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De novo and inherited monoallelic variants in TUBA4A cause ataxia and spasticity

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Keywords:

TUBA4A; ataxia; cerebellar atrophy; de novo; spasticity; tubulinopathy.

Abstract

Alpha-tubulin 4A encoding gene (TUBA4A) has been associated with familial amyotrophic lateral sclerosis (fALS) and fronto-temporal dementia (FTD), based on identification of likely pathogenic variants in patients from distinct ALS and FTD cohorts. By screening a multicentric French cohort of 448 unrelated probands presenting with cerebellar ataxia, we identified ultra-rare TUBA4A missense variants, all being absent from public databases and predicted pathogenic by multiple in-silico tools. In addition, gene burden analyses in the 100,000 genomes project (100KGP) showed enrichment of TUBA4A rare variants in the inherited ataxia group compared to controls (OR: 57.0847 [10.2- 576.7]; $p = 4.02 \times 10^{-07}$). Altogether, we report 12 patients presenting with spasticity and/or cerebellar ataxia and harboring a predicted pathogenic TUBA4A missense mutation, including 5 confirmed de novo cases and a mutation previously reported in a large family presenting with spastic ataxia. Cultured fibroblasts from 3 patients harboring distinct TUBA4A missense showed significant alterations in microtubule organisation and dynamics, providing insight of TUBA4A variants pathogenicity. Our data confirm the identification of a hereditary spastic ataxia disease gene with variable age of onset, expanding the clinical spectrum of TUBA4A associated phenotypes.

Introduction

Microtubules are composed of α/β tubulin heterodimers and play fundamental roles during the development of axons, dendrites and neuronal synapses. The tubulin gene family contains several isotypes, which follow a well-orchestrated spatiotemporal expression pattern.¹ Pathogenic variants in more than half of the 26 known tubulin encoding genes have been described in recent decades as a cause of tubulinopathies. Tubulinopathies represent a wide range of overlapping neurodevelopmental and neurodegenerative disorders, remarkable by the significant variability of clinical presentations.^{2,3,4,5} The alpha-tubulin 4A isoform, encoded by TUBA4A, has been proposed as the cause of familial amyotrophic lateral sclerosis (fALS) type 22 and/or frontotemporal dementia (FTD), based on identification of likely pathogenic variants in unrelated patients from several fALS/FTD cohorts.^{6,7,8} However, other studies state insufficient evidence to support its causal role in fALS and FTD.⁹ Very recently, the p.Glu415Lys TUBA4A variant was identified in a large Italian family with spastic cerebellar ataxia and in an unrelated sporadic patient with de novo occurrence.¹⁰

Herein, we describe 12 patients from 11 unrelated families harbouring ultra-rare missense variants in TUBA4A, including five de novo variants. Most patients presented with a neurodegenerative disorder leading to spastic cerebellar ataxia with variable age of onset and disease severity. We carefully characterized the patients' phenotype by providing detailed clinical data and imaging, when available. Functional studies on microtubule properties were performed on cultured fibroblasts from three patients.

Material and methods

Genetics analysis

Patient recruitment

Patients 1, 2 and 5 were part of a larger French cohort of 448 index cases from unrelated families, presenting with unexplored inherited ataxia and/or spastic paraplegia, screened by clinical exome sequencing using Illumina NextSeq technology (www.illumina.com/trusightone), including 366 families previously described.¹¹

Patients 3, 4, 7, 8 and 12 were recruited through Genematcher Exchange.¹² Patients 3 and 4 were screened by whole genome sequencing (WGS) performed on French sequencing platform using Illumina NovaSeq technology (AURAGEN and SEQOIA platforms).

