

DYT6 Dystonia: A Neuropathological Study

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Key Words

THAP1 · DYT6 · Neuropathology · Isolated dystonia

Abstract

Background: Mutations in the thanatos-associated protein domain containing apoptosis-associated protein 1 gene (*THAP1*) are responsible for adult-onset isolated dystonia (DYT6). However, no neuropathological studies of genetically proven DYT6 cases have been previously reported. **Objective:** We report the first detailed neuropathological investigation carried out on two DYT6 brains. **Methods:** Genetic screening for *THAP1* gene mutations using standard Sanger polymerase chain reaction sequencing identified 2 cases, 1 with a known pathogenic mutation and the other with a novel mutation. A detailed neuropathological assessment of the cases was performed. **Results:** Both DYT6 cases showed no significant neurodegeneration and no specific disease-related pathology. **Conclusions:** No neuropathological features that could be defined as hallmark features of DYT6 dystonia were identified. Our study supports the notion that in isolated dystonia, there is no significant neurodegeneration or morphological lesions that can be identified using routine methods.

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Introduction

The dystonias are a common but heterogeneous group of movement disorders. Mutations in the thanatos-associated protein domain containing apoptosis-associated protein 1 (*THAP1*) gene on chromosome 8p21-q22 have been identified as a cause of adult-onset isolated dystonia (DYT6). This condition has been recently classified as an isolated dystonia in which dystonia is the main clinical manifestation with the exception of tremor [1, 2]. DYT6 dystonia is an autosomal dominantly inherited condition with a heterogeneous phenotypic spectrum reported across genetically diverse populations and ethnicities, with both craniocervical and upper limb onset described [1, 3–5].

Pathophysiological studies of dystonia have concentrated specifically on the basal ganglia owing to the evidence from the combined dystonias, where dystonia occurs in conjunction with other neurological disorders. However, recent studies have also provided evidence of cerebellar abnormalities in isolated dystonias [6, 7]. In humans, *THAP1* is widely expressed in the brain and in a wide variety of extraneural tissues such as the liver, kidney, skeletal muscle, thyroid gland and prostate [8]. In a mouse model, the highest levels of expression are observed in cerebellar Purkinje cells, the dentate gyrus, hippocampal pyramidal cells and the cerebral cortex [8]. In

Table 1. Demographic and clinical details of DYT6 cases

Case	Sex	Ethnicity	Clinical diagnosis	DYT6 variant	Age at onset, years	Age at death, years	Cause of death
1	male	American Caucasian	dystonia, affected	p.R29Q	17	71	seizures
2	male	American Caucasian	dystonia, affected	p.S21F	N/A	59	N/A

N/A = Not available.

rats, the neurodevelopmental expression pattern of a *Thap1* transcript was higher in the brain than in the spinal cord at early stages but later declined to similar levels. In the cerebellum, however, it remained high throughout adulthood [9].

There have been few neuropathological descriptions of isolated dystonia cases. Based on routine neuropathological techniques, most studies did not identify specific abnormalities [10]. Perhaps the most interesting finding in isolated dystonia has been a neuropathological study of genetically confirmed DYT1 cases in which intraneuronal inclusions immunoreactive for ubiquitin, torsin A and lamin A/C were identified in brainstem nuclei, although this observation has never been replicated [11]. The aim of this study is to report a neuropathological study of 2 previously unreported genetically confirmed DYT6 cases. There have been no other neuropathological descriptions of cases with *THAP1* pathogenic mutations to our knowledge.

Materials and Methods

Cases

This project was approved by the Joint Local Research Ethics Committee of the National Hospital for Neurology and Neurosurgery and the UCL Institute of Neurology. Formalin-fixed, paraffin-embedded brain tissue was received from the Brain and Tissue Banks for Developmental Disorders (BTBDD), Baltimore, Md., USA (case 1) and formalin-fixed brain tissue of case 2 was provided by the Harvard Brain Bank. Clinical details were provided by the respective institutions. To our knowledge, neither of the cases had been previously reported. Where possible, the frontal, temporal and parietal cortices, hippocampus, striatum, globus pallidus, thalamus, subthalamic nucleus, cerebellum, midbrain, pons and medulla were investigated.

Genetics

DNA was extracted from frozen brain tissue (cerebellum) using a DNeasy Blood and Tissue Kit (Cat. No. 69504). Cases were sequenced for the sequence variants in the three exons of the *THAP1* gene (ENST00000254250) using standard Sanger sequencing, as previously described [4].

