Non-5q Spinal Muscular Atrophy in a Patient With a Novel BICD2 Missense Variant

Nahee Park BS[1], Michael M Muriello MD[1,2], Donald Basel MD[1,2], Caroline A Kielczewski MS[1,2], Matthew Harmelink MD [1,3]

[1] Medical College of Wisconsin, Milwaukee, WI
[2] Dept of Pediatrics, Section of Medical Genetics, Medical College of Wisconsin, Milwaukee, WI
[3] Dept of Neurology, Section of Child Neurology, Medical College of Wisconsin, Milwaukee, WI

ABSTRACT

Variants in BICD cargo adapter 2 (*BICD2*) cause autosomal dominant spinal muscular atrophy with lower extremity dominance (SMALED2) which is characterized with lower extremity muscle weakness and atrophy. We describe a novel, heterozygous *BICD2* variant (c.1661T>C, [p.Leu554Pro]) in a 21-month-old female patient with a severe phenotypic presentation of the SMALED2 expression including arthrogryposis multiplex congenita, absent deep tendon reflexes, respiratory insufficiency, and cerebral depression. The variant p.Leu554Pro is located just outside of a domain that interacts with the motor protein KIF5A. The detailed neuro-phenotyping as well as a short clinical course is presented here expanding the understanding of *BICD2*-related disease.

Introduction

BICD2 is a highly conserved gene that encodes for protein bicaudal D homolog 2. BICD2 is a cargo adaptor protein involved in the dynein-dynactin motor complex which facilitates binding of transport vesicles and microtubules.[1] Since the identification of *BICD2*, more than 18 publications have been made that describe the disease spectrum.[2-19] Koboldt et al. (2020) published a review describing the phenotypic extremes of *BICD2* variants ranging from the absence of symptoms to arthrogryposis, respiratory failure, and death.[19] This wide range of symptoms suggests variable expressivity and incomplete penetrance.[13] Studies in mouse models suggest genetic modifiers act in a protective fashion which may explain the wide spectrum of symptoms in individuals with *BICD2*-related disease.[20,21]

Of the described *BICD2* variants, most are heterozygous missense variants, although cases of *BICD2* in-frame deletions have been reported.[15,17] Studies have found that individuals carrying heterozygous *BICD2* missense variants typically present with reduced fetal movement and benign or slowly progressive lower extremity muscle weakness and atrophy.[13]

Here we present a five-month-old female with a novel, heterozygous *BICD2* variant (c.1661T>C [p.Leu554Pro]) that has not been reported in ClinVar or GnomAD to date. The patient's presentation of severe arthrogryposis, absent deep tendon reflexes, preserved attentiveness, respiratory insufficiency, feeding difficulties, and cerebral depression is on the severe end of the typical SMALED2 expression spectrum, and we describe a new variant with phenotypic data.[19]

Clinical Report

A 21-month-old African American infant with a history of reduced fetal movement, severe arthrogryposis multiplex congenita (AMC), respiratory insufficiency, and cerebral depression (also known as neonatal encephalopathy) presented at 5 months of age for worsening symptoms. She was born prematurely at 34 weeks and 4 days (birth weight 1.8kg) via cesarean section due to severe joint contractures and hyperextended neck noted prenatally. Postnatal evaluation found severe arthrogryposis (elbows, wrists, fingers, shoulders adducted, knees extended, and hips abducted and dislocated), cervical spine hypermobility, and extreme hyperextension of the neck at rest. At birth, she had dysmorphic features including thin upper lip vermilion, prominent nasolabial fold, ectopic anus, triphalangeal thumb, overlapping fingers, a wide gap between the first and second finger, camptodactyly, and bilateral talipes equinovarus.

At birth, neurologic examination found minimal facial movement with slight myopathic appearance. Gag reflex along with upper and lower extremity stretch reflexes (biceps, supinator, triceps, patella, ankle, plantar) were absent. Muscle tone was low throughout. Overall, movements were limited to minimal distal but not proximal extremity movements. Cranial nerves were intact without tongue fasciculations, and she was responsive to stimuli throughout her extremities and torso. She was unable to be weaned from invasive support due to hypoventilation, and eventually required tracheostomy placement.

