The Egyptian Journal of Neurology, Psychiatry and Neurosurgery

CASE REPORT

Open Access

The p.D417N variant of TUBB4A as a possible cause of hereditary spastic paraplegia: a case report

Enrico Matteoni[1*], Antonio Canosa[1,2,3], Alessandra Tessa[4], Gemma Natale[4] and Salvatore Gallone[5]

Abstract

Background Tubulins are dimeric proteins expressed in all eukaryotic cells, serving as the fundamental building blocks of microtubule filaments. The *TUBB4A* gene encodes the protein β-tubulin. Mutations of *TUBB4A* have been associated with two neurodegenerative diseases with very different clinical characteristics: dystonia type 4 (DYT4) and Hypomyelination with Atrophy of the Basal Ganglia and Cerebellum (H-ABC). Several cases of patients with H-ABC or unclassified leukoencephalopathy show spastic ataxia with or without leukodystrophy in association with mutations in exon 5 of *TUBB4A* gene.

Case presentation We report the case of a 65-year-old woman who has been complaining of progressive difficulty in walking since childhood. The neurological examination revealed, among all, a spastic gait, difficulties in standing on the toes and heels, brisk deep tendon reflexes at upper limbs, clonic patellar reflexes, brisk ankle reflexes, as well as bilateral Babinski and Hoffmann sign, brisk jaw jerk and severe lower limb spasticity. NGS studies for inherited cases of spastic paraplegia revealed a novel variant of unknown significance (VUS) (c.1249A > G, p.D417N), in *TUBB4A*. To the best of our knowledge, this variant has not been reported in the literature or any databases in association with these diseases.

Conclusion We report the case of a patient carrying a variant of uncertain significance (VUS) of the *TUBB4A* gene, showing a progressive, spastic paraparesis, supporting the extension of the phenotypic spectrum up to include spastic paraplegia.

Introduction

Tubulins are dimeric proteins, which are expressed in all eukaryotic cells, serving as the fundamental building blocks of microtubule filaments [1]. Microtubules are notable components of structures like flagella, cilia, and the cytoskeleton, playing a crucial role in creating mitotic spindle fibers. In recent years, mutations in genes encoding various alpha- and beta-tubulin isotypes (*TUBA1A*, *TUBA4A*, *TUBB4A*, *TUBA*, *TUBB2B*, *TUBB3*, *TUBB5*) have been reported in a wide range of developmental brain disorders, including epilepsy, intellectual disability, and different variants of motor developmental delay, known as tubulinopathies [2–4].

The *TUBB4A* gene encodes the protein β-tubulin, which is predominantly found in the brain, specifically

*Correspondence:
Enrico Matteoni
enrico.matteoni@unito.it
[1] Department of Neuroscience "Rita Levi Montalcini", University of Turin, Turin, Italy
[2] Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino, SC Neurologia 1U, Turin, Italy
[3] Institute of Cognitive Sciences and Technologies, C.N.R., Rome, Italy
[4] Molecular Medicine for Neurodegenerative and Neuromuscular Diseases Unit, IRCCS Stella Maris Foundation, 56128 Pisa, Italy
[5] Clinic Neurogenetic Neuroscience Department, University of Turin, Turin, Italy

in regions such as the basal ganglia, the cerebellum, and the white matter. It plays a crucial role in neural cell migration during the embryo development. Mutations in *TUBB4A* have been so far associated with two neurodegenerative diseases [5]: Dystonia type 4 (DYT4, whispering dystonia), characterized by segmental/generalized dystonic phenomena, and Hypomyelination with Atrophy of the Basal Ganglia and Cerebellum (H-ABC), an early-onset leukodystrophy manifesting with a complex phenotype including progressive spastic paraparesis, ataxia, and extrapyramidal disorders.

In this study, we report the case of a patient carrying a novel variant of uncertain significance (VUS) in *TUBB4A* showing a progressive, spastic paraparesis, supporting the extension of the phenotypic spectrum up to include spastic paraplegia.

Case presentation

The patient is a 65-year-old woman who has been complaining of progressive difficulty in walking since childhood (approximately 8–10 years). She reported leg stiffness leading to frequent falls. Additionally, she showed a head tremor, presenting as a yes-yes movement (onset at 50 years), and a disabling depressive mood disorder for many years. The neurological examination revealed a spastic gait, difficulties in standing on the toes and heels, brisk deep tendon reflexes at upper limbs, clonic patellar reflexes, brisk ankle reflexes, as well as bilateral Babinski and Hoffmann sign and brisk jaw jerk, severe lower limb spasticity, normal muscle strength at four limbs, impaired perception of pain and vibration, yes-yes head tremor, upper limb postural and kinetic tremor. The patient refused a full neuropsychological assessment, but major cognitive impairment was not clinically evident.

