Mosaic Dominant TUBB4A Mutation in an Inbred Family With Complicated Hereditary Spastic Paraplegia

Dahlia Kancheva, MSc,1,2,3† Teodora Chamova, MD, PhD,4‡ Velina Guergueltcheva, MD, PhD,4 Vanio Mitev, MD, PhD DSc,3 Dimitar N. Azmanov, MD, PhD,5,6 Luba Kalaydjieva, MD, PhD,6 Ivailo Tournev, MD, PhD DSc,4,7*, and Albena Jordanova, PhD1,2,3*

1Molecular Neurogenomics Group, Department of Molecular Genetics, VIB, Antwerp, Belgium 2Neurogenetics Laboratory, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium 3Department of Medical Chemistry and Biochemistry, Molecular Medicine Center, Medical University-Sofia, Sofia, Bulgaria 4Department of Neurology, Medical University-Sofia, Sofia, Bulgaria 5Department of Diagnostic Genomics, PathWest, QEII Medical Centre, Nedlands, WA, Australia 6Harry Perkins Institute of Medical Research and Centre for Medical Research, The University of Western Australia, Perth, Australia 7Department of Cognitive Science and Psychology, New Bulgarian University, Sofia, Bulgaria

Abstract

Background: Mutations in TUBB4A have been associated with a spectrum of neurological conditions, ranging from the severe hypomyelination with atrophy of the basal ganglia and cerebellum syndrome to the clinically milder dystonia type 4. The presence of movement abnormalities was considered the common hallmark of these disorders.

Methods: Clinical, neurological, and neuroimaging examinations, followed by whole exome sequencing and mutation analysis, were performed in a highly consanguineous pedigree with five affected children.

Results: We identified a novel c.568C>T (p.H190Y) TUBB4A mutation that originated de novo in the asymptomatic mother. The affected subjects presented with an early-onset, slowly progressive spastic paraparesis of the lower limbs, ataxia, and brain hypomyelination, in the absence of dystonia or rigidity.

Conclusions: Our study adds complicated hereditary spastic paraplegia to the clinical spectrum of TUBB4A-associated neurological disorders. We establish genotype–phenotype correlations with mutations located in the same region in the tertiary structure of the protein.

© 2015 International Parkinson and Movement Disorder Society

Key Words: TUBB4A; H-ABC; hereditary spastic paraplegia; mosaicism

TUBB4A encodes a brain-specific member of the β-tubulin family with highest expression in cerebellum, putamen, and white matter.1 The first mutation in TUBB4A, c.4C>G, was identified in a pedigree with dystonia type 4 (DYT4), characterized by adolescent/adult onset of spasmodic dysphonia or generalized dystonia, and normal brain magnetic resonance imaging.1,2 Concurrently, a de novo mutation (c.745G>A) was reported in 11 patients with hypomyelination and atrophy of the basal ganglia and cerebellum (H-ABC). This severe form of leukodystrophy presents with onset in infancy or childhood, developmental delay, dystonia, choreoathetosis, rigidity, progressive spastic tetraplegia, and ataxia.3 Presently, 25 additional mutations have been reported in neurological disorders with pyramidal and cerebellar features, dystonia, and neuroimaging evidence of hypomyelination and atrophy of the cerebellum or basal ganglia.4-12 Dystonia is a unifying sign of H-ABC and DYT4 and has been proposed as a clinical characteristic indicating TUBB4A screening.7,8,12 However, recent reports described patients lacking dystonia or atrophy of the basal ganglia on magnetic resonance imaging, further complicating the diagnostic process.4,7,9,10,12

We describe a novel mutation in TUBB4A resulting in complicated hereditary spastic paraplegia (HSP) with no basal ganglia involvement or cognitive impairment. Surprisingly for this highly consanguineous family, the mode of inheritance was dominant; the mutation had originated de novo in the mosaic mother and had been transmitted to five of the six offspring.

*Correspondence to: Prof. Dr. Albena Jordanova, PhD, Molecular Neurogenomics Group, VIB Department of Molecular Genetics, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium; E-mail: albena.jordanova@molgen.vib-ua.be.† Prof. Dr. Ivailo Tournev, MD, DSc, Department of Neurology, Medical University-Sofia, Sofia 1000, Bulgaria; E-mail: itourn@emhpf.org.

Funding agencies: This study was supported by the Research Fund of the University of Antwerp, Belgium (to A.J.); the Fund for Scientific Research–Flanders, Belgium (to A.J.); the Research Fund of the Medical University-Sofia, Bulgaria (to A.J. and I.T.); the Tom Wahlig Foundation, Jena, Germany (to A.J and I.T.). D.K. received a travel grant from the Boehringer Ingelheim Fund, Germany.

Relevant conflicts of interest/financial disclosures: Nothing to report. Full financial disclosures and author roles may be found in the online version of this article.

1These authors contributed equally to the study.
2These authors contributed equally to the study.

