Reassessment of Rare *TUBA4A* Variants in Patients with Myopathy and Neurodevelopmental Features

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INTRODUCTION & METHODS

TUBA4A variants are recognized in ClinGen and gene panels for adult-onset amyotrophic lateral sclerosis (ALS) & thrombocytopenia. However, recent publications have reported variants present in children with wider phenotypes. Review of internal genome and exome sequencing (WGS/WES) data for patients with unsolved phenotypes overlapping with myopathy or neurodevelopmental features uncovered 4 rare (AF<0.0001) *de novo* variants in *TUBA4A* for deeper review and reconsideration of pathogenicity. We present the evidence supporting these variant associations with pediatric myopathy and discuss the *in silico* data supporting future investigations of their putative pathogenic mechanisms.

Review & Reclassification of Cases

Querying all rare (AF<0.0001) *TUBA4A* variant carriers with phenotypes overlapping with myopathy or neurodevelopmental disorder (NDD)

↓

Filtering out variants known to be benign and/or highly present in "normal" populations

↓

Identifying existing publications and/or publicly available submissions of variant-disease relationship

↓

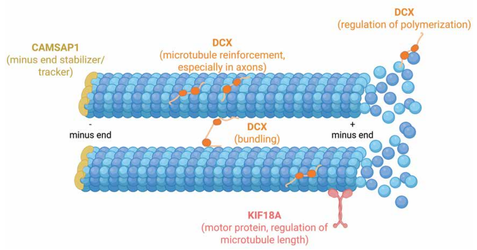
Generating computational and/or predictive evidence of:

- Conservation of the molecular alterations/residue
- Predicted Δ in splicing (SpliceAI, Pangolin)
- Predicted missense impact (AlphaMissense, REVEL, SIFT, PolyPhen)
- Predicted Δ in post-translational modifications (DeepMVP)
- Predicted Δ in tertiary/quaternary protein structure (AlphaFold3)

Proposing reclassification for top variants based on ACMG/AMP guidelines

Population	not in healthy pop *(PP → PM2)*		--
Segregation	co-segregation with disease in multiple affected family members *(PP1+)*		
De novo	--	unconfirmed *(PM6)*	confirmed *(PS2)*
Functional	↓ benign missense % *(PP1)*	hotspot/domain *(PM1)*	functional studies *(PS3)*
Computational	multiple predictions *(PP3)*	residue known as P *(PM5)*	exact Δ known as P *(PS1)*
Other	supporting to moderate evidence		--

Figure 1. *TUBA4A* is a key tubulin whose molecular interactions and post-translational modifications regulate microtubule (MT) dynamics



TUBA4A interaction partners prioritized based on report in the Tubulin Database; Figure created with BioRender

Figure 2. Expression of *TUBA4A* and *CAMPSAP1* is enriched in skeletal muscle compared to other α-tubulin and MT associated protein (MAP) genes (Human Protein Atlas)

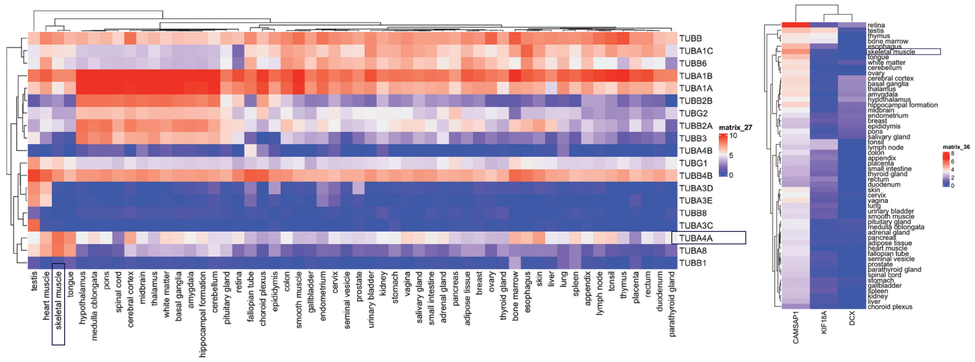


Figure 3. Internally identified myopathy & NDD patients carrying *TUBA4A* variants do not demonstrate a visually differentiable distribution pattern along the gene versus previously reported ALS variants