Electromyography (EMG) and Nerve Conduction Studies (NCS), done in the intensive care unit, demonstrated mildly prolonged peak latencies in the median and ulnar sensory nerves with profound reduced amplitudes in the right ulnar, right radial, and left sural nerves. The rest of the sensory nerve conduction studies were unremarkable. Additionally, the motor nerve conduction studies on the lower extremity demonstrated reduced amplitudes throughout with normal distal latencies and conduction velocities. Nee-

dle electrode examination of the left lower extremity demonstrated diffuse fibrillation potentials and positive sharp waves in all muscles; during activation, reduced recruitment of mildly increased amplitude motor unit potentials was seen in the tibialis anterior and gastrocnemius. This data is shown in Table 1A, 1B, & 1C.

Table 1: NCS and EMG Report

Nerve/ Sites	**Recording Site**	**Onset Latency (ms)**	**Peak Latency (ms)**	**NP Amplitude (uV)**	**PP Amplitude (uV)**	**Segments**	**Distance (cm)**	**Onset Velocity (m/s)**
Right Median – Index Finger								
Wrist	Index	3.28	4.17	35.6	54.6	Wrist-Index	5	15.2
Right Ulnar – Dig V								
Wrist	Digit V	2.86	3.13	0.56	3.9	Wrist – Digit V	2.4	8.4
Right Radial – Wrist								
Forearm	Wrist	1.51	1.82	3.5	4.1	Forearm – Wrist	3	19.9
Left Sural								
Calf	Lateral Malleolus	1.61	1.93	0.74	11.8	Calf – Lateral Malleolus	3.4	21.1
Left Superficial Fibular (Peroneal)								
Lateral Leg	Ankle	1.72	2.29	75.3	47.9	Lateral Leg – Ankle	3	17.5
Left Medial Plantar								
Medial Sole	Medial Malleolus	1.20	1.56	13.2	10.2	Medial Sole – Medial Malleolus	4.6	38.4

Table 1A: Sensory Nerve Conduction Studies: Demonstrated low amplitudes in the right ulnar, right radial and left sural nerves. Various sides were done to avoid the extremities with the worst contractures as well as support apparatus given the patient was in the ICU and maximize anatomical placement of stimulator and recording electrodes; however, given her overlapping fingers and wrist deviation this was difficult.

Nerve/Sites	Recording Site	Distal Latency (ms)	Amplitude (mV)	Distance (cm)	Velocity (m/s)
Right Median – Abductor pollicis brevis					
Wrist	Abductor pollicis brevis	1.77	2.2	2	
Elbow	Abductor pollicis brevis	3.07	2.5	5	38.4
Right Ulnar – Abductor digiti minimi					
Wrist	Abductor digiti minimi	3.54	1.8	2.6	
Below Elbow	Abductor digiti minimi	4.48	0.7	4.5	48
Above Elbow	Abductor digiti minimi	3.54	0.8	4.5	48
Left Fibular (Common Peroneal) – Extensor digitorum brevis					
Ankle	Extensor digitorum brevis	1.61	1.4	2.5	
Fibular Head	Extensor digitorum brevis	4.17	1.0	7	27.4
Left Tibial – Abductor hallucis					
Ankle	Abductor hallucis	2.5	1.5	2.4	
Knee	Abductor hallucis	5.10	1.1	7	26.9

Table 1B: Motor Nerve Conduction Studies: Reduced amplitudes throughout all muscles for age were seen with normal conduction velocities.