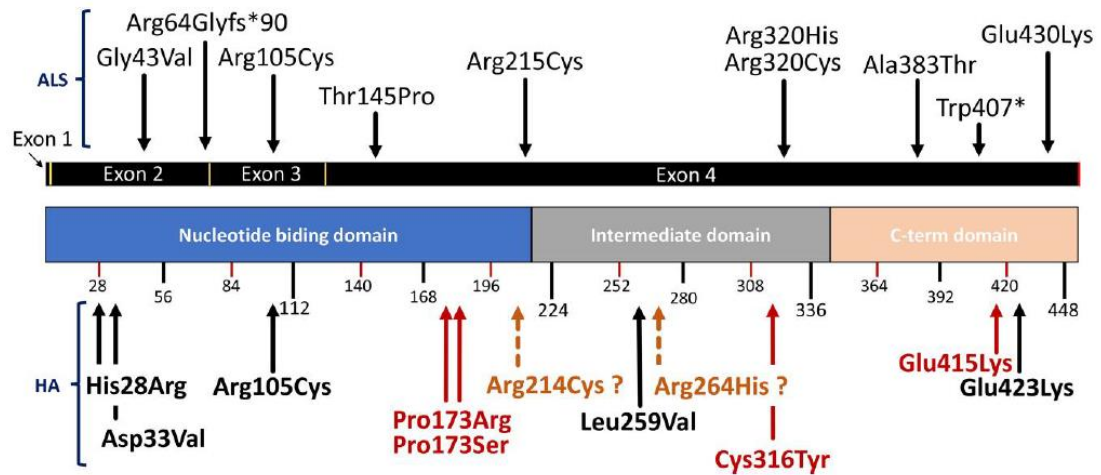


Figure 1 Schematic drawing of the TUBA4A protein. Missense mutations reported below the figure are those identified in this study in a patient presenting with hereditary ataxia while variants reported on top are those already identified in ALS-FTD patients. Confirmed *de novo* variants are indicated in red. Variants indicated in orange with dotted line arrows (p.Arg214Cys and p.Arg264His) were identified during the 100KGP burden analysis in patients with hereditary ataxia who were not included in the cohort due to high frequency of missense affecting the same residue in databases. 100KGP = 100 000 Genomes Project; ALS = amyotrophic lateral sclerosis; FTD = frontotemporal dementia.

Genetic screening of Patients 7, 8 and 12 was performed by whole exome sequencing (WES). Patients 6, 9, 10 and 11 were part of the UK 100 000 Genome Project (100KGP) and were identified through a rare variant gene-based burden case-control analysis (Supplementary Table 1). Variant calling and clinical sequence interpretation was performed following the Broad Institute's Genome Analysis Toolkit (GATK4) best practices and the guidelines from the American College of Medical Genetics. All the *TUBA4A* variants are given following the canonical MANE select Refseq NM_006000.3. Proband and available relatives from each family were tested for variant segregation by Sanger sequencing.

Functional assays

Fibroblast isolation and culture

Studies of microtubule organization and dynamics were performed on cultured fibroblasts from three patients (Patients 1, 2 and 7) and two controls (Patient 1's father and Patient 2's mother) obtained from skin biopsies (Supplementary material).

Western immunoblotting and immunofluorescence

The antibodies used were the mouse anti- α -tubulin [T6199; Sigma- Aldrich; dilution immunofluorescence (IF): 1:5000, dilution western blot (WB): 1:10 000], the rabbit anti-4A α -tubulin (SAB2102603; Sigma-Aldrich; dilution IF: 1:50, dilution WB: 1:1000), the mouse anti-acetylated α -tubulin (acetyl K40) (clone TEU318; Stemcell technologies; dilution IF: 1:2000) and the rat anti-tyrosinated α -tubulin (clone YL1/2; dilution IF: 1:1000). DyLight Fluor and Alexa Fluor secondary antibodies were from Thermo Fisher Scientific and LifeTechnologies, respectively. WB and IF staining and imaging protocols are given in the Supplementary material.

Ethics declaration

All procedures were conducted according to the guidelines of the Declaration of Helsinki and all patients/their guardians included in the study consented to participation according to ethical approval of the recruiting institutions.