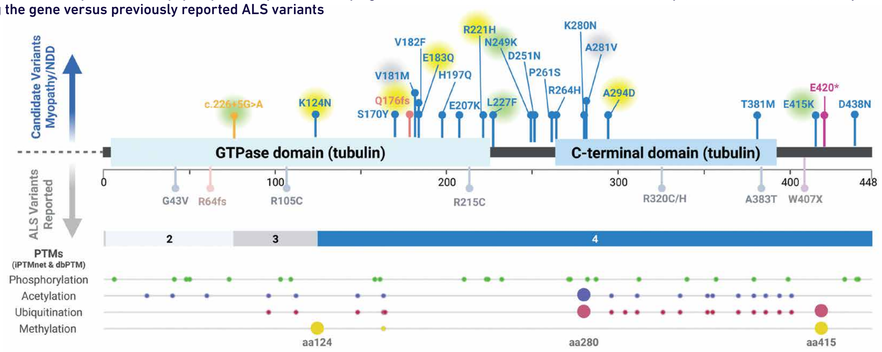


Figure 4. Variant prioritization surfaces top 4 variant candidates for reclassification

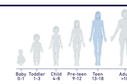
		Cases	Unsolved Myo/NDD	Age (y)	Mutation Taster	Other Predictors	PTM	Predicted change in: Structure	Interactions	Literature/Other Support
Prioritized Variants	**N249K**	1	✓	18	Deleterious	AM: Strong Pathogenic	↑ acetyl ↑ methyl	None	Altered MAP interactions	No publications but ✓ Biopsy immunopositive
	L227F	1	✓	2	Deleterious	AM: Strong Pathogenic	None	None	N/A	✓ Direct
	c.226+5G>A	1	✓	10	Deleterious	SpliceAI: Splice Loss	N/A	N/A	N/A	✓ Indirect
	E415K	1	✓	32	Deleterious	AM: Strong Pathogenic	↑ methyl ↑ sumo	None	Altered MAP interactions	✓ Direct
Variants w/ Puibs	**R221H & A281V** *(also carries p.K280N)*	2	✓ (2/2)	3 36	Deleterious	PrimateAI: Deleterious	None	N/A	N/A	✓ Direct in Other Tissue
	E183Q & A281V	1	✓	11	Deleterious	AM: Pathogenic	None	N/A	N/A	✓ Direct
	K124N	1	✓	24	Benign	AM: Pathogenic	↑ glycosyl ↓ methyl ↓ ubiquitin		N/A	✓ Indirect
Other	**A294D**	1	✓	10.5	Benign	AM: Support Pathogenic	None	N/A	N/A	None
	Q176fs	1	✓	3	Deleterious	NMD due to >10%	None	N/A	N/A	None
↓ pen.	*A281V*	12	3 (75% solved)	0-33	Deleterious	AM: Support Pathogenic	N/A	N/A	N/A	None
	V181M	5	2 (60% solved)	2-44	Deleterious	AM: Support Pathogenic	None	N/A	N/A	✓ Indirect

AM: AlphaMissense; DD: developmental delay; ID: intellectual disability; MT: microtubule; PTM: post-translational modification; *R: age at report (age of onset not available)

Figure 5. Review 4 prioritized cases provided rationale for follow-up and potential gene/variant reclassification

N249K

Not reported
c.747T>G



Onset: Neonate (M 0)
(hypotonia; metatarsus adductus)

Severe, progressive mobility loss
(wheelchair dependence: 9yo)

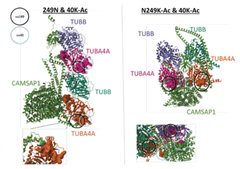
Delayed language/cognition/motor development, intellectual disability, & abnormal attention/emotional state

Diffuse bilateral weakness (upper/lower limbs), broad-based gait then wheelchair dependence, elbow/feet contractures

No ptosis/swallowing defect; mild dysarthria/slurred speech; autophagic vacuoles & TUBA4A+ aggregates in muscle biopsy

***In silico* mechanistic investigation (AlphaFold3)**

- 249N near the regulatory residue 40K & binding interface for αß-tubulin
- Acetylation (Ac) at 40K increases MT stability
- N249K-Ac predicted to alter interaction of αß-dimers with CAMSAP1 (muscle-associated MAP)

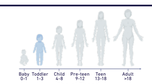


Current Classification: N/A *(missense at same residue is VUS)*
Proposed Classification: Likely Pathogenic

Population: not in ExAC/gnomAD	**Functional:** TUBA4A+ aggregates
Segregation: N/A	**Computational:** multiple predictions
De novo: confirmed	**Other:** same residue variant not benign

L227F

Publications
Only c.679C>T



Onset: Infancy/neonate (F 0)
(hypotonia)

Moderate-to-severe weakness
(lower limbs)