Received: 16 October 2014; Revised: 20 January 2015; Accepted: 26 January 2015

Published online 00 Month 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.26196

BRIEF REPORT
Patients and Methods

The study involved a family from a strictly endogamous Roma/Gypsy group residing in Bulgaria, with five siblings (including a twin pair) diagnosed with HSP (Fig. 1A). Written informed consent was obtained from all participants. The study complies with the ethical guidelines of the institutions involved. The employed clinical and genetic methods can be found online.

Results

Clinical Features

Pregnancies and deliveries were uneventful. The disease onset was in infancy or early childhood, with delay in motor milestones acquisition (Supplemental Data Table S1). Individuals V-2, V-3, and V-4 achieved independent walking at the age of 2 to 3 y, with subsequent progressive gait deterioration and loss of ambulation between 5 and 17 y of age. The two younger siblings (V-5, V-6) never achieved independent ambulation; they have only been able to crawl. Early neuropsychological development was reported as normal; speech was acquired between 1.5 and 2 y. No language or cognitive deterioration was observed.

Recent examination showed a homogeneous phenotype with predominant pyramidal involvement: lower spastic paraparesis, brisk tendon reflexes, and pathological reflexes in the four limbs. Cerebellar symptoms were mild, including stance ataxia in three siblings, slight upper limb dysmetria and dysdiadochokinesia, and broken eye pursuit in all patients. Dystonia or rigidity were not observed. Nerve conduction studies indicated axonal motor and sensory polyneuropathy in the lower limbs. The neuroimaging findings in V-4 and V-6 were identical, characterized by bilateral hyperintense confluent lesions on T2 and Fluid Attenuation Inversion Recovery (FLAIR) sequences in the periventricular white matter and mild cerebellar atrophy. The size of the basal nuclei was normal (Supplemental Data Fig. S1). No abnormalities were seen in the cervical and thoracic spinal cord. Plasma amino acids and urine organic acids were normal.
Genetic Findings

Relatedness and inbreeding estimations based on the whole exome sequencing (WES) data showed higher than expected values for both the patients and their parents, corresponding to a 1st cousin–double 1st cousin union offspring (Supplemental Data Tables S2, S3). Whereas the proportion of affected children, five of six or four of five in the case of identical twins, is relatively high for an autosomal recessive mode of inheritance, this model and autozygosity for the disease-causing mutation were supported by the high level of inbreeding.

Linkage analysis was performed after introducing additional consanguinity loops to approximate the undeclared relatedness (Supplemental Data Fig. S2A, Supplemental Methods). Three suggestive regions were detected, on chromosomes 1p21-13, 14q24-32, and 20p12-q11 (LODmax = 1.62) (Supplemental Data Fig. S2B).

Next, the WES data were filtered for homozygous variants within these regions that co-segregated with the disease in the individuals analyzed and had less than 5% frequency in public databases (1000 genomes, Exome Variant Server) and 104 in-house genomes. The 10 remaining variants were checked by Sanger sequencing for co-segregation with the disease in the entire family. Surprisingly, none showed segregation. Furthermore, the same filtering approach was applied for variants outside the homozygous regions. Only four additional homozygous variants were retrieved, which also did not co-segregate.

These negative results prompted us to reconsider the inheritance model by selecting for rare (<1%) heterozygous variants, shared between the affected individuals and not present in the parents. Two variants fulfilled these criteria: c.239C>T (p.T80M) in PLEK2 (NM_016445.1) and c.568C>T (p.H190Y) in TUBB4A (NM_006087.1, Supplemental Data Fig. S3). Segregation analysis excluded c.239C>T in PLEK2, and supported the c.568C>T variant in TUBB4A (Fig. 1A). Although only the reference allele was observed in the 48 WES reads of IV-2, a low variant allele peak was clearly detectable in the Sanger sequencing traces (Supplemental Data Fig. 3B), pointing to mosaicism in the mother. Mutant-allele specific polymerase chain reaction (PCR)-based assay showed that the alternative allele was present in approximately 10% of the blood mononuclear cells’ DNA of the mother (Fig. 1B and 1C; Supplemental Data Methods).

The c.568C>T variant was not detected in 72 ethnically matched controls and was predicted to be “disease causing” (Mutation Taster) and “probably damaging” (Polyphen2). p.H190 is located in the H5-helix of TUBB4A and is highly conserved (Supp. Figure S4, phyloP score = 5.1). Mapping the p.H190 position on the 3D structure of the αtubulin dimer placed it at the interface between homologous subunits (α-α, β-β), involved in the lateral contacts between microtubule protofilaments (Supplemental Data Fig. S5).

Discussion

We report a novel c.568C>T TUBB4A mutation in a consanguineous family affected by early-onset complicated HSP with regional hypomyelination, mild cerebellar atrophy, and no dystonia, basal ganglia atrophy, or cognitive dysfunction. Despite the high level of inbreeding in the pedigree, the ratio of affected children and our failure to detect the expected autozygous mutation prompted us to reconsider the inheritance model.