EMG Summary Table									
	Spontaneous/Insertional					Morphology			Recruitment
Muscle	Insertional Activity	Fibrillation	Positive Sharp Waves	Fasciculations	Other	Amplitude	Duration	Polyphasia	Pattern
L. Tibialis Anterior	Normal	1+	None	None	None	1+	Normal	Normal	1-
L. Gastrocnemius (Medial Head)	Normal	1+	None	None	None	1+	Normal	Normal	1-
L. Vastus medialis	Normal	1+	None	None	None	Normal	Normal	Normal	1-
L. Adductor Longus	Normal	1+	None	None	None	Normal	Normal	Normal	1-
L. Iliopsoas	Normal	1+	None	None	None	Normal	Normal	Normal	1-

Table 1C: Needle Electrode Examination: During the needle electrode evaluation, diffuse fibrillation potentials were seen as well as reduced recruitment with large amplitude motor unit potentials in the tibialis anterior and gastrocnemius with other muscle unit morphology being normal.

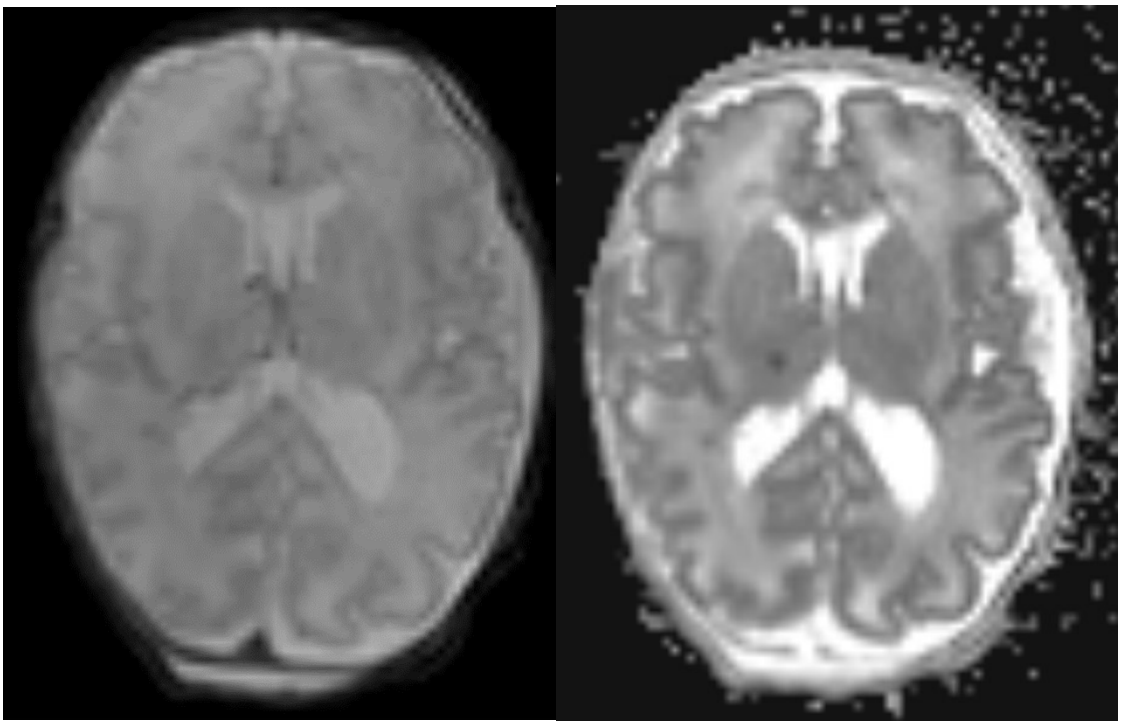

Figure 1: MRI Brain (obtained at age 1 day old) demonstrating mild ventriculomegaly as well as the punctate foci of restricted diffusion. The punctate foci were not felt to be significant to indicate another etiology or birth related injury.

The patient's brain magnetic resonance imaging (MRI) revealed punctate lesions in the right thalamus and left caudate with mild enlargement of the left lateral ventricle (Figure 1). The punctate lesions were thought to be clinically insignificant due to their small size.