Immunohistochemistry

Paraffin-embedded tissue sections (7 μ m) were stained using hematoxylin and eosin (HE), Gallyas silver and Luxol fast blue/cresyl violet. Immunohistochemistry (IHC) was performed as previously described [11, 12]. Antibodies used included the following: ubiquitin (1:200; Dako, Ely, UK), tau (1:600, AT8; Autogen Bioclear, Calne, UK); GFAP (1:1,000; Dako), A β (1:200; Dako), α -internexin (1:75, 967654; Abcam), α -synuclein (1:50; Novacastra, Newcastle, UK), α -synuclein (1:1000; BD Transduction Biolabs, Oxford, UK), p62 (1:100; BD Bioscience, Oxford, UK), CD68 (1:150; Dako) and TDP-43 (1:2000; Proteintech, Manchester, UK).

Neuropathological Assessment

A detailed neuropathological study was carried out on both cases for the brain regions available. Semiquantitative assessment of neuronal loss and gliosis was performed using HE staining, GFAP and α -internexin IHC, respectively. The midbrain and pons were stained with antibodies to ubiquitin and p62 to assess the presence of intraneuronal inclusions in the periaqueductal gray matter, pedunculo-pontine nucleus, cuneiform nucleus and reticular formation where available [11]. The A β cortical plaques were assessed based on the criteria of the Consortium to Establish a Registry for Alzheimer's disease [13]. Other concomitant pathological conditions, including tau, cerebral amyloid angiopathy, TAR DNA-binding protein (TDP-43), α -synuclein pathology and small vessel disease, were assessed using published criteria [13–17].

Results

Clinical and Genetic Data

Demographic and clinical data are summarized in table 1.

Case 1 was a Caucasian male with an onset of dystonia at the age of 17 which became severe. Later in life he developed seizures and chronic obstructive pulmonary disease and was thought to have multi-infarct dementia. Genetic testing was negative for DYT1 dystonia. He died following two seizures at the age of 71. A single heterozygous mutation, p.R29Q, in the coding region of *THAP1* was found. This mutation has previously been described as pathogenic in DYT6 [5, 8].

Case 2 was a 59-year-old male with a clinical diagnosis of dystonia. No further clinical details were available. A

Table 2. Summary of neuropathological findings in DYT6 cases

Case	Macroscopic features	A β pathology		Tau pathology	Lewy body pathology	SVD	CAA	TDP-43 pathology
		D	M					
1	Mild-to-moderate cerebral atrophy Mild cortical and hippocampal atrophy Patchy acute ischemic lesions	+++	+	+	-	+	+	-
2	No abnormalities	+	-	-	-	-	++	-

Macroscopic features were derived from the neuropathology report issued by the BTDBB for case 1 and by direct observation for case 2. - = Absent; + = mild; ++ = moderate; +++ = severe; SVD = small vessel disease; CAA = cerebral amyloid angiopathy; D = diffuse; M = mature.

Table 3. Semiquantitative summary of neuropathological changes in DYT6 cases

Regions	Case 1								Case 2								
	neuronal loss	gliosis	A β		Lewy bodies	tau	CAA	TDP-43	neuronal loss	gliosis	A β		Lewy bodies	tau	CAA	TDP-43	
			D	M							D	M					
Cortex																	
Frontal	-	+	+++	+	-	-	+	N/P	-	-	NP	NP	N/P	N/P	N/P	N/P	N/P
Motor	N/A	N/A	N/A		N/A	N/A	N/A	N/A	-	-	N/P		N/P	N/P	N/P	N/P	N/P
Temporal	-	+	+	-	-	+	+	N/P	-	-	-	-	N/P	-	N/P	N/P	N/P
Parietal	N/A	N/A	N/A		N/A	N/A	N/A	N/A	-	-	+	-	-	-	++	N/P	N/P
Hippocampal formation	N/A	N/A	N/A		N/A	N/A	N/A	N/A	-	-	N/P		-	-	N/P	N/P	N/P
Caudate	-	-	N/P		N/P	-	N/P	-	-	-	N/P		N/P	-	N/P	-	-
Putamen	-	+	N/P		N/P	-	N/P	-	-	-	N/P		N/P	-	N/P	-	-
Globus pallidus	-	+	N/P		N/P	-	N/P	-	-	-	N/P		N/P	-	N/P	-	-
Thalamus	N/A	N/A	N/A		N/A	N/A	N/A	N/A	-	-	N/P		N/P	-	N/P	N/P	N/P
Subthalamic nucleus	N/A	N/A	N/A		N/A	N/A	N/A	N/A	-	-	N/P		-	-	N/P	N/P	N/P
Substantia nigra	N/A	N/A	N/A		N/A	N/A	N/A	N/A	+	-	-		-	-	N/P	N/P	N/P
Locus coeruleus	N/A	N/A	N/A		N/A	N/A	N/A	N/A	-	-	N/P		-	N/P	N/P	N/P	N/P
Pontine nuclei	N/A	N/A	N/A		N/A	N/A	N/A	N/A	-	-	N/P		-	N/P	N/P	N/P	N/P
Dorsal motor nucleus of vagus	-	++	N/P		-	-	N/P	N/P	N/A	N/A	N/A		N/A	N/A	N/A	N/A	N/A
Twelfth nerve nucleus	-	++	N/P		-	-	N/P	N/P	-	-	N/P		-	N/P	N/P	N/P	N/P
Inferior olive	-	++	N/P		-	-	N/P	N/P	-	-	N/P		-	N/P	N/P	N/P	N/P
Cerebellar Purkinje cells	+	+	N/P		N/P	-	N/P	N/P	+	+	N/P		N/P	-	N/P	N/P	N/P
Dentate nucleus	-	+	N/P		N/P	-	N/P	N/P	-	-	N/P		N/P	-	N/P	N/P	N/P

