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DYT-TUBB4A (DYT4 Dystonia)

New Clinical and Genetic Observations

Julien F. Bally, MD, Sarah Camargos, MD, PhD, Camila Oliveira dos Santos, BSc, Drew S. Kern, MD, MS, Teresa Lee, MD, Francisco Pereira da Silva-Junior, MD, Renato David Puga, MSc, Francisco Cardoso, MD, PhD, Egberto Reis Barbosa, MD, PhD, Rachita Yadav, PhD, Laurie J. Ozelius, PhD, Patricia de Carvalho Aguiar, MD, PhD, and Anthony E. Lang, MD

Correspondence
Dr. Lang
Lang@uhnresearch.ca

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Abstract

Objective

To report 4 novel *TUBB4A* mutations leading to laryngeal and cervical dystonia with frequent generalization.

Methods

We screened 4 families including a total of 11 definitely affected members with a clinical picture resembling the original description.

Results

Four novel variants in the *TUBB4A* gene have been identified: D295N, R46M, Q424H, and R121W. In silico modeling showed that all variants have characteristics similar to R2G. The variants segregate with the disease in 3 of the families with evidence of incomplete penetrance in 2 of them. All 4 variants would be classified as likely pathogenic. The clinical picture particularly included laryngeal dystonia (often the site of onset), associated with cervical and upper limb dystonia and frequent generalization. Laryngeal dystonia was extremely prevalent (>90%) both in the original cases and in this case series. The hobby horse gait was evident in only 1 patient in this case series.

Conclusions

Our interpretation is that laryngeal involvement is a hallmark feature of DYT-TUBB4A. Nevertheless, *TUBB4A* mutations remain an exceedingly rare cause of laryngeal or other isolated dystonia.

From the Edmond J. Safra Program in Parkinson's Disease and the Morton and Gloria Shulman Movement Disorders Clinic (J.F.B., A.E.L.), Toronto Western Hospital and University of Toronto, Ontario, Canada; Department of Neurology (J.F.B.), University of Geneva and University Hospitals of Geneva, Switzerland; Department of Internal Medicine (S.C., F.C.), Universidade Federal de Minas Gerais, Belo Horizonte; Hospital Israelita Albert Einstein (C.O.d.S., R.D.P., P.d.C.A.), Sao Paulo, SP, Brazil; Departments of Neurology (D.S.K., T.L.) and Neurosurgery (D.S.K.), University of Colorado School of Medicine; Aurora; Department of Neurology and Neurosurgery (F.P.d.S.-J., E.R.B., P.d.C.A.), Universidade Federal de Sao Paulo, SP, Brazil; and Department of Neurology (R.Y., L.J.O.), Massachusetts General Hospital, Boston. Dr. Bally is currently at Service of Neurology, Department of Clinical Neurosciences, Lausanne University Hospital and University of Lausanne, Switzerland.

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Glossary

CADD = combined annotation-dependent depletion; **CD** = cervical dystonia; **gnomAD** = Genome Aggregation Database; **H-ABC** = hypomyelination with atrophy of the basal ganglia and cerebellum; **SD** = spasmodic dysphonia.

DYT-TUBB4A,[1] formerly known as DYT4 or whispering dysphonia, was first described in 1985 by Parker[2] in a large family of UK origin who immigrated to Australia. Onset of dystonia was usually in the third decade or earlier. Patients commonly presented with spasmodic dysphonia (SD) and/or a cranio-cervical dystonia progressing to generalized dystonia. A peculiar "hobby horse" gait (described in 5 of 9 Australian cases) and extrusional tongue movements have been emphasized.[3] In a subsequent description of 8 patients from this family, 6 had dysphonia and 6 had cervical dystonia (CD).[4] In 2013, 2 independent groups of researchers almost simultaneously identified a heterozygous missense mutation, c.4C>G;p.R2G, in exon 1 of the *TUBB4A* gene as causative in members of the original family.[4,5] *TUBB4A*, located on chromosome 19p, codes for the protein beta-tubulin 4A, a neuronally expressed tubulin[5] that is an essential component of microtubules that form the cytoskeleton and serve diverse cellular functions.[6] Sequencing of *TUBB4A* in close to 400 unrelated patients with dystonia (among whom 124 had SD) revealed another missense variant, p.A271T, in a possibly familial case of segmental dystonia with SD.[5]

