a 138-kb deletion of 22q22.21 (18847961 to 18986108) consistent with carrier status of hyperprolinemia type I. He had a negative hypotonia panel (spinal muscular atrophy, myotonic dystrophy, Prader-Willi syndrome, uniparental disomy 14), and his newborn screen and metabolic evaluation were normal.

Rapid WES trio identified a homozygous VUS, c.3125 C>T (p.Pro1042Leu), in the *TBCD* gene. Mitochondrial genome sequencing was unrevealing. Our genetics and neurology teams considered this homozygous variant as likely pathogenic given that his clinical presentation was consistent with PEBAT.

Outcome

This infant was extubated but remained dependent on positive pressure and never developed clinical seizures. Owing to his poor neurological prognosis, his family decided to redirect care and allow natural death shortly after counseling on exome results (see Supplemental Information for detailed ventilator management).

Patient 2

Clinical course

This patient was born at 33 weeks via emergent Caesarean section for fetal bradycardia. She was noted to have hypotonia, a weak cry, and upper extremity contractures at birth. She required admission to the neonatal intensive care unit for feeding difficulties but was discharged home at three weeks. At age two months, she was hospitalized for acute respiratory failure secondary to rhino/enterovirus and *Escherichia coli* urosepsis and was placed on mechanical ventilation. She developed epileptic encephalopathy refractory to multiple antiseizure medications. She had a prolonged hospital course during which she ultimately needed a tracheostomy with home ventilatory support and a gastrostomy tube.

Her physical examination at age 19 months was significant for microcephaly with head circumference of 43.1 cm (<3rd percentile, -2.72 standard deviation) with no dysmorphism. Her fingers were contracted with mild overlap bilaterally. She had camptodactyly of all toes with bilateral hammertoe deformity and prominent arches. She had poor head control and was areflexic. Electromyographic testing was indicative of severe neurogenic lesion affecting the motor neurons and or its axons.

Imaging

Noncontrast brain MRIs at 10 and 19 weeks of life were notable for progressive cerebral volume loss, callosal thinning, a small area of bilateral posterior insular/perisylvian polymicrogyria, and a suggestion of optic nerve hypoplasia/atrophy (Fig 1E and F).

Genetic investigation

Her family history was significant for a full sister who died at 18 days of life with hypotonia and poor feeding; parents could not exclude consanguinity (Fig 1B). Her newborn screening and chromosomal microarray were normal. Hypotonia, neuromuscular, and epilepsy gene panels revealed several VUS and recessive variants, which were not considered diagnostic (see Supplemental Information for detailed extensive genetic testing). WES trio identified a homozygous VUS, c.3125C>T (p.Pro1042Leu) in the *TBCD* gene. Genetics and neurology both determined that the *TBCD* variant was the most compelling explanation for her presentation.

Outcome

This patient remains alive at 30 months with significant neurocognitive delays, refractory epilepsy, tracheostomy, and ventilator dependence.

Discussion

PEBAT causes a wide spectrum of clinical characteristics, even between siblings with the same pathogenic variants. The most common features described are developmental delay, intellectual disability, seizures, and microcephaly. This presentation has a wide differential diagnosis including Pompe disease; myotonic dystrophy; neuromuscular, mitochondrial, and peroxisomal disorders; and inborn errors of metabolism such as very-long-chain acyl-CoA deficiency disorder.

Published cases before April 2022 have been summarized in Table. The physical examination, imaging, electrodiagnostics, and clinical course of the patients presented here are consistent with PEBAT. Variability on imaging has been reported, but these two cases had strikingly similar findings. In addition to the commonly reported and published radiological findings (hypomyelination, cerebral atrophy, and callosal thinning), we encountered optic nerve atrophy and small areas of polymicrogyria.

Both individuals were found to have a novel variant in *TBCD* that has not been previously published in the medical literature. Available population and computational data support pathogenicity of the c.3125C>T (p.Pro1042Leu) TBCD variant. Population data from gnomAD identified this variant in two of 248,360 total alleles tested, conferring a mean allele frequency (MAF) of 0.00008%. The highest mean allele frequency was 0.0018% in the European (non-Finnish) population. No healthy homozygotes have been reported. This finding suggests that this variant is extremely rare in the general population and provides moderate support for pathogenicity according to the classification guidelines established by the American College of Medical Genetics and Genomics and the Association of Pathology.[15] This variant has also been reported in ClinVar three times, twice as a VUS and once as likely pathogenic in a compound heterozygote by a German laboratory. Furthermore, the implicated base pair and the encoded amino acid are highly conserved across primates with a PhyloP score of 0.89. The variant discussed here has a Combined Annotation Dependent Deletion (CADD) score of 25.3 and is predicted to be damaging by SIFT and Polyphen, providing further support for pathogenicity according to the guidelines.