The patient underwent genetic analysis which revealed a heterozygous, missense variant in *BICD2* (NM001003800.1, c.1661T>C, p.Leu554Pro). This variant was not present in the ClinVar, GnomAD, or Pubmed databases. Follow up familial testing did not identify the variant in the patient's mother. The father was not available for analysis and his clinical history was unable to be obtained. This variant was classified as a variant of uncertain significance according to ACMG guidelines.[22] There were no clinically relevant copy number variants (CNVs) or structural changes on karyotype. A compilation of further testing done did not reveal significant findings as shown in Table 2.

Supplemental Clinical Report

The patient's clinical progression was tracked with age. At 21-months-old, she had made some improvement without any regressions. Per mother, the patient was able to flex and extend her left elbow but could not move her right elbow. Her contractures were less fixed with slight improvement compared to 1 year prior. However, the patient's motor tone, motor strength, reflexes, and respiratory status were unchanged. Additionally, she started to make non-specific babbling but did not advance cognitively beyond that stage of language. She did not have any history of seizures. Between her 21st and 26th month, she started to say "Hi" without any other developmental advancement despite frequent therapy services.

Methods

Clinical Exome sequencing (WES) assay was used. Next Generation Sequencing (NGS) technologies were used to cover the coding regions of targeted genes. Genomic DNA was extracted from the patient's specimen, captured using Agilent Clinical Research Exome hybridization probes, and sequenced using Illumina's Reversible Dye Terminator (RDT) platform NovaSeq 6000 using 150 by 150 bp paired-end reads (Illumina, San Diego, Ca, USA). Median coverage of the target region was 120X with 98% of target bases covered by at least 20 reads. Data analysis and interpretation were performed using an internally developed software Titanium-Exome. The identified variant was confirmed by targeted Sanger sequencing.

Table 2: Compilation of further testing is shown above with respect to age.

Genetic Testing (Age: 1 day old)	Prevention Genetics Pediatric Disease Panel with exome-wide CNV analysis: - BICD2 c.1661T>C p.Leu554Pro heterozygous variant of unknown significance (VUS). Not maternally inherited. - NEB c.7710T>A p.Asp2570Glu heterozygous variant of unknown significance (VUS). - No copy number variants detected.
Echocardiogram (Age: 1 day old)	Structurally normal heart. - Patent ductus arteriosus with a trivial shunt, left-to-right. - PDA peak gradient = 18 mmHg. - Normal left ventricular cavity size, wall thickness, and systolic function. - Left ventricular ejection fraction (apical 4-chamber) = 66 %. - Normal right ventricular cavity size, wall thickness, and systolic function. - Patent foramen ovale -small interatrial communication -left-to-right shunt. - No pericardial effusion.
Renal Ultrasound (Age: 1 day old)	Normal renal ultrasound including the bladder with trace left pelviectasis, likely physiologic.
FISH Cytogenetics Study Report (Age: 4 days old)	Aneuploidy not detected, female FISH pattern.
Spine MRI (Age: 11 days old)	- Mild prominence of the central canal measuring up to 2.2 mm from T9 through T12. Otherwise unremarkable exam. - Note: This is a limited spine examination performed for detection and follow-up of syringohydromyelia. Other spinal cord, bony spine and soft tissue abnormalities may be missed on this protocol. If there is persistent clinical concern, dedicated MRI of the spine would be of value in further assessment.
Brain MRI and Venogram (Age: 25 days old)	- Stable appearance of small hematoma in left frontal white matter, not significantly changed since study dated 3/16/2020. No evidence of abnormal vascular flow voids in the vicinity of this hematoma to suggest an underlying vascular malformation. No new focus of intracranial hemorrhage is evident. - Punctate foci of T1 hyperintensity and T2 hypointensity in right periatrial white matter appear stable. These are suggestive of foci of nonspecific subacute insult. - Bilateral parieto-occipital convexity subdural fluid collections (larger on left) appear slightly decreased in size since prior study. There is no intracranial mass effect. Basal cisterns are preserved. - Unchanged mild enlargement of the left lateral ventricle. No evidence of transependymal flow. - Normal MR venography of the brain. No evidence of dural venous sinus thrombosis.
XR Legs Bilateral (Age: 39 days old)	Changes of arthrogryposis with bowing deformity. Significant periosteal change is noted involving both femora and both tibia with bowing deformity. The more significant changes to the distal metaphysis of the femur on the left and the left and right distal tibiae and fibulae are suggestive of older posttraumatic change with healing.
Ultrasound Hips (Age: 40 days old)	- Left Hip: Shallow left acetabulum with undercoverage of the left femoral head in the neutral and flexed positions. - Right Hip: Shallow right acetabulum with undercoverage of the right femoral head in the neutral and flexed positions.
Brain MRI (Age: 56 days old)	- No convincing residual subdural collections. - Punctate susceptibility adjacent to left frontal horn is smaller. - Stable left trigone larger than right without CSF flow. - Overall, no acute interval change.
Creatine Kinase (Age: 6 days old)	64 (Within Normal Limits)