In 2013, *TUBB4A* mutations were also shown to result in hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC).[7] This disorder, first described in 2002,[8] derives its name from characteristic abnormalities demonstrated on brain MRI, whereas brain MRI is normal in DYT-TUBB4A. The clinical spectrum of this severe illness, which expresses during infancy and causes a hypomyelinating leukodystrophy, comprises not only dystonia but also developmental delay, choreoathetosis, rigidity, opisthotonus, oculogyric crises, progressive spastic tetraplegia, ataxia, and, more rarely, seizures.[8] Originally, a single missense mutation in *TUBB4A*, p.D249N, was reported in 11 unrelated patients.[7] However, many other mutations in *TUBB4A* associated with H-ABC have since been described in this gene.[9] Noteworthy, mutations in the *TUBB4A* gene more commonly cause H-ABC than DYT-TUBB4A.[1] It has been suggested that these disorders represent different ends of a spectrum, with H-ABC at the severe end and DYT-TUBB4A at the milder end, with movement disorders being the common feature.[9] Supporting this concept is the report of 4 unrelated patients with *TUBB4A* mutations with imaging findings consistent with H-ABC syndrome who had severe generalized dystonia and complete aphonia, reminiscent of DYT-TUBB4A phenomenology, but also with a variety of other neurologic features.[10]

On the other hand, *TUBB4A* mutations are an extremely rare cause of isolated dystonia in the absence of other neurologic signs. In a study of >700 patients with isolated dystonia, the authors were unable to identify any possibly pathogenic sequence alteration,[11] while another group, screening close to 500 patients with isolated dystonia, found 1 rare, potentially pathogenic, in-frame deletion in an Italian patient with CD.[12] Thus, routine genetic testing of *TUBB4A* in individuals with isolated dystonia is currently not recommended.[11,12]

Here, we report a total of 11 patients from 4 families with 4 novel *TUBB4A* variants manifesting isolated dystonia. In silico modeling showed that all variants have characteristics similar to R2G and A271T.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

Brazilian families were screened as part of a research project on the genetics of dystonia, approved by the institutional review board of each participating center. The Canadian and American families were screened after the probands sought medical attention at our movement disorders centers. All participants provided written informed consent.

Mutation Screening and In Silico Analysis

Probands were screened by next-generation sequencing with a dystonia panel (TruSeq Custom Amplicon assay 1.5, Illumina for families 1 and 2) or whole-exome sequencing (family 3). Detailed sequencing methods are provided as supplemental material (supplemental Methods, available on Dryad, doi.org/10.5061/dryad.34tmpg4gt). All variants were validated by Sanger sequencing. Family 4 was tested with a commercial next-generation sequencing gene panel (Invitae Dystonia Comprehensive Panel[13]). Allele frequencies were assessed in population databases (Genome Aggregation Database [gnomAD],[14] AbraOM,[15] and BIPMed-WES-db[16]). In silico predictions of pathogenicity were performed with combined annotation-dependent depletion (CADD)[17]; other functional prediction programs used included SIFT, PolyPhen, M-CAP, Meta-SNP, and Mutation Taster.[18,19] Amino acid conservation was assessed through Clustal Omega.[20]

TUBB4A: Homology Modeling

To verify the effect of variants on the 3D structure and function of beta-tubulin-4A (NP 006078) wild-type and variant models, we performed homology modeling by in silico analysis using Phyre2 server[21] and University of California San Francisco Chimera 1.13 software.[22] Residues not aligned were modeled by ab initio approach.[21] For the ligand binding sites prediction, 3DLigandSite server was used.[23,24]

Table 1 Clinical Features and Variants Found in the 3 Reported Families

Family	Participant	Age at last examination, y	Sex	Family history	Age at onset, y	Site of onset	Current distribution	Neuroimage	*TUBB4A* (NM_006087)	Scaled CADD score
1	1.II.1 (proband)	34	Male	No	6	Upper limb	Generalized: predominantly cervical, larynx (SD) and right upper limb	Normal	c.G883A (p.D295N)	24.6
	1.II.2 (brother)	30	Male		Not affected	NA	NA	Not done	c.G883A (p.D295N)	
	1.I.1 (father)	64	Male		Not affected	NA	NA	Not done	c.G883A (p.D295N)	
	1.I.2 (paternal aunt)	57	Female		Not affected	NA	NA	Not done	c.G883A (p.D295N)	
	1.I.3 (paternal aunt)	58	Female		Not affected	NA	NA	Not done	c.G883A (p.D295N)	
2	2.II.1 (proband)	50	Female	Yes	30	Upper limb and larynx (SD)	Cervical, larynx and upper limbs	Normal	c.G137T (p.R46M)	31
	2.II.2 (brother)	45	Male		21	Larynx (SD)	Generalized: cervical, larynx, trunk, upper and lower limbs	Normal	c.G137T (p.R46M)	
3	3.III.6 (proband)	Deceased (37)	Female	Yes	10	Larynx (SD)	Generalized: cervical, larynx, trunk, upper and lower limbs	Normal	c.G1272C (Q424H)	25.2
	3.III.5 (brother)	36	Male		21	Larynx (SD)	Larynx and right upper limb (WC)	Not done	c.G1272C (Q424H)	
	3.II.3 (mother)	45	Female		18	Cervical (transitory)	Right upper limb (WC) and questionable cervical	Not done	c.G1272C (Q424H)	
	3.II.1 (maternal aunt)	Deceased (last seen at age 57)	Female		25	Right upper limb and cervical	Generalized: craniofacial, larynx (SD), cervical, upper and lower limbs	Not done	c.G1272C (Q424H)	
	3.III.2 (maternal cousin)	~35	Female		24	Larynx, face, cervical, upper limbs	Generalized: especially craniofacial and upper limbs	Not done	c.G1272C (Q424H)	
	3.III.4 (maternal cousin)	Deceased (age 13)	Female		7	Cervical, then left lower limb	Generalized: very severe	Not done	Not tested	
	3.III.3 (maternal cousin)	≈35	Female		Questionable if affected (no complaints)	Right upper limb (WC)	Right upper limb (WC): mild phenotype	Not done	Wild Type	
	4.II.2 (proband)	64	Female	No	2	Larynx, then lower limbs	Generalized: craniofacial, larynx (SD), cervical, trunk, upper and lower limbs, plus ambulatory difficulties similar to the hobby horse gait	a	c.C361T (R121W)	26.5