Indeed, further analyses indicated that the mode of inheritance was autosomal dominant, in which the clinically unaffected mother is a somatic and germline mosaic for a de novo pathogenic variant, which she has transmitted to the affected children. TUBB4A mosaicism has been reported and should be considered in families suspected for mutations in this gene.

Impaired microtubule dynamics and stability are common functional defects caused by TUBB4A mutations, most of which are positioned in the heterodimer interface or near the guanine nucleotide-binding pocket (Supplemental Data Fig. S5). The p.H190 homologous residue in α-tubulin, p.H192, has been predicted to bind a zinc ion and facilitate the zinc-induced lateral interactions in tubulin sheets. These interactions are thought to regulate microtubule dynamics and affect microtubule assembly.


The introduction of proline into the M-loop possibly destabilizes the lateral contacts. The p.E410K mutation is positioned in the C-terminal outer surface of TUBB4A. The homologous p.E410K mutation in TUBB3 affects a kinesin-binding site and shows alterations in microtubule dynamics similar to p.R262H.

A comparison of the clinical phenotypes associated with these five TUBB4A mutations (Table 1) shows a disease onset within the first years of life, with impaired early motor development observed in seven of nine reported patients. Lower limb spasticity, cerebellar involvement (clinically manifested or detectable by neuroimaging), and white matter changes are consistently observed in all cases. Basal ganglia
involvement, considered until recently an invariable feature of TUBB4A-associated phenotypes, was documented in six patients as either dystonic hyperkinesia (4/9), basal ganglia atrophy (1/9), or both (1/9).4,6,7,10 An additional patient has been reported with globular appearance of the basal ganglia and spontaneous nonpurposeful movements of the extremities.6 Patients without dystonia or basal ganglia atrophy had mutations in the vicinity of p.H190,4,6,7,10 suggesting that this location is related to lower risk of basal ganglia involvement. In contrast to our patients, in whom cognitive ability was unaffected as regards both early development and recent performance on formal testing, impairment of different severity was present in all of the nine patients.4-7,9,10

In conclusion, the phenotype in our family is compatible with complicated HSP with early onset, slow progression, spasticity, cerebellar involvement, and mild hypomyelination. These manifestations are present in other patients with mutations affecting the same region in the tertiary protein structure. Noteworthy, spasticity is present in most reported cases, with TUBB4A mutations independent of their localization. Our findings confirm that basal ganglia involvement and cognitive impairment are not mandatory features of TUBB4A-associated disorders, and screening of this gene should be undertaken in patients presenting with complicated forms of HSP. Furthermore, our genetic data emphasize the importance of correct assumption of the inheritance model for the identification of pathogenic defects.

Acknowledgments: We thank all study participants for their cooperation. We thank the VIB Genetic Service Facility (http://www.vibgeneticservicefacility.be/) for the Sanger sequencing, P. De Rijk and B. Smets for the bioinformatic support, N. Ivanova for the preliminary genetic analysis, and M. Ivanova for the biochemical analysis.

References


TABLE 1. Clinical manifestations in patients with mutations in the vicinity of the p.H190 residue within the 3D-structure of TUBB4A

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>5 Siblings</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Inheritance pattern</td>
<td>Autosomal dominant</td>
<td>De novo</td>
<td>De novo</td>
<td>De novo</td>
<td>De novo</td>
</tr>
<tr>
<td>Age at onset (range)</td>
<td>1-12 mo</td>
<td>12 mo</td>
<td>2 mo</td>
<td>3 mo</td>
<td>12 mo</td>
</tr>
<tr>
<td>Age at last assessment</td>
<td>20-29 y</td>
<td>11 y</td>
<td>1 y</td>
<td>3 y</td>
<td>9 y</td>
</tr>
<tr>
<td>Delayed motor milestones</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Delayed language acquisition</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Age at loss of ambulation</td>
<td>0-17 y</td>
<td>Never walked</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>Spasticity UL/LL</td>
<td>-/+</td>
<td>+/ND</td>
<td>+/ND</td>
<td>-/+</td>
<td>+/ND</td>
</tr>
<tr>
<td>Weakness UL/LL</td>
<td>-/+</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Ataxia</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Dystonia</td>
<td>-</td>
<td>Spontaneous but nonpurposeful movements of the extremities</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Choreoathetosis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rigidity</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tremor</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Dysarthria</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Ocular and oculomotor abnormalities</td>
<td>Saccadic eye movements, strabismus</td>
<td>ND</td>
<td>Nystagmus</td>
<td>Nystagmus</td>
<td>Lack of smooth pursuit</td>
</tr>
<tr>
<td>Cognitive impairment</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Axonal polyneuropathy</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Course</td>
<td>2/2</td>
<td>Static—slowly progressive</td>
<td>Progressive</td>
<td>Progressive</td>
<td>ND</td>
</tr>
<tr>
<td>Brain MRI</td>
<td>Hypomyelination</td>
<td>Periventricular</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Basal ganglia atrophy</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cerebellar atrophy</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

UL/LL, upper/lower limbs; ND, no data; y, years; mo, months; w, weeks. *The location of the spasticity is not specified.


Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.