BRIEF REPORT

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

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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.

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.

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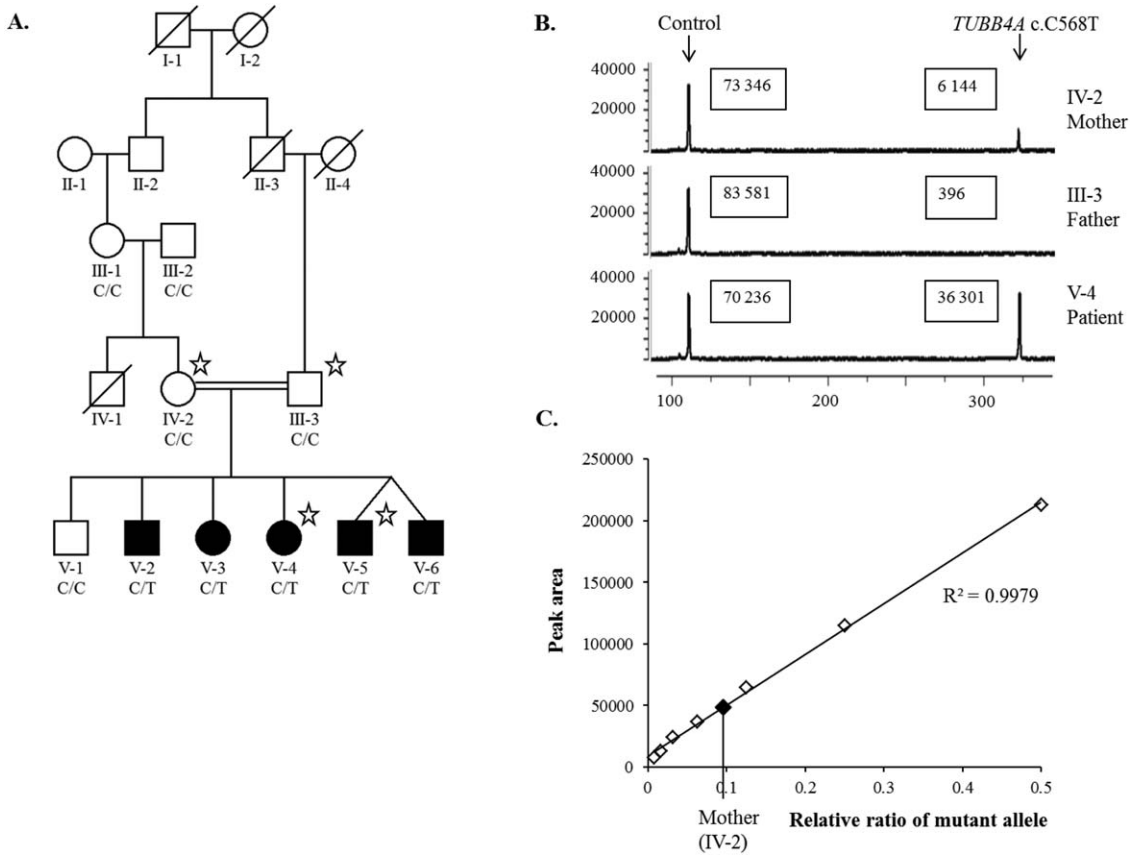

FIG. 1. Pedigree with the c.568C>T (p.H190Y) mutation in the *TUBB4A* gene. (**A**) Pedigree structure of the studied family. The individuals on whom WES was performed are indicated with a star; the genotype for the c.568C>T mutation is shown under the individual symbol. (**B**) Electropherograms of the allele-specific c.568C>T PCR-based assay, visualized by the MAQ-S software. The allele specific PCR product is 325 bp. A control PCR fragment (113 bp) is showing a relatively equal starting amount of genomic DNA for all of the reactions. The *x*-axis shows the size of the fragment in bp, and the *y*-axis shows the fluorescence intensity. In squares next to each peak are shown the values of the area under the respective peak. (**C**) Standard curve for the relative c.568C>T allele quantity**,** derived from serial dilutions of DNA from patient V-4, and a control, in which 50%, 25%, 12.5%, 6.25%, 3.13%, and 1.56% of the DNA are from the patient. For the calculations, the area under the peak was used. The mother, IV-2, carries 9.54% of mutant allele in her peripheral blood mononuclear cells' DNA. WES, whole exome sequencing; PCR, polymerase chain reaction.