Walking difficulties due to leg stiffness were reported in the father (deceased due to complications of Chronic Obstructive Pulmonary Disorder), two paternal uncles (deceased for unknown reasons), and the patient's sister (deceased from colon cancer) who could not be examined directly. However, their clinical records were consistent with spastic paraplegia. (shown in Fig. 1A).

Over time, several diagnostic assessments were performed, including an MRI (Magnetic Resonance Imaging) of the brain and cervical spinal cord, which showed mild hyperintensity of the white matter next to the anterior horns of the lateral ventricles (presumably of vasculopathy origin). Laboratory tests encompassing white and red cell counts, erythrocyte sedimentation rate, antiphospholipid antibodies, serum protein electrophoresis, assessment of liver, renal, and thyroid function, vitamin B12, folic acid, glucose levels, and urine analysis resulted in normal. Additionally, genetic testing for mutations of the *SPAST* genes was performed, yielding negative results.

NGS (Next-Generation Sequencing) studies for inherited cases of spastic paraplegia (see Appendix 1 for the gene list) revealed a novel variant of unknown significance (VUS) (c.1249A > G, p.D417N), in *TUBB4A* (CADD score 25.80; CADD score is a tool for scoring the deleteriousness of single nucleotide variants). To the best of our knowledge, this variant has not been reported in the literature or any databases in association with these diseases, although two pathogenic variants have been identified in the same exon [6–8].

In silico studies suggested a 99% probability that p.D417N was pathogenic (with a sensitivity and

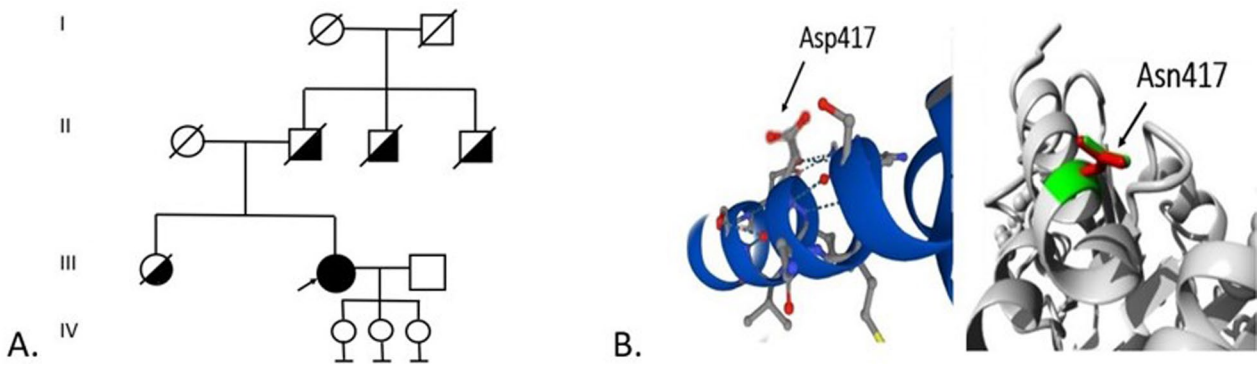

Fig. 1 **A** Pedigree of the family. Squares indicate males and circles indicate females. The family tree shows the affected member (fully black circle), members with gait impairment who did not undergo genetic/clinical analysis (half black circles and squares), and asymptomatic members (blank). Circles and squares with the bar represent deceased subjects The circle with the arrow indicates the proband. Roman numerals indicate the different generations. **B** In silico evaluations of the variant identifified (on the right) compared with the normal protein (on the left). The position of the substitution D417N is indicated with an arrow

specificity of 14% and 99%, respectively) based on PolyPhen-2, Provean, Mutation Taster, and UMD-Predictor Pro site prediction [9, 10].

Whole exome sequencing was conducted on the patient's DNA using the NextSeq500 Illumina platform as reported elsewhere. The interpretation of variants followed the guidelines of the American College of Medical Genetics (ACMG). Variants with a frequency exceeding 1% in the gnomAD database, those with CADD scores < 20, classified as benign/probably benign according to ACMG criteria, and those of uncertain significance in autosomal recessive genes are filtered out [11, 12].

Discussion

We report a patient presenting with childhood-onset slowly progressive spastic paraparesis associated with head and upper limb tremors from adulthood. Laboratory and neuroimaging studies did not reveal any significant finding, whereas we identified a p.D417N in the C-terminal domain of the β-tubulin 4A protein (*TUBB4A* gene). Residues from 380 to 425 (exon 5) are highly conserved both across different species (as highlighted by interspecies conservation analysis performed using Polyphen-2 software) and various tubulin proteins.