Continued

Table 1 Clinical Features and Variants Found in the 3 Reported Families *(continued)*

Family	Participant	Age at last examination, y	Sex	Family history	Age at onset, y	Site of onset	Current distribution	Neuroimage	*TUBB4A* (NM_006087)	Scaled CADD score
	4.II.3 (brother)	61	Male		22	Left upper limb	Generalized: cervical, trunk, bilateral upper limbs	Not done	c.C361T (R121W)	

Abbreviations: CADD = combined annotation-dependent depletion; NA = not applicable; SD = spasmodic dysphonia; WC = writer's cramp.
[a] Mild diffuse global atrophy, mild scattered deep and subcortical cerebral white matter disease.

We analyzed structural and functional effects caused by the variants identified in our families with dystonia and by variants previously reported in patients with DYT-TUBB4A (p.R2G and p.A271T)[5] and p.D295H, a rare variant described in GnomAD.

Data Availability

All data and supplemental data are available on Dryad, doi.org/10.5061/dryad.34tmpg4gt.

Results

We have identified heterozygous novel *TUBB4A* (NM_006087) single nucleotide variants in 4 families with isolated dystonia, including a singleton case, 2 sib pairs, and a multigeneration family (family 3). All variants change highly conserved amino acids and are predicted to be deleterious by in silico analysis (CADD scores 24.6, 31, 25.2, and 26.5 for families 1, 2, 3, and 4, respectively). None of them was found in population databases. Clinical summaries and mutation data are presented in table 1 and pedigrees in figure 1.

Next-Generation Sequencing

Probands from 3 of the families were screened for mutations using a dystonia gene panel. The only potentially pathogenic variants identified in *TUBB4A* included a singleton case harboring p.D295N and 2 sib pairs segregating p.R46M and p.R121W. Exome sequencing was performed on individuals 3.II.1 and 3.III.5 (figure 1, bottom left) from family 3. Examination for shared rare heterozygous variants (minor allele frequency <0.0001) revealed 17 single nucleotide variants (doi.org/10.5061/dryad.34tmpg4gt). After filtering of these variants for CADD score, family segregation, disease phenotype, and variants not observed in our internal dystonia exomes (n > 500) or GnomAD, 2 variants remained: p.A180V in *C19ORF47* and p.Q424H in *TUBB4A* (supplemental table 1). *C19ORF47* has an unknown function, is conserved only in rodents, and has a low level of expression in the brain (Genotype-Tissue Expression portal[25]). Thus, the variant p.Q424H in *TUBB4A* is the most likely candidate variant to cause the phenotype in this family.

Protein Modeling

To assess the pathogenicity of the mutations, protein models were created for wild-type and variant primary sequences. The best template structure selected was C2p4nB (beta-tubulin-chain 2B, TUBB2B_Q6B856) according to the coverage (96% of the sequence) and confidence (100%) of the model. The predicted structure was a core of beta-sheets surrounded by alpha-helices as illustrated in figure 2.