- = Absent; + = mild; ++ = moderate; +++ = severe; D = diffuse plaques; M = mature plaques; N/A = not available; N/P = not performed; CAA = cerebral amyloid angiopathy.

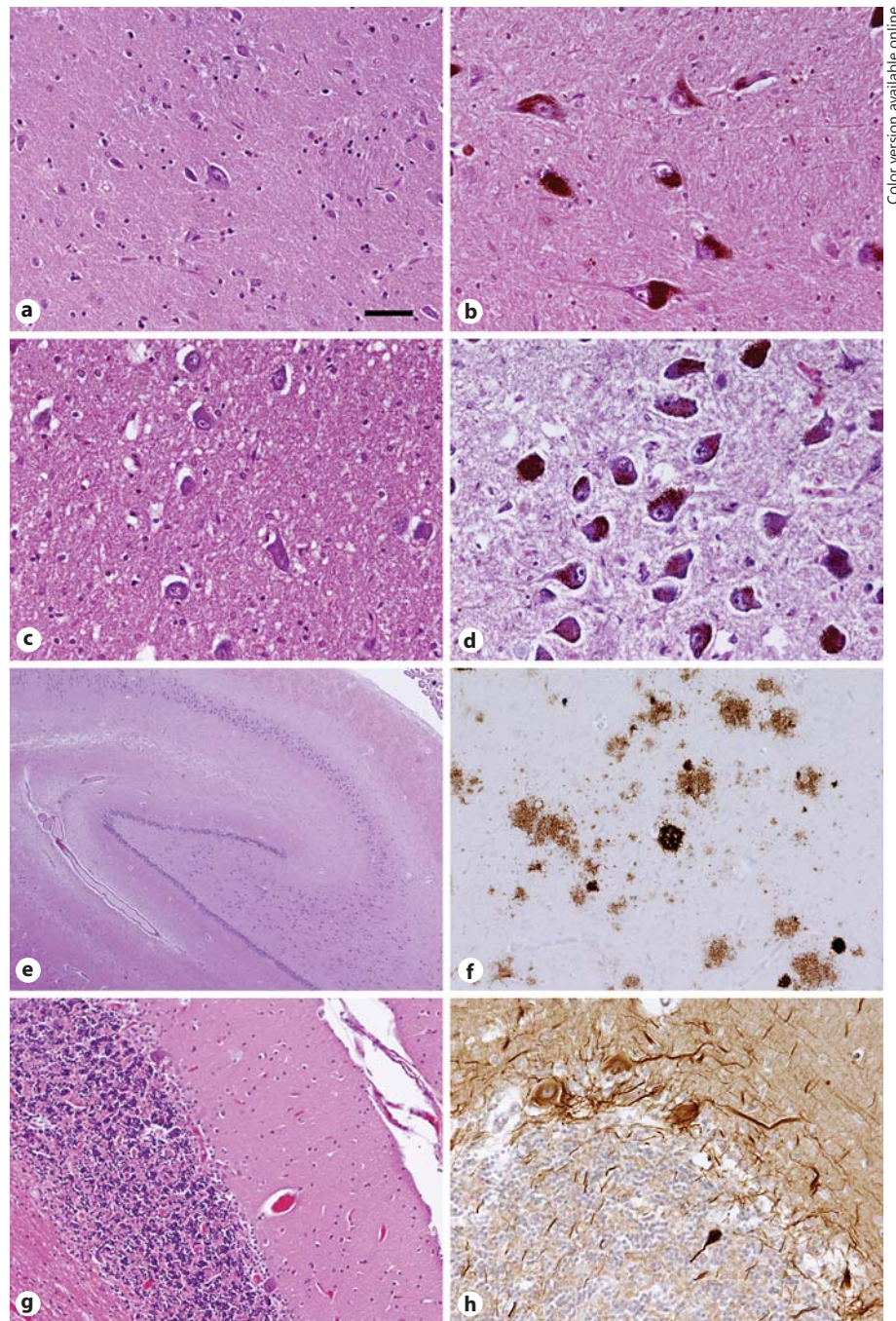
novel heterozygous variant, p. S21F, predicted to be disease causing (Mutation Taster score: 155, PolyPhen score: 0.981 and SIFT score: 0.01) was identified. The amino acids involved in each case are conserved across several species.

