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Screening study of *TUBB4A* in isolated dystonia

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Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Abstract

Mutations in *TUBB4A* have been identified to cause a wide phenotypic spectrum ranging from hereditary generalized dystonia with whispering dysphonia (DYT4) to the leukodystrophy hypomyelination syndrome with atrophy of the basal ganglia and cerebellum (H-ABC). To test for the contribution of *TUBB4A* mutations in different ethnicities (Spanish, Italian, Korean, Japanese), we screened 492 isolated dystonia cases for mutations in this gene and for the first time determined *TUBB4A* copy number variations in 336 dystonia patients. A potentially pathogenic rare 3bp-in-frame deletion was found in a patient with cervical dystonia but no copy number variations were detected in this study, suggesting that *TUBB4A* mutations exceedingly rarely contribute to the etiology of isolated dystonia.

Keywords

TUBB4A; Dystonia; Leukodystrophy; H-ABC

1. Introduction

Mutations in *TUBB4A* (chr19:6,494,319–6,502,584) have been identified to cause a wide spectrum of neurological diseases ranging from hereditary generalized dystonia with whispering dysphonia (DYT4) [1,2] to the more severe leukodystrophy hypomyelination syndrome with atrophy of the basal ganglia and cerebellum (H-ABC) [3]. To date, only one large Australian family has been described with dystonia unequivocally linked to the missense variant NM006087.3:c.4C > G in the *TUBB4A* gene [1,2]. However, at present, screening of dystonia patients has mainly been restricted to Caucasian samples and neither revealed the NM006087.3:c.4C > G DYT4-associated mutation, nor any novel *TUBB4A* variant [1,2,4,5]. To further elucidate whether *TUBB4A* mutations are limited to a single ethnicity (here Caucasian), as may be the case for rare variants [6], we additionally

genotyped samples of patients of Asian descent. Furthermore, we here also examined for the first time the contribution of gene dosage alterations in *TUBB4A* in a large dystonia sample.

2. Methods

For *TUBB4A* Sanger sequencing of all 4 exons and exon/intron boundaries (primer sequences used as published [2]), we included 37 Korean, 191 Japanese, 101 Italian and 163 Spanish patients with isolated dystonia, all of whom were diagnosed with focal, segmental or generalized dystonia (Table 1) and were negative for mutations in exon 5 of the *TOR1A* gene. For gene dosage analysis, we included 336 dystonia patients with an age at onset of ≤30 years and/or a positive family history, and patients diagnosed with spasmodic dysphonia. The multiethnic samples could not be included due to limited DNA amounts.

To detect copy number variations, TaqMan copy number assays (Hs02186957_cn, Hs00446221_cn, and Hs02319378_cn (Applied Biosystems)) for exons 2, 3 and 4 of the *TUBB4A* gene were used. Due to the short length of exon 1, a forward (5′–CCAGCCCCCTCCATCATC-3′), a reverse primer (5′-GGGCACGCGTCACG-3′) and a FAM- and NFQ-Quencher-labeled probe (5′–CCGGTCACCCTCCCGCTCC-3′) against the non-coding region of exon 1 were designed. TaqMan Copy Number Reference Assay (Applied Biosystems) targeting the telomerase reverse transcriptase (TERT) was used as reference.

3. Standard protocol approvals, registrations, and patient consents

The study was approved by the local ethics committee at the University of Lübeck (04–155). All participants gave written informed consent for participation in the research study.

4. Results

TUBB4A screening in isolated dystonia patients from different populations (Korean, Japanese, Italian, Spanish) (Table 1) revealed one rare, in-frame-deletion at position NM006087.3:c.1015_1017del (rs756023196) (NP_006078.2:p.S339del) in a male Italian patient with cervical dystonia and an age at onset of 21 years (Supplementary Fig. S1). Unfortunately, this patient was lost to follow-up and it was not possible to assess disease progression after his last examination aged 35 years. This variant was found in one allele among 66,714 alleles of European (Non-Finnish) samples (MAF 1.499e-05) in the Exome Aggregation Consortium Browser (ExAC at http://exac.broadinstitute.org/) and has a CADD PHRED score of 14.95 (CADD score calculation at http://cadd.gs.washington.edu).

TUBB4A exon copy numbers were unremarkable in all tested 336 dystonia patients.

5. Discussion

Considering the influence of ethnicity on mutation frequencies, we screened ~500 multiethnic patients with isolated dystonia and found one rare, potentially pathogenic in-frame deletion (NM006087.3:c.1015_1017delAGC) in an Italian patient with cervical dystonia and no mutations in our Asian patients. This finding is reminiscent of the situation

in DYT-Tor1A (DYT1) dystonia, where it has already been shown that in-frame deletions (NM_000113.2:c.907_909delGAG) can be a major cause of dystonia [7,8].

Unfortunately, the patient in our study was lost to follow-up for additional confirmation. *In-silico* analyses using the CADD PHRED score of 15 did not unequivocally confirm this variant as being pathogenic. Nevertheless, to finally evaluate pathogenicity of the *TUBB4A* variant, further *in-vitro* analyses are needed.

Although previous studies have highlighted the contribution of gene dosage changes to the mutational spectrum of several genes causative of movement disorders [9], no copy number variations were detected in the *TUBB4* gene in this study.

Our data as well as other studies suggest that mutations in *TUBB4A* exceedingly rarely contribute to the etiology of isolated dystonia [4,5].

Consequently, routine genetic testing of *TUBB4A* in individuals with isolated dystonia is not recommended but mutational analysis in patients with complex forms of dystonia may be warranted in order to further explore the role of this gene and of microtubule dysfunction in these disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2017.06.001.

Table 1

Clinical and demographic data of patients included in the study.

Population		All patients	Focal dystonia	Segmental dystonia	Generalized dystonia
Korean	**No. of patients**	37	25	4	8
	Female	31	21	4	6
	AAO	40.0 (±22.6)	49.5 (±16.5)	23.5 (±28.0)	18.5 (±18.9)
Japanese	**No. of patients**	191	127	37	27
	Female	89	54	21	14
	AAO	40.1 (±15.6)	39.7 (±14.3)	46.2 (±14.4)	35.7 (±19.4)
Italian	**No. of patients**	101	95	6	0
	Female	57	53	4	0
	AAO	46.8 (±15.8)	46.3 (±15.7)	53.5 (±17.4)	0
Spanish	**No. of patients**	163	162	0	1
	Female	122	121	0	1
	AAO	51.3 (±14.8)	51.3 (±14.9)	0	53.0

AAO – age at onset.