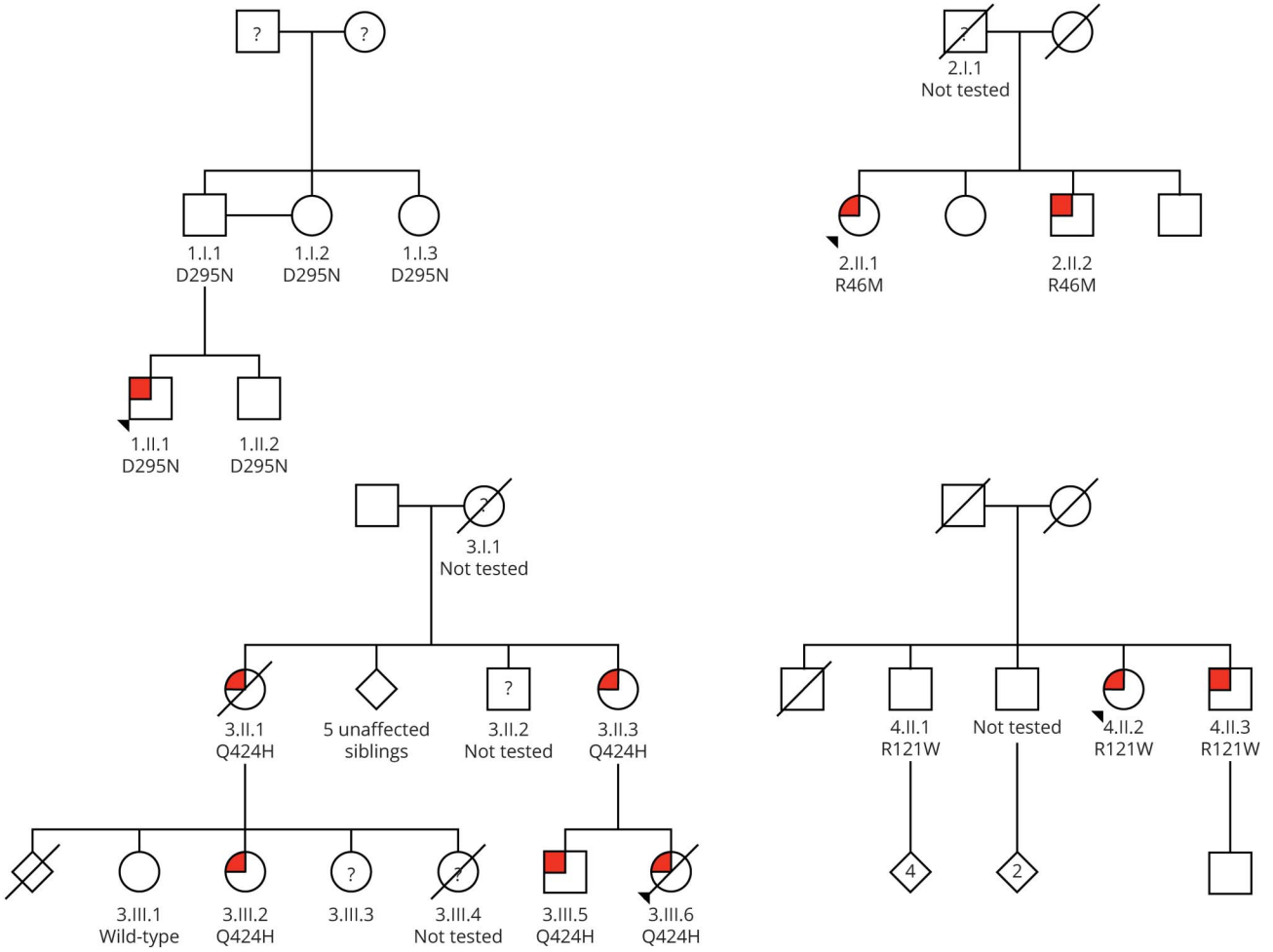
Table 2 shows the predicted structural modifications of each variant found in our families, as well as in the previously described mutation R2G[5] and in the rare variant D295H described in gnomAD through TUBB4A homology modeling. Figure 3 presents the structural and interaction modifications in variants R46M, D295N, Q424H, and R121W compared to the wild-type TUBB4A model. Supplemental figure S1 (doi.org/10.5061/dryad.34tmpg4gt) shows the wild-type TUBB4A model (left) and the loss of hydrogen bonds in the R46M, D295N, and R2G TUBB4A models (right; see also first row of table 2). Supplemental figure S2 shows the modification of the M-loop region in the R46M and R121W models.

The 3DLigandSite server predicted clusters of ligand binding sites with heterogens (GTP, magnesium, or zinc) for each model on the basis of the alignment with known 3D structure domains (table 3). The functions of the predicted sites were annotated according to the Universal Protein Resource (UniProtKB) P04350, Conserved Domain Database (National Center for Biotechnology Information) cd02187, and previous studies.[26,27] Variants R46M, D295N, Q424H, R121W, and A271T were predicted to lose the binding site in residue N99 (alpha/beta domain interface), and specifically in the R121W model, this residue lost all interactions with other residues (supplemental figure S5, doi.org/10.5061/dryad.34tmpg4gt). Except for R121W, the variants were predicted to acquire a zinc-binding site in residue 280. Variants R46M, D295N, R2G, and A271T were predicted to acquire a GTP-binding site in residue 72. The 3D surface models are shown in supplemental figure S3.