The C-terminal domain is involved in calcium binding, which is important for microtubule assembly. In our case, the p.D417N mutation involves the substitution of an acidic residue (aspartic acid) with its basic counterpart (asparagine), suggesting that this substitution may contribute to the disassembly of microtubules by disrupting the cationic binding site normally present at this level under normal conditions (shown in Fig. 1B).

Over the years, mutations in the *TUBB4A* gene have been associated with whispering dysphonia (DYT4) and hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) [13]. DYT4 dystonia is typically characterized by onset between 13 and 37 years of age, autosomal dominant inheritance, and dystonia mainly involving laryngeal (resulting in whispering dysphonia) and cervical muscles [14].

On the other hand, H-ABC typically shows an earlier age of onset (usually during puberty) is also characterized by autosomal dominant inheritance and includes a broad spectrum of symptoms, such as widespread leukodystrophy, hypomyelination, atrophy of the basal ganglia and cerebellum, spasticity, tremors, intellectual disability, and gait impairment and exhibits a progressive course [15].

Several cases of patients with H-ABC or unclassified leukoencephalopathy show spastic ataxia with or without

Table 1 Clinical, genetic, and neuroradiological features of patients with TUBB4A gene mutations near D417N

	Blumkin et al.[6]	Sasaki et al.[5]	Hattori et al.[4]	Our patient
Number of described patients	1	2	1	1
Family history	–	–	–	+
Age at onset (range)	1 year	3–10 years	3 years	8–10 years
Gait impairment	+	+ + + (2/2)	+	+ + +
Spasticity	–	+ (2/2)	+	+ + +
Nystagmus	Lack of smooth pursuit	–	NR	–
Dysarthria	+	+ (2/2)	NR	+
Dysmetria	NP	NR	NR	+
Ataxia	–	+ + (2/2)	+	++
Tendon reflexes	+	+ + (2/2)	+	+ + +
Extrapiramidal signs	+ (tremor)	+ (tremor, 2/2)	–	+ (tremor)
Babinski sign	NR	+ (2/2)	NR	+
Psychiatric involvement	+ (Disruptive behaviour)	NR	NR	+ (Disruptive behaviour)
Cognitive abnormalities	+	+ (2/2)	+ + +	NP
Abnormal MRI findings	+ Hypomielination in parietal lobe and mild cerebellar atrophy	+ Hypomielination in supratentorial cerebral white matter (T2-weighted sequences) and mild cerebellar atrophy (2/2)	+ Supratentorial hypomyelination and progressive atrophy of the basal ganglia and cerebellum	+ Mild pallor of the white matter in the anterior horns of the lateral ventricles
Progressive course	+	+ (2/2)	+	+
CADD score	NR	NR	NR	25.80
Mutation	E410K	E410K	M388N	D417N

NP not performed, NR not reported. Severity = + to +++; - absent/normal, + present

leukodystrophy in association with mutations in exon 5 of the *TUBB4A* gene (shown in Table 1). This finding strengthens the hypothesis that the VUS identified in our proband may be disease-related. MutScore analysis showed that variants affecting residues from 388 to 418 have a 99% likelihood of being pathogenic [16]. As highlighted in Table 1, these variants share similar phenotypic presentations, with some minor differences. Although the 3D modeling showed a possible impact on protein function, a major limitation of our study is that segregation analysis in our kindred was not possible because the other potentially affected subjects had already died from other diseases.

Conclusion

In conclusion, we report a novel VUS of *TUBB4A* in a case of childhood-onset spastic paraparesis without apparent involvement of the white matter, supporting the expansion of the phenotypic spectrum of *TUBB4A* up to include spastic paraplegia.

Abbreviations

NGS	Next-generation sequencing
DYT4	Dystonia type 4
H-ABC	Atrophy of the basal ganglia and cerebellum
MRI	Magnetic resonance imaging
VUS	Variant of unknown significance
ACMG	American College of Medical Genetics
CADD score	Combined annotation dependent depletion score

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41983-024-00905-w.

Additional file 1.

Acknowledgements

The authors are grateful to Dr. Filippo Santorelli and his team for the helpful collaboration. AT is supported in part by the Italian Ministry of Health Grant RC2023 and RC 5×1000

Author contributions

E.M.: clinical data collection and article writing. A.C.: review of the article. S.G., A.T, G.N.: genetic analysis.