The variant was visualized in the 3-dimensional protein structure (Figure 2) using the AlphaFold prediction[28,29] and the YASARA software.[30]

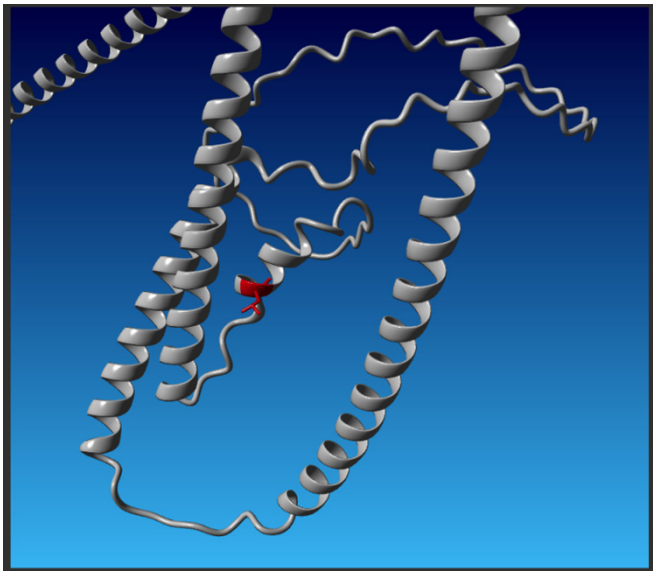

Figure 2: Heterozygous *BICD2* variant (c.1661T>C, p.Leu554Pro) visualized in the 3-dimensional protein structure using the AlphaFold prediction[28,29] and the YASARA software[30]

Discussion

In this case report, we discuss a patient with a novel mutation with a severe phenotypic presentation of the typical SMALED2 expression including severe arthrogryposis, absent deep tendon reflexes, preserved attentiveness, respiratory insufficiency, and cognitive impairment. Genetic testing identified a novel heterozygous variant in *BICD2* (p.Leu554Pro), which fit her phenotype and led to her diagnosis of autosomal dominant spinal muscular atrophy, lower extremity dominant, type 2B. Finally, we demonstrate a 21-month course with minimal improvements but no declines in either motor or cognitive functioning.

Spinal muscular atrophy (SMA), a leading genetic cause of infant mortality, is a congenital neuromuscular disorder that results in a loss of motor neurons often leading to progressive skeletal muscle weakness and atrophy.[23] However, less data is available for non-SMN1 associated disease. Autosomal dominant SMA with lower extremity dominance (SMALED), which is characterized by skeletal muscle weakness and atrophy in the lower extremities, was later identified to be associated with 2 loci: SMALED1 and SMALED2.[19] In 2013, the molecular basis of SMALED2 was identified to be due to a variant in *BICD2*.[24] Since 2019, the Online Mendelian Inheritance in Man (OMIM) database further classified SMALED2 into two categories: autosomal dominant spinal muscular atrophy type 2A and 2B (OMIM #615290 & 618291).[19] Type 2A describes a milder form of the disease while Type 2B describes a more severe form of the disease.[19]

Previously described *BICD2* variants include heterozygous missense changes and in-frame deletions. With these molecular changes, a wide phenotypic spectrum has been described, ranging from an asymptomatic patient with subclinical findings at the age of 71 years old, to multiple independent families with severe AMC, respiratory insufficiency, and early death.[13]