Discussion

Here, we describe 4 novel variants in the *TUBB4A* gene in 3 unrelated families with dystonia, 1 from Brazil (Portuguese

Figure 1 Pedigree Trees



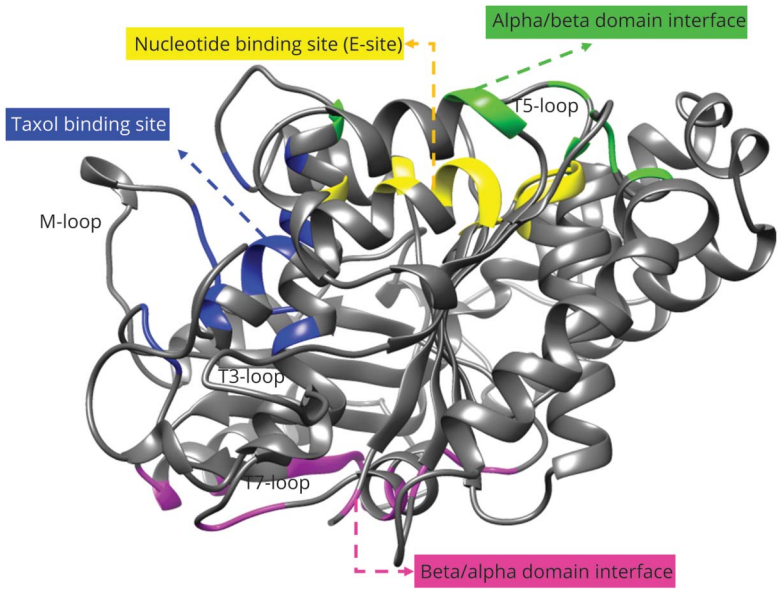
Pedigree trees for families 1 (top left), 2 (top right), 3 (bottom left), and 4 (bottom right) with the definitely affected members in red and unaffected ones in white. Question marks indicate questionably affected members in families 2 and 3; case 3.III.4 from family 3 was clinically definitely affected but not genetically tested and hence has a question mark. A question mark in family 1 indicates cases without information. Whenever a case was genetically tested (whether clinically affected or not), the variant in *TUBB4A* gene (D295N, R46M, Q424H, and R121W for families 1, 2, 3, and 4, respectively) or wild-type is indicated. Exome sequencing was performed on cases 3.II.1 and 3.III.5.

and African descent), 1 from Canada (French descent), and 1 from the United States (Czech and Norwegian descent), as well as in a singleton affected case also from Brazil. All variants change highly conserved protein regions, and in silico and protein modeling analyses suggest that they are deleterious. These are the first 3 families with dystonia segregating *TUBB4A* mutations with disease since the original family was described,[4,5] thus confirming this dystonia locus.

In total, we describe 11 definitely affected cases (10 mutation positive, 1 mutation status unknown, see table 1 and figure 1). The dystonia varied between mild and very severe (in 1 case, leading to death at a very young age). The mean age at onset was 17 (range 2–30) years (median 21 years). The site of onset was most often the larynx (n = 6), arm (n = 5), or neck (n = 4), with the distribution extending from segmental to generalized. Final sites involved included the larynx (n = 8, 1 unknown) with both adductor (predominant) and possibly abductor (in case 1.II.1) types of SD, neck (n = 10), and upper limb (n = 10, 1 unknown), with isolated writer's cramp in the most mildly affected cases (n = 2) and more severe arm dystonia in the others. Generalized dystonia[28] was found in 8 of 11 cases. The famous hobby horse gait described in the original Australian family was seen in only 1 of our cases (case 4.II.2); rather, SD associated with CD was the most consistent features of DYT-TUBB4A: 6 of 8 cases in the Australian family and 7 of 10 (plus one unknown, case 3.III.4, who was the most severe of all and therefore likely presented with SD and CD) cases in this series.

There was a marked variability of the progression of this disorder, especially emphasized by the early death in case 3.III.4 and the late striking progression in case 4.II.2, contrasting with the more benign course in some others.

Figure 2 *TUBB4A* (NP 006078) Wild-Type Protein Model



Created with Phyre2 server based on C2p4nB (TUBB2B) structure with colored domains: nucleotide binding site (E-site, GTP/GDP) (yellow), alpha/beta domain interface (green), beta/alpha domain interface (magenta), and Taxol binding site (blue).

Overall, the most consistent feature was laryngeal involvement, present in >90% of the reported case (all 9 cases of the original family[3] and in 8 of 10 [plus 1 unknown] of our cases). Compared with other genetic causes of isolated dystonia, laryngeal involvement is found in only 14% of DYT-*TOR1A* (DYT1),[29] in 44% of DYT-*THAP1* (DYT6),[30] in 53% of DYT-*KMT2B* (DYT28),[31] and occasionally in DYT-*GNAL* (DYT25).[32] Rarely is laryngeal involvement the presenting feature in these patients, in contrast to DYT-TUBB4A. Thus, laryngeal involvement, highlighted in the descriptions of the original family and present in the isolated case with the p.A271T variant,[5] is a hallmark feature of DYT-TUBB4A (DYT4).