Delayed language (2-3 words) & motor skill (sit-stand defect, Gower's sign, not running), normal cognition at 25 mo

Hypotonia, hyporeflexia of patella/Achilles, wide-based gait, & hip dysplasia

No ptosis, swallowing difficulties, or dysarthria present

Other patient reports & phenotypic comparisons

- Other patients reported with congenital myopathy[6] & functional evidence is currently in pre-print[5]
- Multiple unrelated patients with de novo mutations and biopsies with autophagic vacuoles & P62/TUBA4A+

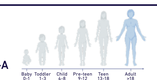
	Prior Report (n=4)	Baylor Case (n=1)
Onset:	Neonatal/Infancy	+ Neonatal/infancy
Weakness:	All limbs/generalized	+ All limbs/generalized
Gait:	Waddling gait	+ Wide-based gait
Progression:	Stagnant or mild ↑	*N/A*

Current Classification: N/A
Proposed Classification: Pathogenic

Population: not in ExAC/gnomAD	**Functional:** *in vitro* effect
Segregation: N/A	**Computational:** multiple predictions
De novo: confirmed in 4/5 pts	**Other:** ≥5 unrelated patients

E415K

Publications
Only c.1243G>A



Onset: Childhood (M 7)
(Motor problems; trouble riding bike)

Progressive spastic disease
(wheelchair dependence: 28yo)

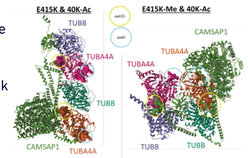
Normal development; onset of progressive leg weakness, spasms, falls in teens plateauing in 20s & progressing in 30s

Hyperreflexia; dystonia; spastic/short gait then wheelchair; activity-induced hand tremors; type 2B fiber atrophy

Nystagmus; depression, ADHD, hallucinations, and insomnia; bladder abnormalities; medical history of neuronopathy

***In silico* investigation (AlphaFold3) & prior reports**

- 415E: methyl/ubiquitin site
- E415K predicted to destabilize α-tubulin & ↑ probability of methyl/SUMO addition
- Other pts (AAO 10-30yr): weak lower limbs, gait & bladder defects, nystagmus[4,14]
- E415K-me predicted to alter interaction with CAMSAP1

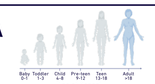


Current Classification: N/A *(missense at same residue is VUS)*
Proposed Classification: Pathogenic

Population: absent in ExAC/gnomAD	Functional: N/A
Segregation: Multigeneration ancestry	**Computational:** multiple predictions
De novo: confirmed in 4/5 pts	Other: --

c.226+5G>A

Not reported



Onset: Teenage (M 18)
(exact age unconfirmed)

Very mild severity
(NMDAS SII = 2/45; total = 2/145)

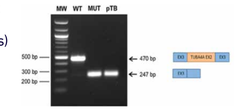
Normal development; onset of mild symptoms indicating undefined mitochondrial myopathy in late teens

Muscle strength & tone within normal range; patient reports exercise/engagement with swim practice

Insomnia, dyslexia, history of abnormal EKG, bronchitis, reactive airway disease, and constipation

Comparison to nearby exon skipping variant (226+4A>G) associated with adult ALS

- Slow-progressing ALS patient diagnosed at 57 (death at 71 from pneumonia complications)
- Phenotype of lower limb wasting/ weakness, which spread to upper limbs (mainly distal)
- Assay confirmation of exon 2 skipping affecting the GTPase domain



- Recommend follow-up & study of potential for hypomorphic TUBA4A to confirm mechanism and threshold(s) for impact

Current Classification: N/A
Proposed Classification: VUS / Likely Pathogenic

Population: absent in ExAC/gnomAD	Functional: N/A
Segregation: N/A	**Computational:** multiple predictions
De novo: confirmed in 2/2 pts	**Other:** exon skipping nearby variant

KEY CONCLUSIONS AND DISCUSSION POINTS

- We report earlier age of onset associated with *TUBA4A* myopathy compared to later age of onset, on average, in ALS & spastic ataxias
- Though sample sizes are small, we note relatively consistent phenotypic profiles in patients carrying predicted damaging missense variants
- Our results support the inclusion of *TUBA4A* as a gene of interest for neonatal or juvenile patients presenting with hypotonia and/or progressive weakness
- *In silico* analysis provide hypotheses for future investigations of the pathogenic mechanisms underlying *TUBA4A* variants and to lay a foundation for considering the potential complexity likely underlying related genotype-phenotype relationships

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