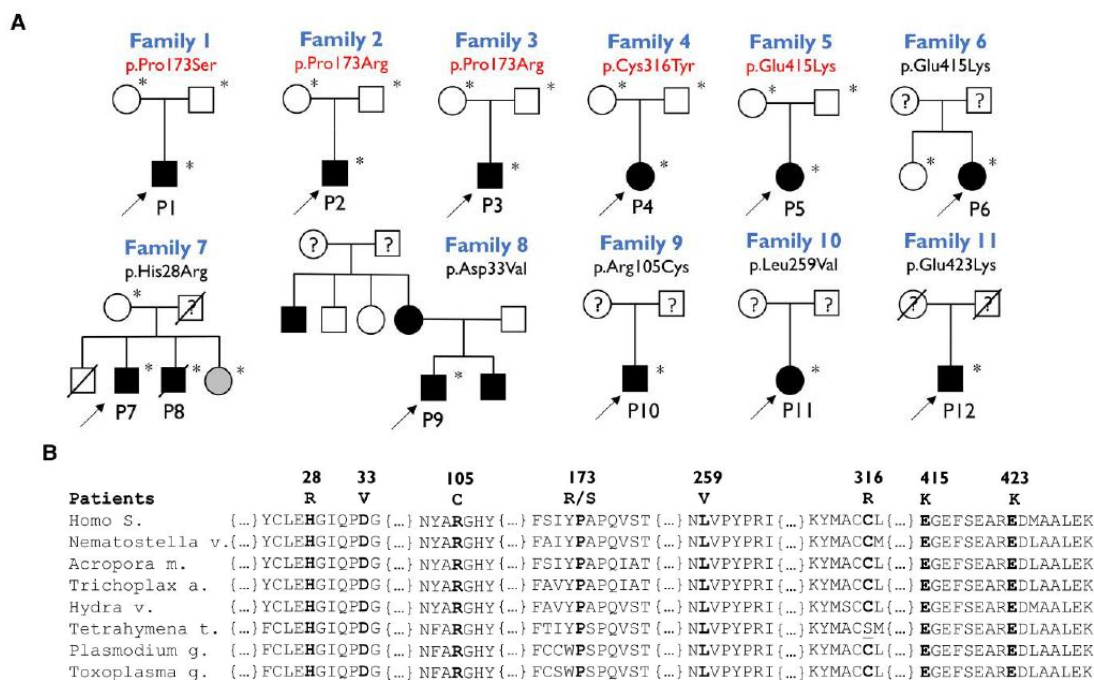


Figure 2 Pedigrees of the reported families and protein conservation study. (A) Pedigrees of reported families. Affected individuals are represented by black symbols. Index cases are indicated by an arrow. Individuals tested for *TUBA4A* variant are indicated by an asterisk (*). Apart from the younger unaffected sister of Patients 7 and 8, who was found to carry the p.His28Arg (in grey), all tested unaffected individuals were negative for the variant. Confirmed *de novo* variants are indicated in red. **(B)** Protein sequence conservation throughout eukaryotic kingdoms. *Nematostella v.*, *Acropora m.*, *Hydra v.* and *Trichoplax a.* are distant metazoa. *Tetrahymena t.*, *Plasmodium g.* and *Toxoplasma g.* are protists (alveolata). Mutant residues are indicated on top.

Results

Monoallelic *TUBA4A* missense variants identified in 12 ataxia patients

WES/WGS allowed for the identification of nine pathogenic or possibly pathogenic monoallelic *TUBA4A* missense variants in 12 patients with ataxia, from 11 unrelated families, including two previously reported variants (p.Arg105Cys and p.Glu415Lys) and five *de novo* (missense) variants identified by trio analysis (Figs 1 and 2). Clinical exome sequencing screening was performed in a multicentric cohort of 448 unrelated probands presenting with inherited cerebellar ataxia. Three *de novo* *TUBA4A* variants (p.Pro173Ser, p.Pro173Arg and p.Glu415Lys) were identified in Patients 1, 2 and 5, respectively, who presented with early-onset cerebellar ataxia (24, 22 and 10 years, respectively) with or without spasticity. Through GeneMatcher exchange, five additional patients (from four families) presenting with a similar neurodegenerative disorder and carrying a rare *TUBA4A* missense variant, were identified (Patients 3, 4, 7, 8 and 12). Interestingly, Patient 3 harbours the same *de novo* p.Pro173Arg identified in Patient 2, in whom an earlier onset of cerebellar ataxia was noticed (2 years), similar to the age of onset of Patient 4 (5 years), who was found to carry the *de novo* variant p.Cys316Tyr. The two *TUBA4A* variants p.His28Arg (brothers Patients 7 and 8) and p.Glu423Lys (Patient 12) should be considered of unknown significance (VUS) since parental segregation could not be performed due to late onset of the disease (46, 45 and 62 years, respectively).