Although most dystonia genes show reduced penetrance, because families with DYT-TUBB4A are rare, overall penetrance estimations might not be reliable. The p.R2G *TUBB4A* mutation appears to be highly penetrant in the original family with DYT4.[4,5] This effect could be specific to this variant, while other variants might be under the influence of modifiers. There are only 2 other previously described cases with DYT-TUBB4A dystonia, but neither had DNA available from relatives for segregation analysis, so penetrance could not be assessed.[5,12]

In the singleton case and in family 4, variants p.D295N and p.R121W, respectively, were also present in unaffected members; this could either be noncausal or due to reduced penetrance, which is a common feature for most dystonia genes.[33] Because almost no unaffected family members were tested in families 2 and 3, no determination about penetrance can be made; however, examining the pedigree structure, we found almost equal numbers of affected and unaffected individuals as well as affected individuals in all generations, consistent with high penetrance of a dominant disorder, similar to what was described in the original family.[5,33]

Considering the clinical characteristics, particularly the marked laryngeal dystonia, as well as the in silico data including pathogenicity predictors (CADD scores >20), the high conservation of the amino acids across species, and our protein structure modeling analysis (see below), coupled with the fact that all of the variants are novel, the 4 variants we identified in *TUBB4A* would be classified as likely pathogenic according to the American College of Medical Genetics and Genomics guidelines for the interpretation of sequence variants.[34] In the case of p.D295N and R121W, there are 2 very rare variants described in gnomAD[14] at the same codon (p.D295H, allele frequency 8.8×10^{-6}; and p.R121Q, allele frequency 3.9×10^{-6}). Both in silico analysis and various functional prediction programs (SIFT, PolyPhen, M-CAP, Meta-SNP Mutation Taster, and CADD[18,19]) suggest that these variants are pathogenic (supplemental table 2, doi.org/10.5061/dryad.34tmpg4gt). No clinical information is provided in gnomAD, but given the age at onset and phenotypic expression associated with mutations in this gene, it is unlikely that the p.D295H and R121Q carriers expressed dystonia, thus supporting reduced penetrance of mutations at these residues. However, in vitro assays similar to recently published studies[35–37] will be necessary to definitively define the pathogenicity of the variants described here. It should be noted that in family 3, a variant in *C19ORF47* also segregates with the disease, and although nothing is known about the function of this gene and no missense variants have been reported to cause disease in ClinVar,[38] it is possible this variant could, by itself or

Table 2 Predicted Structural Modifications of Each Variant Found in our Families, in the Previously Described Mutation R2G, and in the Rare Variant D295H Described in GnomAD Through TUBB4A Homology Modeling

	Singleton case (family 1) D295N	Sib pair (family 2) R46M	Multigeneration (family 3) Q424H	Sib pair (family 4) R121W	D295H	R2G
Loss of hydrogen bonds MET1-CYS129[a]	Yes	Yes				Yes
Loss of hydrogen bonds MET164-ASP197	Yes	Yes				
Loss of hydrogen bonds GLU198-MET164[b]	Yes				Yes	
Other	Acquisition of a hydrogen bond between 295 and 296 residues[c]	Loss of a hydrogen bond between residues LEU42 and GLU45[d]	Novel hydrogen bond between GLN423 and ASP427[e]	Loss of interaction between alpha-helix H3 and loop 3 (GLY104-TRP101)[h]		
Other	Loss of the hydrogen bond GLY132-GLU133[b]	Interaction changes between residues ARG52, ARG62, and GLU3	Gain of a hydrogen bond between H12 and loop H8-B7: residues 264 and 421[f]	Loss of interaction of ASN99 (binding site with alpha-tubulin) with 140 and 178 residues (GDP/GTP binding site)		
Other		Loss of part of the alpha-helix structure: residues 277–279 GSQ[g]	Residue 426 (in H12) interacts with residue 375 instead of 372	Loss of interaction between alpha-helix H3 and H4 [h]		
Other				Loss of interaction of H3 with H11 and H12 alpha-helices [h]		
Other				Alteration of M-loop conformation		

Abbreviation: gnomAD = Genome Aggregation Database.
[a] Interaction region between the N-terminal and intermediary domain, supplemental figure S1, doi.org/10.5061/dryad.34tmpg4gt.
[b] Involved in the interaction between domains.
[c] Figure 3A.
[d] Modification of the alpha-helix structure in this region (figure 3B).
[e] Extension of the H12 alpha-helix structure to residue 426 modifying interaction between H12, H11, and loop H8-B7.
[f] Figure 3C.
[g] Supplemental figure-S2, doi.org/10.5061/dryad.34tmpg4gt.
[h] Supplemental figure S5, doi.org/10.5061/dryad.34tmpg4gt.

in combination with the *TUBB4A* variant, influence the phenotype.