The TBCD protein is mainly composed of α-helices in antiparallel configuration.[6] In Fig 2, our protein model localizes the p.Pro1042Leu variant to one of these α-helices on the protein surface. Flex et al. simulated variant impact on protein structure. For most variants in their cohort, they observed a considerable decrease in structured residues due to transition from helix to coil. Reduced flexibility was seen at the other residues. All variants exhibited a statistically significant reduction of the solvent accessibility surface. These predictions were confirmed through functional studies in skin fibroblasts and HeLa cells.[6,10] One known pathogenic variant is a proline to leucine amino acid substitution (p.Pro1122Leu) located 80 bp downstream of our variant (p.Pro1042Leu). Flex et al. reported this substitution to be damaging to the protein structure and that it was associated with the most severe clinical phenotype. Considering the location within an α-helix and the specific amino acid substitution, it is reasonable to directly compare our variant with the p.Pro1122Leu variant. Such a variant is likely to destabilize the helix or disrupt interactions with other binding partners.

TABLE.
Summary of the Current Published Cases of PEBAT in the Literature

Variable (s)	Ocampo et al.		Flex et al.[6]	Miyake et al.[10]	Edvardson et al.[5]	Pode-Shakked et al.[11,*]	Ikeda et al.[8]	Gronborg et al.[7]	Zhang et al.[14]	Stephen et al.[12,†]	Isik et al.[9,‡]	Chen et al.[4]
	Case 1	Case 2	7 Cases	8 Cases	4 Cases	3 Cases	2 Cases	8 Cases	1 Case	1 Case	2 Cases	2 Cases
Variants (TCBD gene)	c.3125C>T [NM_005993.4]	c.3125C>T [NM_005993.4]	c.3365C>T c.2981C>T c.3313G>A c.1876G>A c.1130G>A c.771+1_771+10del c.1121 C>T c.686 T>G	c.1564-12 C>G c.2314 C>T c.1160 T>G c.2761 G>A c.2280 C>A c.3365 C>T c.2810 C>G	c.1423 G>A c.1757 C>T	c.1423 G>A c.1757C>T c.3192-2A>G	c.2825 G>A	c.3099 C>G	c.230 A>G; Del exons 28-39	c.1423G>A	c.202 C>T c.880 C>T	c.1340 C>T c.817+2T>C
Sex	M	F	4/7 M	3/8 M	4/4 F	3/3 M	2/2 F	3/8 M	M	M	One male and one female	2/2 F
Developmental delay	+	+	7/7	Present 6/8 NR 2/8	4/4	3/3	2/2	8/8	+	+	2/2	2/2
Intellectual disability	+	+	7/7	Present 5/8 NR 3/8	4/4	3/3	2/2	8/8	+	+	2/2	2/2
Encephalopathy	+	+	Absent in 3/7 NR 4/7	NR	NR	NR	NR	8/8	NR	+	NR	2/2
Microcephaly	+	+	4/7	8/8	4/4	3/3	2/2	Present 7/8 NR 1/8	-	+	Present 1/2 NR 1/2	NR
Hypotonia	+	+	Present 2/7 Absent 1/7 NR 4/7	6/8	4/4	3/3	2/2	8/8	+	+	2/2	2/2
Hyporeflexia	Hyporeflexia	Hyporeflexia	NR	NR	Brisk 2/4 NR 2/4	Hyporeflexia 2/3 NR 1/3	NR	Hyporeflexia 1/8 NR 7/8	Hyperreflexia	Hyperreflexia	Hyporeflexia 2/2	Hyporeflexia 1/2 NR 1/2
Seizures	-	+	Present in 6/7 NR 1/7	6/8	4/4	3/3	2/2	8/8	+	+	1/2	2/2
MRI/CT changes	Supratentorial volume loss. Hypomyelination with cerebral volume loss. Posterior insular/perisylvian polymicrogyria	Cerebral volume loss. Hypomyelination. Bilateral posterior insular/ perisylvian polymicrogyria	Most prominently hypomyelination and cortical white matter loss	Imaging only performed in 4 cases but most prominently with progressive atrophy of the cerebral cortex and enlargement of the ventricles	Varied, but mostly remarkable for generalized atrophy	Diffuse white matter volume loss with mild dilation of the ventricles	Cerebral atrophy	Cerebral cortical atrophy with white matter volume loss	Progressive brain atrophy, widened cortical sulci, and enlarged lateral ventricles	Diffuse cortical atrophy, white matter volume loss, atrophic corpus callosum in 3/3 of patients	Periventricular leukomalacia	Thinning of corpus callosum, diffuse cerebral atrophy, sulcal widening, enlargement of ventricles and cerebral cortical sulci
EMG/NCS changes	Diffuse denervation changes in the muscles	Severe neurogenic lesion affecting the motor neurons and/or its axons	NR	NR	NR	Severe motor axonal neuropathy in one case. Other cases not reported	One case with diffuse reduced nerve conduction velocity andreduced compound muscle action potential. Other case was not reported	Only reported in 2 cases with noted spinal muscular atrophy-like pathology and signs of denervation	NR	Axonal motor neuropathy	Sensorimotor axonal polyneuropathy	NR
Thin corpus callosum	+	+	7/7	Present 5/8 NR 3/8	4/4	3/3	NR	Present 5/8 NR 3/8	+	+	2/2	2/2
Respiratory failure	+	+	0/7	5/8	0/4	1/3	2/2	8/8	NR	-	NR	1/2
Outcome	Deceased	Alive with tracheostomy/ vent dependence	NR	Three deceased from respiratory complications	All alive	All alive	One case deceased. The other patient alive with tracheostomy dependence.	All deceased from respiratory failure	NR	Alive	NR	1 deceased at 36 months; 1 alive at 33 months