Subsequently, *TUBA4A* was independently investigated as a candidate gene for the clinical indication of hereditary ataxia within the latest available rare disease 100KGP dataset, i.e. 35 008 rare disease families, including 1103 hereditary ataxia probands used as cases and 20 904 non-neurologic probands used as controls.

According to American College of Medical Genetics (ACMG) guidelines, variants were defined as rare when minor allele frequency was below 1×10^{-5} , which corresponds to a heterozygote carrier frequency of 1/50 000 individuals. The signal was based on six *TUBA4A* distinct missense variants within the cases versus two in controls, leading to a significant burden of *TUBA4A* rare variants assessed via a two-sided Fisher's exact test (i.e. exomiser variant score of ≥ 0.80), [6/1103 cases versus 2/20 904 controls, odds ratio (OR) = 57.0847 (10.2–576.7), $P = 4.02 \times 10^{-7}$]. The p.Arg214Cys and p.Arg264His variants identified in cases were not retained due to the high allele frequency of additional missense variants affecting the same residues in databases (84 and 21 heterozygous carrier on GnomAD v4, respectively). However, pathogenicity of the p.Arg214Cys and p.Arg264His variants could not be fully excluded and further investigations are needed. Interestingly, a p.Arg215Cys variant has already been identified in a fALS patient.⁶The four other *TUBA4A* missense variants identified in Patients 6, 9, 10 and 11 were absent from control cohorts. Interestingly, Patient 6 was found to have the same variant identified in Patient 5 (p.Glu415Lys). The p.Arg105Cys variant was identified in Patient 10, who presented with very late onset (60 years) pure cerebellar ataxia associated with severe cerebellar atrophy and cerebellar cognitive and affective syndrome (CCAS). This variant was previously reported in a large Dutch family exhibiting an heterogeneous clinical presentation related to FTD.⁸Two other missense variants, p.Asp33Val and p.Leu259Val, were identified in a proband from a family of four members affected with ataxia (Patient 9) and in a sporadic case presenting with early onset spastic ataxia (Patient 11), respectively. These two variants remain of unknown significance as parents or relatives were unavailable for segregation analyses. All identified variants affect evolutionarily highly conserved residues (in most eukaryota, Fig. 2) and were predicted as pathogenic across a series of *in silico* prediction tools. All variants were absent from several databases including GnomAD v.4.0 and Regeneron database (Supplementary Table 2).

Clinical findings

Our multicentric cohort consists of 12 patients from 11 unrelated families, five females and seven males, whose age at last evaluation ranged from 20 to 67 years. Table 1 provides an overview of the main clinical findings. All patients presented with gait disturbance/ataxia with a highly variable age of onset ranging from 2 years to 60 years (mean age of onset: 26 years). Spasticity and spastic gait were reported in more than half of the patients (7/12, 58.3%). Neurological examination also revealed increased deep-tendon reflexes in two-thirds of the cohort (8/12, 66.6%), while dysarthria, ankle clonus and bladder dysfunction were reported in less than half of the patients (5/12, 41.6%). Ophthalmological evaluation revealed nystagmus or abnormal eye movements in half of the patients (6/12, 50%) and cognitive decline was noted in one-third of the cases (4/12, 33.3%). Interestingly, a neurodevelopmental disorder was noticed only for Patients 2 and 3, who carried the same p.Pro173Arg variant and presented with learning disabilities. Apart from two cases who showed amyotrophy or upper limb muscular weakness (2/12, 16.6%), there was no evidence of lower motor neuron involvement in the other patients. Brain imaging was performed for 11 cases and showed cerebellar atrophy in seven cases ranging from mild to severe (7/11, 63%) (Supplementary Fig. 1). No abnormal signal was noticed at the level of cerebellar nuclei on MRI images of these seven patients. Most identified patients (8/12, 66.6%) presented with a slowly or moderately progressive disease (data not available for four patients) (Supplementary Table 3).