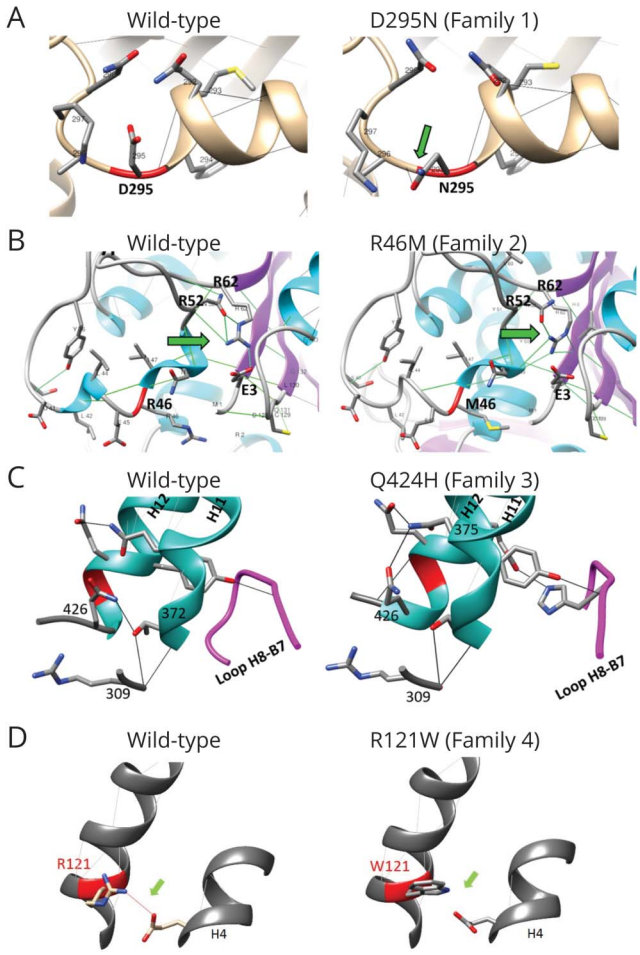
Tubulins (alpha and beta) have 3 domains: N-terminal domain (GTP binding), intermediary domain (with Taxol-binding site, involved in microtubule stabilization), and C-terminal domain with 2 alpha-helices (H11 and H12), which form a crest on the outside surface of the microtubule protofilament 6.

The correct interaction between residues of the protein core is key to the monomer stability and efficiency of the nucleotide-binding domain. The interaction between residues that participate in the rotation of the N-terminal and second domains, which occurs after microtubule depolymerization and nucleotide hydrolysis, is essential for the function of beta-tubulin.[26] The loss or modification of hydrogen bonds (as observed in the R46M, R121W, D295N, and Q424H models) may contribute to the destabilization of beta-tubulin after activation or oligomerization in a head-to-tail arrangement between alpha- and beta-tubulin.

Loop regions, T3, T5, T7, and M loops are important for the interaction between beta-tubulin and other structures (supplemental figure S4, doi.org/10.5061/dryad.34tmpg4gt).[39,40] The loop H8-B7 is in the region of the beta/alpha interface domain. Variant Q424H modified the interaction of H12 with H11 and loop H8-B7 (figure 3C). H12 and H11 alpha-helices are in the C-terminal of beta-tubulin and are important for the assembly of M loops between protofilaments, as well as for the interaction with motor proteins.[26] In the R121W variant model, interactions between H3 and these alpha-helices were lost (supplemental figure S5).

In silico modeling indicates an alpha-helix structure in the region of residues 277 to 279, but this was not confirmed by

Figure 3 Structural and Interaction Modifications in Variants R46M, D295N, Q424H, and R121W Compared to Wild-Type TUBB4A Model



Positions of altered residues are indicated in red; hydrogen bonds are shown by lines between side chains; and green arrows point to the main changes. (A) Variant D295N (family 1) acquired a hydrogen bond between residues 295 and 296. (B) Variant R46M (family 2) modified the interactions among arginine 52 (R52), arginine 62 (R62), and glutamic acid 3 (E3) and changed the alpha-helix structure. (C) Variant Q424H (family 3) modified its interactions with residues 372 and 375 and extended the alpha-helix structure to residue 426; it also acquired a novel hydrogen bond between H12 alpha-helix and loop H8-B7. (D) Variant R121W (family 4) lost interaction between 121 and 158 residues (H3 and H8 alpha-helices).

crystallographic studies.[41] However, this region mediates lateral interactions between protofilaments; therefore, variant R46M may have functional consequences. The helix H3 in the N-terminal GTPase domain of a beta-tubulin subunit (containing R46M and R121W) contacts the M loop of the intermediary domain of an adjacent beta subunit. The M loop also contains a zinc-binding site, involved in the assembly of beta-beta subunits. The Taxol domain stabilizes the M loop, reducing dynamic instability of beta-tubulin[26,39,40] (figure 2 and figure S2, doi.org/10.5061/dryad.34tmpg4gt).

In relation to the ligand binding sites, the R46M, R121W, D295N, and Q424H models are predicted to lose sites in the N-terminal region that are involved in GTP binding and polymerization (doi.org/10.5061/dryad.34tmpg4gt). In the R121W model, the loss of the N99 residue interactions with active sites of alpha/beta interface and GTP binding can impair the assembly of the tubulin dimer and conformation of the E site (supplemental figure S5).