Abbreviations:
+ = Present
− = Absent
CT = Computed tomography
EMG = Electromyogram
F = Female
hom = Homozygous

M = Male
MRI = Magnetic resonance image
NCS = Nerve Conduction Studies
NR = Not reported
PEBAT = Progressive encephalopathy with brain atrophy and thin corpus callosum.
The three cases described by Tian et al. reflected patients with autism spectrum disorder and were not included in our summary table as they did not match the characteristics of our cases or those published in the literature.

* Pode-Shakked et al. cases 2 and 3 had been previously reported as cases 7 and 8 in Miyake et al.
† Only the proband was included in this table as affected siblings did not have molecular confirmation.
‡ Only cases 4 and 5 of Isik et al. present PEBAT cases.

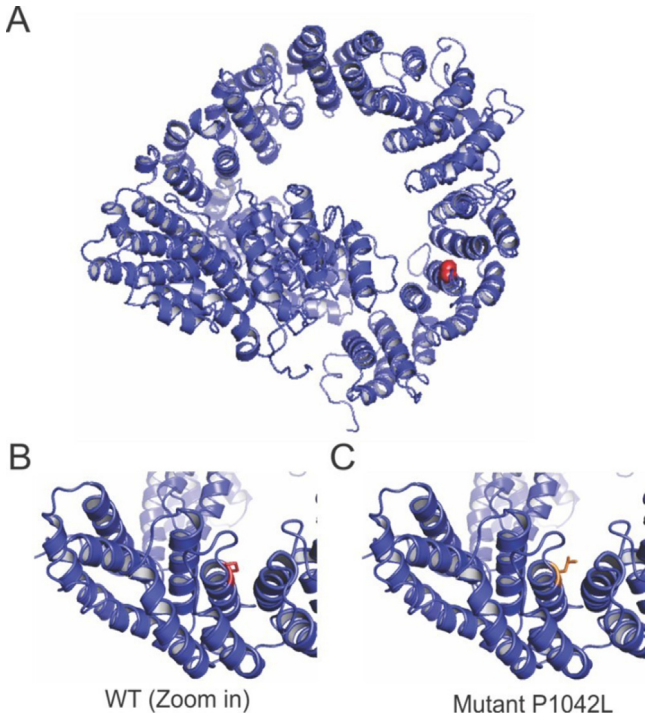

FIGURE 2. Structure model of the tubulin-specific chaperone protein D (TBCD) protein. (A) The modeled structure of the full-length TBCD. The ribbon diagram of the structure is shown in blue, and the red sphere indicates the amino acid position of P1042. (B) The zoomed-in image of the wild-type TBCD. The red sticks indicate P1042. (C) The mutant residue P1042L is animated with orange sticks. The structure model of TBCD was predicted using the homology modeling by Phyre2 (The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC) with intensive mode.[16] The confidence in the model: 1156 residues (97%) modeled at >90% confidence. The images are displayed with Pymol (Shrodinger LCC., 2015), and the variant is altered by Coot.[17] The color version of this figure is available in the online edition.