Table 1 Clinical and genetic findings of 12 patients reported in this study

General	Family 1	Family 2	Family 3	Family 4	Family 5	Family 6	Family 7		Family 8	Family 9	Family 10	Family 11
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12
Gender/ethnicity	M/Caucasian	M/Caucasian	F/Caucasian	F/Caucasian	F/Caucasian	F/Caucasian	M/Caucasian	M/Caucasian	M/Caucasian	M/Caucasian	F/India	M/Caucasian
Age at last examination	36 y	47 y	20 y	32 y	32 y	36 y	60 y	51 y	42 y	65 y	20 y	62 y
Genetics findings												
DNA	c.517C>T	c.518C>G	c.518C>G	c.947G>A	c.1243G>A	c.1243G>A	c.83A>G	c.83A>G	c.98A>T	c.313C>T	c.775C>G	c.1267G>A
Protein	p.Pro173Ser	p.Pro173Arg	p.Pro173Arg	p.Cys316Tyr	p.Glu415Lys	p.Glu415Lys	p.His28Arg	p.His28Arg	p.Asp33Val	p.Arg105Cys	p.Leu259Val	p.Glu423Lys
Inheritance	De novo	De novo	De novo	De novo	De novo	Unknown	Not maternal	Not maternal	Unknown	Dominant	Unknown	Unknown
Clinical findings												
Initial symptoms	Ataxia	Spastic ataxia	Ataxia	Ataxia	Spastic ataxia	Ataxia	Spastic gait	Spastic gait	Ataxia	Ataxia	Ataxia	Spastic ataxia
Age of onset	24 y	22 y	2 y	5 y	10 y	17 y	46 y	45 y	20 y	60 y	10 y	54 y
Developmental delay	-	LD	LD	-	-	-	-	-	-	-	-	-
Ataxia (SARA score)	+ (16.5)	+ (21)	+ (NA)	+ (11)	+ (17)	+ (NA)	-	-	+ (NA)	+ (23)	+ (12)	+ (NA)
Dysarthria	+	+	+	+	Mild	-	?	?	-	+	+	?
Eye movements	Saccadic pursuit	Nystagmus	?	+	Nystagmus	Nystagmus	N	N	N	N	Nystagmus	N
UL weakness	-	+	-	-	-	?	-	-	-	-	-	+
LL weakness	-	+	++	-	+	?	-	+	-	-	-	+
Spasticity	-	+	+	-	+	-	+	+	-	-	++	+
Extensor planter reflex	-	+	++	-	+	-	+	+	-	-	+	-
Increased deep tendon reflex	-	+	+	+	+	-	+	+	-	-	+	+
Ankle clonus	-	+	+	+	-	-	+	+	-	-	+	-
Cognitive decline	-	-	NA	+	-	-	+	+	-	CCAS	-	-
Bladder dysfunction	-	-	-	-	+	-	+	+	-	-	+	-
Amyotrophy	-	-	-	-	-	-	-	-	-	-	-	+
EMG	Myopathic signs	Normal	UL elongated motor potential	Normal	Elongated motor potential	NA	Perturbed	Perturbed	NA	NA	Normal	NA
Brain MRI	CA+	CA+	CA+	CA++	N	NA	N	Vascular insult	CA	CA++	CA++	N
Other features	Increased CPK	-	Scoliosis	-	-	-	Cirrhosis	Cirrhosis	Hepatic cyst	-	-	-
Disease progression	Moderate	Moderate	Slow	Slow	slow	NA	Moderate	Fast	NA	Slow	Slow	Slow

All variants are reported based on RefSeq NM_006000.3. + = present; - = absent; AD = autosomal dominant; CA = cerebellar atrophy; CCAS = cerebellar cognitive and affective syndrome; CPK = creatin phosphokinase; DD = developmental delay; F = female; LD = learning disability; LL = lower limbs; M = male; N = normal; NA = not available; SARA = Scale for the Assessment and Rating of Ataxia; UL = upper limbs; y = years.