In a review by Koboldt et al. (2020), all 99 individuals with *BICD2* presented with weakness in distal muscles of the lower limbs. Upper limb (UL) weakness was significant, although distal UL was more prominent than proximal UL. 60% of families had contractures, 49% had hip dislocations, 34% had arthrogryposis multiplex congenita (AMC), 55% had talipes equinovarus, 60% had spinal deformities, 17% had respiratory problems, and 49% showed at least one or more signs of central nervous system involvement including increased reflexes (29%) or brain abnormalities (24%).[19] Phenotypically, our patient exhibited all of these symptoms including severe AMC, hip dislocation, hyperextension of neck, and talipes equinovarus, in addition to respiratory insufficiency.

The patient's brain MRI revealed punctate lesions in the right thalamus and left caudate with mild enlargement of the left lateral ventricle (Figure 1). The punctate lesions were thought to be clinically insignificant due to their small size. Similar cases regarding abnormal cerebral white matter have been identified. For instance, a patient with moderate thinning of the corpus callosum and two minor lesions on the posterior part of bilateral second ventricles ultimately passed away due to respiratory failure.[13] Another patient with complete absence of cerebral white matter and cortex with significant enlargement of the ventricular system developed epilepsy and hydrocephalus requiring shunt placement.[17] Although our patient did not present with epilepsy or hydrocephalus, all three of these patients provide evidence that cerebral atrophy may occur in severe SMALED2B diseases.

Our patient's EMG revealed features consistent with sensorimotor neuropathy (Table 1A, 1B, & 1C). Similar cases regarding EMG results have been identified. For instance, in another case, EMG data revealed fibrillation potentials, positive sharp waves, and motor units with markedly increased amplitude.[6] Additionally, in that case, the patient's muscle biopsy showed neurogenic fiber atrophy. While our patient did not undergo a muscle biopsy for comparison, this example shows the association with

BICD2 variant and chronic neurogenic process with spinal motor neuron involvement. Interestingly, in both the article by Bansagi et al (2015) and in our patient, they report no clinical sensory abnormalities though, in our patient, her age and cognitive impairments may limit this evaluation.

Molecular Analysis

The heterozygous *BICD2* variant (c.1661T>C, p.Leu554Pro) was plotted using UniProtKB entry Q8TD16 and was compared with nearby variants within the mutational hotspot region. This figure displayed three coiled-coil domain structures of the BICD2 protein which were categorized into Coiled Coil 1 (CC1), Coiled Coil 2 (CC2), and Coiled Coil 3 (CC3). The p.Leu554Pro variant is located just outside of the CC2 domain and within a region that has been shown to interact with the motor protein, kinesin-1 (isoform KIF5A) in mice.[25] Mutations in *KIF5A* cause decreased motor neuron survival which led to impaired axonal and dendritic outgrowth and impaired anterograde axonal transport in mice.[26,27] A nearby in-frame 3bp deletion, p.Asn546Del is also just outside of CC2 within a region that interacts with molecular motor kinesin-1.[17] The individual with that variant, like our patient, exhibited extreme phenotypic expression with severe AMC, muscle weakness, cerebral cortical atrophy.

While it has been difficult to predict phenotypic expression of *BICD2* variants, Koboldt et al. attempted to correlate variant location with clinical features. Variants in CC2 and CC3 seemed to associate with severe phenotypes including AMC, brain abnormalities, and respiratory issues.[19] CC2 domain variants tended to exhibit fewer foot deformities and spine deformities. Similar to other patients with variants near domain CC2, our patient exhibited severe phenotypic expressions, supporting the genotype-phenotype conclusion identified by Koboldt et al.