Funding

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Since the NGS data used in the study are derived from the usual diagnostic pathway, there was no need for additional ethical approval. All analyses were performed using publicly available GWAS datasets. The GWAS studies cited in this research have received approval from their respective ethical review boards and have complied with informed consent requirements from participants. Informed consent was completed by the patient for the genetic analysis. The patient also consented to using the data from the analysis for research purposes.

Consent for publication

The patient has authorized the use of her data (clinical and genetic) at the same time as signing the informed consent to perform the genetic analysis. The PDF version of the consent signed by the patient is available on request at any time.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Received: 30 April 2024 Accepted: 17 October 2024
Published online: 01 November 2024

References

1. Kubo T, Tani Y, Yanagisawa HA, Kikkawa M, Oda T. α- and β-tubulin C-terminal tails with distinct modifications are crucial for ciliary motility and assembly. J Cell Sci. 2023;136(16):jcs261070. https://doi.org/10.1242/jcs.261070.
2. Gonçalves FG, Freddi TAL, Taranath A, Lakshmanan R, Goetti R, Feltrin FS, et al. Tubulinopathies. Top Magn Reson Imaging. 2018;27(6):395–408.
3. Maillard C, Roux CJ, Charbit-Henrion F, Steffann J, Laquerriere A, Quazza F, et al. Tubulin mutations in human neurodevelopmental disorders. Semin Cell Dev Biol. 2023;137:87–95.
4. Binarová P, Tuszynski J. Tubulin: structure, functions and roles in disease. Cells. 2019;8(10):1294. https://doi.org/10.3390/cells8101294.
5. Curiel J, Rodríguez Bey G, Takanohashi A, Bugiani M, Fu X, Wolf NI, et al. TUBB4A mutations result in specific neuronal and oligodendrocytic defects that closely match clinically distinct phenotypes. Hum Mol Genet. 2017;26(22):4506–18.
6. Hattori A, Ando N, Fujimoto S, Kobayashi S, Ishikawa T, Togari H. A boy with hypomyelination with atrophy of the basal ganglia and cerebellum. No To Hattatsu. 2010;42(1):42–4.
7. Sasaki M, Takanashi J, Tada H, Sakuma H, Furushima W, Sato N. Diffuse cerebral hypomyelination with cerebellar atrophy and hypoplasia of the corpus callosum. Brain Dev. 2009;31(8):582–7.
8. Blumkin L, Halevy A, Ben-Ami-Raichman D, Dahari D, Haviv A, Sarit C, et al. Expansion of the spectrum of TUBB4A-related disorders: a new phenotype associated with a novel mutation in the TUBB4A gene. Neurogenetics. 2014;15(2):107–13.
9. Bhat GR, Sethi I, Rah B, Kumar R, Afroze D. Innovative in silico approaches for characterization of genes and proteins. Front Genet. 2022;13: 865182. https://doi.org/10.3389/fgene.2022.865182.
10. Choudhury A, Mohammad T, Anjum F, Shafie A, Singh IK, Abdullaev B, et al. Comparative analysis of web-based programs for single amino acid substitutions in proteins. PLoS ONE. 2022;17(5): e0267084. https://doi.org/10.1371/journal.pone.0267084.
11. Nykamp K, Anderson M, Powers M, Garcia J, Herrera B, Ho YY, et al. Sherloc: a comprehensive refinement of the ACMG-AMP variant classification criteria. Genet Med. 2017;19(10):1105–17.
12. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–24.
13. Miyatake S, Osaka H, Shiina M, Sasaki M, Takanashi J, Haginoya K, et al. Expanding the phenotypic spectrum of TUBB4A-associated hypomyelinating leukoencephalopathies. Neurology. 2014;82(24):2230–7.
14. Bally JF, Camargos S, Oliveira Dos Santos C, Kern DS, Lee T, Pereira da Silva-Junior F, et al. DYT-TUBB4A (DYT4 Dystonia): new clinical and genetic observations. Neurology. 2021;96(14):e1887–97.
15. Krajka V, Vulinovic F, Genova M, Tanzer K, Jijumon AS, Bodakuntla S, et al. H-ABC- and dystonia-causing TUBB4A mutations show distinct

pathogenic effects. Sci Adv. 2022;8(10):eabj9229. https://doi.org/10.1126/sciadv.abj9229.
16. Quinodoz M, Peter VG, Cisarova K, Royer-Bertrand B, Stenson PD, Cooper DN, et al. Analysis of missense variants in the human genome reveals widespread gene-specific clustering and improves prediction of pathogenicity. Am J Hum Genet. 2022;109(3):457–70.

Publisher's Note