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 presumed autosomal recessive mode of inheritance. We tested a de novo dominant 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[13]) and "probably damaging" (Polyphen2[14]). p.H190 is located in the H5-helix of TUBB4A[15] and is highly conserved (Supp. Figure S4, phyloP score = 5.1). Mapping the p.H190 position on the 3D structure of the αβ-tubulin 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[3] 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).[7,8] 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.[15] These interactions are thought to regulate microtubule dynamics and affect microtubule assembly.[16]

In the 3D structure of TUBB4A, four missense mutations map in the vicinity of p.H190: p.R156L[5], p.R262H[6,7,9], p.R282P[10], and p.E410K[4,7]. p.R262 is located close to the intra-dimer surface. Homologous mutations have been identified in TUBB3 (p.R262H)[16] and TUBB1A (p.R264H)[17]. Tischfield et al.[16] demonstrated that p.R262H results in impaired microtubule dynamics, resistance to destabilizing agents, and loss of kinesin on the microtubule plus-ends.[16] p.R282P is located in the M-loop, which extends from the opposite side of the protein and stabilizes the lateral contact with the adjacent subunit.[10] The M-loop, H5-, and H12-helices together form the tightest part of this interface.[15] The introduction of proline into the M-loop possibly destabilizes the lateral contacts.[10] 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.[16]

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

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

Mutations		p.H190Y This study	p.R262H Ferreira et al., 2014	p.R262H Miyatake et al., 2014	p.R262H Shimojima et al., 2014	p.E410K Blumkin et al., 2014	p.E410K Miyatake et al., 2014	p.R156L Purnell et al., 2014	p.R282P Pizzino et al., 2014
Number of patients		5 Siblings	1	1	1	1	2	1	2
Inheritance pattern		*Autosomal dominant*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*
Age at onset (range)		1-12 mo	12 mo	2 mo	3 mo	12 mo	12 mo	2 mo	2 y; 5 y
Age at last assessment		20-29 y	11 y	1 y	3 y	9 y	23 y; 41 y	4 y	55y; 50 y
Delayed motor milestones		+	+	+	+	+	+	+	-
Delayed language acquisition		-	+	+	+	+	+	+	-
Age at loss of ambulation		0-17 y	Never walked	ND	ND	-	12 y; 25 y	Never walked	-
Spasticity UL/LL		-/+	+[a]	+[a]	-	-/+	+	-/-	-/+
Weakness UL/LL		-/+	ND	ND	ND	-	ND	ND	+/ND
Ataxia		+	ND	ND	-	-	+	-	+
Dystonia		-	Spontaneous but	-	-	+	+	-	+
Choreoathetosis		-	nonpurposeful movements of the extremities	-	-	-	-	-	-
Rigidity		-	-	-	-	-	+	-	-
Tremor		-	-	ND	ND	+	+	-	1/2
Dysarthria		-	ND	ND	ND	+	+	-	+
Ocular and oculomotor abnormalities		Saccadic eye movements, strabismus	ND	Nystagmus	Nystagmus	Lack of smooth pursuit	ND	Nystagmus; strabismus	-
Cognitive impairment		-	+	+	+	+	+	+	+
Axonal polyneuropathy		2/2	-	ND	ND	ND	ND	ND	-
Course		Static—slowly progressive	Progressive	Progressive	ND	Progressive	Progressive	Static	Progressive
Brain MRI	Hypomyelination	Periventricular	+	+	+	+	+	+	+
	Basal ganglia atrophy	-	Globular appearance??	+	-	-	+/-	-	-
	Cerebellar atrophy	+	+	-	+	+	+	+	+

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

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. ■

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Supporting Data

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