Lastly, although the variant did not meet American College of Medical Genetics criteria for "likely pathogenic," we feel that the data supports the pathogenicity of the variant. This is in alignment with multiple in silico scores which predict a deleterious or damaging effect of the amino acid substitution. Additionally, there was absence of any other notable variants reported on exome analysis with clinical features that could fit the diagnosis of *BICD2*-related disease. Therefore, additional tests such as molecular or metabolic testing were not pursued. However, as with current testing capabilities, it is possible that a variant not detected by exome sequencing or a variant in a gene of uncertain significance was excluded from the analysis and could be contributing to the patient's phenotype.

Conclusion

We describe a novel *BICD2* variant (c.1661T>C, [p.Leu554Pro]) in a patient with a severe phenotypic presentation of the typical SMALED2 expression including arthrogryposis multiplex congenita, respiratory insufficiency, and cerebral depression. While it is challenging to predict how a genotype will present phenotypically, these case studies provide evidence that specific amino acid residues of the BICD2 protein must be essential in organizing and maintaining normal intracellular transport processes.

Future Direction

Since the genetic variant is a VUS, a study on the patient's fibroblasts to evaluate for the presence of Golgi-fragmentation may help us understand the impact of the patient's genetic variation. Additional studies such as muscle or nerve biopsies could provide additional support for a deleterious effect on protein function.

Acknowledgements

We thank the patient and her family for their participation and cooperation in this case report.

Conflict of Interest

The authors report no conflicts of interest.

Author Contributions

NP, MM, DB, CK and MH all participated in the creation, review and submission of the manuscript.

Corresponding Author

Nahee Park
8701 W Watertown Plank Rd, Milwaukee, WI 53226
Phone number: 414-955-8296
npark@mcw.edu

References

1. Martinez Carrera, L, Gabriel, E, Donohoe, C,et al. Novel insights into SMALED2: BICD2 mutations increase microtubule stability and cause defects in axonal and NMJ development. *Human molecular genetics* 2018; *27*(10), 1772–1784.

2. Neveling K, Martinez-Carrera LA, Hölker I, et al. Mutations in BICD2, which encodes a golgin and important motor adaptor, cause congenital autosomal-dominant spinal muscular atrophy. *Am J Hum Genet* 2013; 96: 946–954.

3. Peeters K, Litvinenko I, Asselbergh B, et al. Molecular defects in the motor adaptor BICD2 cause proximal spinal muscular atrophy with autosomal-dominant inheritance. *Am J Hum Genet* 2013; 92: 955–964.

4. Oates EC, Rossor AM, Hafezparast M, et al. Mutations in BICD2 cause dominant congenital spinal muscular atrophy and hereditary spastic paraplegia. *Am J Hum Genet* 2013; 92: 965–973.

5. Rossor AM, Oates EC, Salter HK, et al. Phenotypic and molecular insights into spinal muscular atrophy due to mutations in BICD2. *Brain* 2015; 138: 293–310.

6. Bansagi B, Griffin H, Ramesh V, et al. The p.Ser107Leu in BICD2 is a mutation 'hot spot' causing distal spinal muscular atrophy. *Brain* 2015; 138(pt 11):391.

7. Fiorillo C, Moro F, Brisca G, et al. Beyond spinal muscular atrophy with lower extremity dominance: cerebellar hypoplasia associated with a novel mutation in BICD2. *Eur J Neurol* 2016;23:19–21.

8. Ravenscroft G, Di Donato N, Hahn G, et al. Recurrent de novo BICD2 mutation associated with arthrogryposis multiplex congenita and bilateral perisylvian polymicrogyria. *Neuromuscul Disord* 2016; 26:744–748.

9. Rudnik-Schoneborn S, Deden F, Eggermann K, et al. Autosomal dominant spinal muscular atrophy with lower extremity predominance: A recognizable phenotype of BICD2 mutations. *Muscle Nerve* 2016;54:496–500.

10. Synofzik M, Martinez-Carrera LA, Lindig T, et al. Dominant spinal muscular atrophy due to BICD2: a novel mutation refines the phenotype. *J Neurol Neurosurg Psychiatry* 2014;85:590–592

11. Theunissen TEJ, Sallevelt S, Hellebrekers D, et al. Rapid resolution of blended or composite multigenic disease in infants by wholeexome sequencing. *J Pediatr* 2017;182:371–374.e2

12. Unger A, Dekomien G, Güttsches A, et al. Expanding the phenotype of BICD2 mutations toward skeletal muscle involvement. *Neurology* 2016;87:2235–2243.