Protein modelling

The effect of missense variants on alpha tubulin protein structure was investigated using homology modelling of TUBA1A experimentally validated models. Steric clashes were predicted for most reported variants and were particularly relevant for p.Asp33Val, p.Pro173Arg and p.Cys316Tyr. The proline impacted by p.Pro173Arg/Ser variant interacts with the two C-terminal alpha helices, thereby conferring an important role in protein tertiary structure. Interestingly, p.Pro173Arg/Ser and p.Arg105Cys are located close to residues involved in GTP binding domain and could lead to defects in tubulin GTP interaction (Supplementary Fig. 2). The C-terminal p.Glu415Lys and p.Glu423Lys variants, which lie outside the globular structure, led to introduction of positively charged lysine in an enriched polar negatively charged residue region. Most of the reported variants were predicted by DynaMut software to destabilize the protein, and lead to differential vibrational entropy energy between wild-type and mutant ($\Delta\Delta S_{Vib} ENCoM$) as recently predicted for ALS-related *TUBA4A* variants17(Supplementary Table 3).

Microtubule functional studies

Microtubule studies were performed on cultured fibroblasts from Patients 1, 2 and 7 (carrying the p.Pro173Ser, p.Pro173Arg and p.His28Arg variants, respectively). Fibroblasts from Patient 1's father and Patient 2's mother were used as controls ($n = 2$). Using an immunostaining approach, we first investigated aspects of the endogenous form of alpha4A-tubulin protein in fibroblasts of patients and did not observe any abnormal distribution, such as granules or modifications in soluble levels of these isoforms (Fig. 3C). Protein expression level of total alpha-tubulin and alpha4A tubulin isoform quantified by WB on fibroblast lysates from all tested patients were comparable to controls (Fig 3A and B). By contrast, the ratio of alpha4A-tubulin isoform versus total alpha-tubulin in microtubules assessed by an immunostaining approach, was significantly increased in fibroblasts from Patients 2 (p.Pro173Arg) and 7 (p.His28Arg), while it was significantly decreased in Patient 1 (p.Pro173Ser) (Fig. 3C and D). These differences in the incorporation rate of the alpha4A-tubulin isoform in

tested patients may therefore result from defects of alpha4A-tubulin protein incorporation in microtubules rather than lower TUBA4A protein expression.

Acetylated and tyrosinated tubulin levels were quantified in patients' fibroblasts by immunostaining, as these post-translation modifications (PTMs) play a crucial role in regulating microtubule stability.^{18,19,20}

We noticed a significant increment of microtubule tyrosination levels in all three patients' fibroblasts when compared to controls (Fig. 3E and F) as well as significantly decreased microtubule acetylation levels in fibroblast from Patients 2 (p.Pro173Arg) and 7 (His28Arg) (Fig. 3G and H). Surprisingly, no difference in microtubule acetylation levels was observed in fibroblast from Patient 1 (p.Pro173Ser) (Fig. 3G and H).

Discussion

***TUBA4A* variants are associated with inherited ataxia phenotype**

We report a cohort of unrelated patients harbouring heterozygous pathogenic ($n = 1$), likely pathogenic ($n = 4$) or possibly pathogenic ($n = 4$) variants in *TUBA4A* presenting with inherited cerebellar ataxia with or without spasticity. The clinical presentations were distinct from the fALS22 and FTD presentations previously associated with heterozygous *TUBA4A* variants.⁶ Association between cerebellar ataxia and a *TUBA4A* variant was recently suggested, based on p.Glu415Lys variant segregation in a large family presenting with spastic ataxia [logarithm of the odds (LOD) score = 3.6], as well as *de novo* occurrence in a sporadic patient with ataxia.¹⁰ Identification of an additional five unrelated patients carrying *de novo* missense mutations, including three impacting the same residue (Pro173) clearly indicates *TUBA4A* causal role in the disease. In our cohort, the age of onset of neurological signs was highly variable, ranging from 2 years to 60 years (mean age: 26 years). According to our data, the estimated frequency of *TUBA4A* pathogenic variants among patients with inherited ataxia from the French (3/448) and UK 100KGP (4/1103) cohorts is 0.45% (7/1551).

A dominant negative effect of *TUBA4A* variants?

Functional studies performed on cultured fibroblasts from three patients showed significant differences in the incorporation level of alpha4A-tubulin isoforms in microtubules. Increased microtubule tyrosination levels and decreased acetylation levels noticed in Patients 2 and 3 support impact on microtubule stability (Fig. 3).^{18,20} These results could be consistent with an already suggested dominant negative mechanism of *TUBA4A* mutants.^{6,10,17} However, contribution of a partial loss-of-function or a gain-of-function mechanism cannot be formally excluded.