Although functional studies of *TUBB4A* variants, including R2G and A271T, showed variable results,[36,37,42] they all point to disorganization of microtubule dynamics, with findings including lack of typical radial tubulin networks, diminished interaction between beta- and alpha-tubulin, neuronal or oligodendrocyte morphological changes, abnormalities in motor protein binding to microtubules, and disrupted mitochondrial transport. In silico modeling reveals that variants R46M, R121W, D295N, and Q424H have characteristics similar to R2G and A271T, consistent with the idea that they also have functional consequences on microtubule stability.

This study describes 11 affected members in 4 different families with isolated dystonia with 4 distinct *TUBB4A* mutations that are likely to be pathogenic. The clinical picture particularly included laryngeal dystonia (often the site of onset), associated with cervical and upper limb dystonia and frequent generalization. Despite the very high prevalence of laryngeal dystonia in DYT-TUBB4A, mutations in *TUBB4A* remain an exceedingly rare cause of laryngeal dystonia or other isolated dystonia; only a single family and 2 isolated cases have been described before the present case series.[4,5,11,12]

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Disclosure

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Table 3 Changes in the Ligand Binding Sites Predicted by 3Dligandsite for Variant Models[24]

Function	Residues[a]	Amino acid	R46M	D295N	D295H	Q424H	R121W	R2G	A271T
Nucleotide-binding site (GTP/GDP) E-site (N-terminal domain)	10–12	GQC							
	15	Q							
	72	T	Gain	Gain				Gain	Gain
	138	S							
	140–144	GGGTG							
	181	E	Loss						
	204	N							
	222	Y		Loss					
	226	N							
Alpha/beta domain interface	16	I	Loss						
	67	D							
	69	E							
	70	P					Loss		
	71	G					Loss		
	97	A							
	98	G					Loss		
	99	N	Loss	Loss		Loss	Loss		Loss
	100/103/169/177/179	N/K/V/D/V							
	181	E	Loss						
Magnesium binding-site, Mg2+	16	I	Loss						
	67/69/97/169/177	D/E/A/V/D							
Beta/alpha domain interface	2/131	R/Q							
	245/247/251/256	G/N/R/N							
	258–260	VPF							
	322–324	SMK							
	327	D							
	347–351	NNVKT							
Nucleotide binding-site (GTP/GDP) N-site (alpha-tubulin)	246	L			Loss				
	252	K							
Zinc binding site, ZN2+	280	Q	Gain	Gain	Gain	Gain		Gain	Gain
M-loop region	281–282	YR							

[a] Numbering of residues according to *TUBB4A* NP_006078.

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Appendix Authors

Authors	Location	Contributions
Julien F. Bally	University of Toronto, Ontario, Canada; University of Geneva, Switzerland	Research project: organization, execution; statistical analysis: review and critique; manuscript: ; writing of the first draft
Sarah Camargos	Universidade Federal de Minas Gerais, Belo Horizonte, Brazil	Research project: conception; statistical analysis: review and critique; manuscript: review and critique
Camila Oliveira dos Santos	Hospital Israelita Albert Einstein, Sao Paulo, Brazil	Research project: execution; statistical analysis: execution; manuscript: writing of the first draft
Drew S. Kern	University of Colorado School of Medicine, Aurora	Research project: execution; manuscript: review and critique
Teresa Lee	University of Colorado School of Medicine, Aurora	Research project: execution
Francisco Pereira da Silva-Junior	Universidade Federal de Sao Paulo, Brazil	Research project: execution; statistical analysis: review and critique; manuscript: review and critique
Renato David Puga	Hospital Israelita Albert Einstein, Sao Paulo, Brazil	Statistical analysis: design, execution, review, and critique; manuscript: review and critique
Francisco Cardoso	Universidade Federal de Minas Gerais, Belo Horizonte, Brazil	Research project: execution; manuscript: review and critique
Egberto Reis Barbosa	Universidade Federal de Sao Paulo, Brazil	Research project: execution; statistical analysis: review and critique; manuscript: review and critique
Rachita Yadav	Massachusetts General Hospital, Boston	Statistical analysis: design; statistical analysis: execution, review, and critique; manuscript: review and critique
Laurie J. Ozelius	Massachusetts General Hospital, Boston	Research project: conception and execution; statistical analysis: review and critique; manuscript: review and critique
Patricia de Carvalho Aguiar	Hospital Israelita Albert Einstein, Sao Paulo, Brazil; Universidade Federal de Sao Paulo, Brazil	Research project: conception, organization, and execution; statistical analysis: design, review, and critique; manuscript: writing of the first draft, review, and critique
Anthony E. Lang	University of Toronto, Ontario, Canada	Research project: conception and organization; statistical analysis: review and critique. manuscript: review and critique

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