13. Storbeck M, Eriksen BH, Unger A, et al. Phenotypic extremes of BICD2-opathies: from lethal, congenital muscular atrophy with arthrogryposis to asymptomatic with subclinical features. *Eur J Hum Genet* 2017;25:1040–1048.

14. Souza PVS, Pinto W, Aivazoglou LU, et al. Distal myopathy due to BICD2 mutations. *Clin Neurol Neurosurg* 2018;165:47–49.

15. Trimouille A, Obre E, Banneau G, et al. An in-frame deletion in BICD2 associated with a non-progressive form of SMALED. *Clin Neurol Neurosurg* 2018;166:1–3.

16. Ahmed AA, Skaria P, Safina NP, et al. Arthrogryposis and pterygia as lethal end manifestations of genetically defined congenital myopathies. *Am J Med Genet A* 2018;176:359–367.

17. Koboldt DC, Kastury RD, Waldrop MA, et al. In-frame de novo mutation in BICD2 in two patients with muscular atrophy and arthrogryposis. *Cold Spring Harb Mol Case Stud* 2018;4:a003160.

18. Yoshioka M, Morisada N, Toyoshima D, et al. Novel BICD2 mutation in a Japanese family with autosomal dominant lower extremitypredominant spinal muscular atrophy-2. *Brain Dev* 2018;40:343–347.

19. Koboldt D, Waldrop M, Wilson R, et al. The Genotypic and Phenotypic Spectrum of BICD2 Variants in Spinal Muscular Atrophy. *Ann Neurol* 2020; 87: 487-496.

20. Hosseinibarkooie S, Peters M, Torres-Benito L et al: The power of human protective modifiers: PLS3 and CORO1C unravel impaired endocytosis in spinal muscular atrophy and rescue SMA phenotype. *Am J Hum Genet* 2016; 99: 647–665.

21. Riessland M, Kaczmarek A, Schneider S et al: Neurocalcin delta suppression protects against spinal muscular atrophy in humans and across species by restoring impaired endocytosis. *Am J Hum Genet* 2017; 100: 297–315.

22. 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. Genetics in medicine: official journal of the American College of Medical Genetics 2015, 17(5), 405–424.

23. Kolb S, Coffey C, Yankey J, et al. Natural History of Infantile-Onset Spinal Muscular Atrophy. Ann Neurol 2017; 82(6): 883-891.

Neveling K, Martinez-Carrera L, Hölker I, et al. Mutations in BICD2, which encodes a golgin and important motor adaptor, cause congenital autosomal-dominant spinal muscular atrophy. Am J Hum Genet 2013; 92(6):946-954.

Splinter D, Tanenbaum ME, Lindqvist A, et al. Bicaudal D2, dynein, and kinesin-1 associate with nuclear pore complexes and regulate centrosome and nuclear positioning during mitotic entry. PLoS Biology 2010. 8: e1000350.

Karle K, Möckel D, Reid E, et al. Axonal transport deficit in a KIF5A$^{(-/-)}$ mouse model. Neurogenetics 2012; 13: 169–179.

Xia C, Roberts E, Her L, et al. Abnormal neurofilament transport caused by targeted disruption of neuronal kinesin heavy chain KIF5A. J. Cell Biol 2003; 161:55–66.

24. Jumper, J et al. Highly accurate protein structure prediction with AlphaFold. Nature (2021).

25. Varadi M, et al. AlphaFold Protein Structure Database: massively expanding the structural coverage of protein-sequence space with high-accuracy models. Nucleic Acids Research (2021).

26. Krieger E, Vriend G (2014) Bioinformatics 30, 2981-2982 PMID24996895

27. Frasquet M, et al. Clinical Spectrum of BICD2 Mutations. European Journal of Neurology 2020;27(7):1327-1335