Alpha 4A tubulin: a particular isoform

De novo variants impacting proline 173 in *TUBA1A* or *TUBB2B* (p.Pro173Leu) have already been associated with global neurodevelopmental disorders with seizure and multiple brain malformations, distinct from the isolated spastic ataxia found in Patients 1–3.^{2,21} The expression pattern of TUBA4A isoform has been shown to exhibit age-related increments in fetal human and in adult mice brains.^{1,6} In contrast with most tubulin isoforms, a minor involvement of *TUBA4A* during neurogenesis may allow a normal brain development, as was suggested for *TUBB4A* associated with adult onset dystonia 4 (DYT4).³

Alpha4A tubulin contains the longest C-terminal polyglutamic side chain across the tubulin family, which is highly involved in microtubule-associated protein (MAPs) binding and in tubulin PTMs.18,19,22The p.Glu423Lys and p.Glu415Lys identified variants could disrupt MAP association or PTMs, as suggested for a *de novo* C-terminal variant in *TUBB2A* identified in early onset spastic ataxia families.23

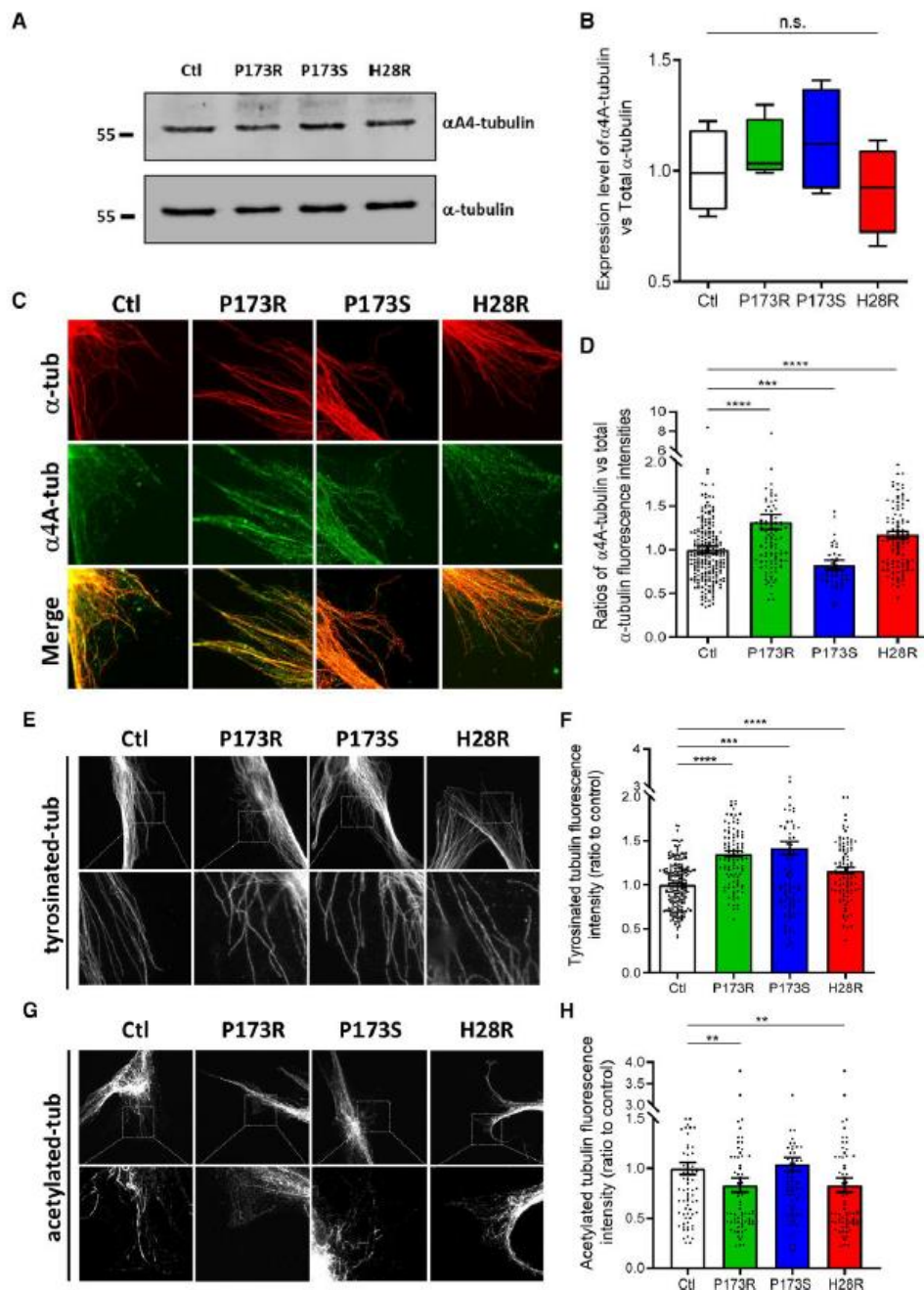


Figure 3 Microtubule functional studies on cultured fibroblasts from three patients. Microtubule organization was analysed on cultured fibroblasts from three patients (carrying p.Pro173Arg, p.Pro173Ser and p.His28Arg, respectively) and two controls subjects. For the immunostaining panel, all images were processed and analysed using Fiji software (National Institutes of Health). For each condition, $n = 30$ fibroblasts per patient and control were analysed. Microtubules were delineated using the segmented line tool on the total alpha-tubulin channel. For all fluorescence intensity measurements, the background signal was measured in an adjacent area of microtubules and subtracted. For microtubule acetylation and tyrosination level assessment, individualized microtubules, chosen in peripheral regions of fibroblasts, were quantified. Statistical analyses were done with Prism 6, GraphPad Software. Data distribution was assumed to be normal, but this was not formally tested. P-values are represented as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. (A) Alpha4A-tubulin immunoblots of lysates from cultured fibroblasts. Total alpha-tubulin was used as a loading control. (B) Alpha4A-tubulin expression level quantification from blots shown in A. (C) Representative immunostaining of total alpha-tubulin and alpha4A-tubulin isoform in cultured patient fibroblasts. (D) Quantification of alpha4A-tubulin incorporation