Conclusions

These individuals highlight the importance of considering rapid WES when faced with a neurodegenerative clinical course to avoid extensive non-cost-effective genetic evaluations. Although there is no treatment for PEBAT, earlier diagnosis can provide more tailored counseling for families on prognosis and recurrence risk.

Furthermore, these cases also highlight the gap that currently exists between VUS and available resources to investigate and reclassify such variants. Genetic testing has become routine in many places to help aid in diagnosis and management of critically ill patients. However, the rate at which VUSs are reported arguably outpaces the resources it takes to confirm or refute their pathogenicity. Many critically ill patients do not have time to wait for VUS to be reclassified, leaving their care teams to assess possible diagnoses as best they can. For the patients in this case report, both neurology and genetics critically compared these patients with those reported in the literature and determined that their clinical course was compelling for PEBAT. Although further data through functional studies and/or segregation analyses within other families would help confirm pathogenicity, our team considers this variant to be pathogenic.

Acknowledgments

We thank the families of both cases for allowing us to care for their children.

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.pediatrneurol.2022.11.006.

References

1. Tischfield MA, Cederquist GY, Gupta Jr ML, Engle EC. Phenotypic spectrum of the tubulin-related disorders and functional implications of disease-causing mutations. Curr Opin Genet Dev. 2011;21:286–294.
2. Kapitein LC, Hoogenraad CC. Building the neuronal microtubule cytoskeleton. Neuron. 2015;87:492–506.
3. Lewis SA, Tian G, Cowan NJ. The alpha- and beta-tubulin folding pathways. Trends Cell Biol. 1997;7:479–484.
4. Chen CL, Lee CN, Chien YH, Hwu WL, Chang TM, Lee NC. Novel compound heterozygous variants in TBCD gene associated with infantile neurodegenerative encephalopathy. Children (Basel). 2021;8:1140.
5. Edvardson S, Tian G, Cullen H, et al. Infantile neurodegenerative disorder associated with mutations in TBCD, an essential gene in the tubulin heterodimer assembly pathway. Hum Mol Genet. 2016;25:4635–4648.
6. Flex E, Niceta M, Cecchetti S, et al. Biallelic mutations in TBCD, encoding the tubulin folding cofactor D, perturb microtubule dynamics and cause early-onset encephalopathy. Am J Hum Genet. 2016;99:962–973.
7. Gronborg S, Risom L, Ek J, et al. A Faroese founder variant in TBCD causes early onset, progressive encephalopathy with a homogenous clinical course. Eur J Hum Genet. 2018;26:1512–1520.
8. Ikeda T, Nakahara A, Nagano R, et al. TBCD may be a causal gene in progressive neurodegenerative encephalopathy with atypical infantile spinal muscular atrophy. J Hum Genet. 2017;62:473–480.
9. Isik E, Yilmaz S, Atik T, et al. The utility of whole exome sequencing for identification of the molecular etiology in autosomal recessive developmental and epileptic encephalopathies. Neurol Sci. 2020;41:3729–3739.
10. Miyake N, Fukai R, Ohba C, et al. Biallelic TBCD mutations cause early-onset neurodegenerative encephalopathy. Am J Hum Genet. 2016;99:950–961.
11. Pode-Shakked B, Barash H, Ziv L, et al. Microcephaly, intractable seizures and developmental delay caused by biallelic variants in TBCD: further delineation of a new chaperone-mediated tubulinopathy. Clin Genet. 2017;91:725–738.
12. Stephen J, Nampoothiri S, Vinayan KP, et al. Cortical atrophy and hypofibrinogenemia due to FGG and TBCD mutations in a single family: a case report. BMC Med Genet. 2018;19:80.
13. Tian G, Lewis SA, Feierbach B, et al. Tubulin subunits exist in an activated conformational state generated and maintained by protein cofactors. J Cell Biol. 1997;138:821–832.
14. Zhang Y, Zhang L, Zhou S. Developmental regression and epilepsy of infancy with migrating focal seizures caused by TBCD mutation: a case report and review of the literature. Neuropediatrics. 2020;51:68–71.
15. 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.
16. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ. The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc. 2015;10:845–858.
17. Emsley P, Cowtan K. Coot: model-building tools for molecular graphics. Acta Crystallogr D Biol Crystallogr. 2004;60:2126–2132.