level in microtubules from patient fibroblasts shown in C. (E) Representative immunostaining of tyrosinated tubulin in cultured patient fibroblasts. (F) Quantification of tyrosinated tubulin in microtubules shown in E. (G) Representative immunostaining of acetylated tubulin in cultured patient fibroblasts. (H) Quantification of acetylated tubulin in microtubules shown in G. n.s. = not significant.

Is ALS a clinical continuum with spastic paraplegia and ataxia?

Our patients carrying monoallelic *TUBA4A* variants presented with a progressive neurological disorder associating cerebellar degeneration and predominant upper motor neuron lesions leading to spasticity, and are distinct from classical ALS patients, who present simultaneous upper and lower motor neuron impairment. Hereditary spastic paraplegia (HSP) and primary lateral sclerosis (PLS) are characterized by pure upper motor neuron damage leading to spasticity and are difficult to distinguish from atypical forms of ALS due to important overlaps.²⁴ It is not clear whether these atypical forms are distinct types of disease or are milder ends of an ALS phenotypic spectrum.²⁵ However, it is interesting to notice that pathogenic variants in several genes (e.g. *ALS2*, *SPG11*, *KIF5A*) have been associated with either ALS, PLS or HSP phenotypes.^{26,27,28} Additionally, other genes, such as *SOD1*, have been associated with either ALS, by a dominant gain-of-function mechanism, or HSP, by a recessive loss-of-function, supporting the hypothesis of a clinical continuum.²⁹

Conclusion

Our study confirms the identification of an inherited ataxia phenotype associated with *TUBA4A* rare variants. Disease onset and severity prediction remain unclear and no obvious genotype/phenotype correlation was identified. The highly heterogeneous phenotype associated with *TUBA4A* variants is reminiscent of variability seen in other neurodegenerative disorders, such as multiple system proteinopathy disorders that include ALS and FTD in the clinical spectra.³⁰ Drawing a distinctive line between *TUBA4A* patients with ALS-FTD or ataxia can be challenging based on the limited number of cases and functional studies reported so far. Further studies are needed for a better understanding of *TUBA4A* pathophysiology.

Data availability

Data are available upon reasonable request.

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Competing interests

The authors report no competing interests.

Appendix 1

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