Neuropathological Assessment

A summary of the pathological findings is given in table 2 with details of the regional histological assessment provided in table 3.

Macroscopic Findings

Macroscopic findings were derived from the report provided by the BTDBB for case 1 and by direct observation of the brain slices provided for case 2. In case 1 mild-to-moderate generalized cerebral atrophy was described. The blood vessels of the circle of Willis showed moderate atherosclerosis with no vascular anomalies. The cortical ribbon was of slightly reduced thickness and the hippocampal formation was mildly atrophic. Patchy areas of acute ischemia were observed in the deep



Color version available online

Fig. 1. Histological examination of the putamen (**a**), subthalamic nucleus (**c**), locus coeruleus (**d**) and hippocampus (**e**) of case 2 showed no appreciable neuronal loss, although there was mild loss of pigmented neurons in the substantia nigra (**b**). In case 1, there were frequent cortical diffuse and sparse mature A β deposits (**f**, frontal cortex). There was mild Purkinje cell depletion in the cerebellum (**g**) with occasional axonal torpedoes (**h**). **a-e**, **g** HE stain. **f** A β IHC. **h** α -Internexin IHC. Scale bar in **a** represents 500 μ m in **e**, 100 μ m in **f** and **g** and 50 μ m in **a-d** and **h**.

gray matter structures. Case 2 showed no macroscopic abnormalities. The cortical ribbon, caudate, putamen, thalamus, subthalamic nucleus, globus pallidus, hippocampus, cerebellum, pons and medulla were all unremarkable. There were no focal lesions and no brain atrophy was observed.

Histology

The histological features are illustrated in figure 1. In case 1 the cortex, cerebellum, dentate nucleus, basal ganglia, substantia nigra and medulla were well preserved with no detectable neuronal loss, with the exception of mild Purkinje cell depletion with occasional axonal tor-

pedoes (fig. 1g, h). There was mild-to-moderate gliosis in several brain regions (table 3). A β deposits were most frequent in the frontal cortex where there were frequent diffuse and sparse mature plaques (fig. 1f). There was also mild cerebral amyloid angiopathy. Tau pathology of Alzheimer type (neurofibrillary tangles, neuropil threads and abnormal neurites) was mild in the temporal cortex but was not observed in subcortical regions. Formal analysis of the A β Thal phase and Braak and Braak tau staging was not possible due to the limited regions available for examination (brain regions available for study and observations in each case are provided in table 3). There was no α -synuclein pathology in the form of either Lewy bodies or glial cytoplasmic inclusions and no TDP-43 immunoreactive inclusions were found. There was mild small vessel disease but no further evidence of vascular lesions. We sought to identify pathological neuronal cytoplasmic inclusions in the midbrain and pons as described previously in DYT1 dystonia cases using ubiquitin and p62 IHC; however, such inclusions were not identified.

In case 2 no neuronal loss was observed except for mild loss of pigmented neurons in the substantia nigra (fig. 1b) and mild Purkinje cell depletion. Gliosis was restricted to the cerebellar cortex where it was of mild degree. Alzheimer pathology in the form of cortical A β deposition was observed in the parietal cortex where there were sparse diffuse deposits. There was moderate cerebral amyloid angiopathy in the parietal lobe. No tau immunoreactive inclusions were found in any region. Careful examination of the midbrain and pons showed no p62- or ubiquitin-labelled neuronal cytoplasmic inclusions. No α -synuclein or TDP-43 pathology was found and there was no evidence of small vessel disease.

Discussion

The molecular genetics of isolated dystonia syndromes have been the subject of extensive analysis and a number of genes associated with autosomal dominant inheritance have now been identified. Despite these genetic advances which shed light on the disease pathogenesis, there are few neuropathological descriptions of isolated dystonia in the literature and the neuropathology of DYT6 has not previously been described. In this study we undertook detailed neuropathological assessment of 2 DYT6 cases with confirmed *THAP1* mutations and could find no disease-specific morphological abnormalities.

Both cases had missense mutations in the THAP1 domain of *THAP1*, p. R29Q and a novel mutation p. S21F, respectively. The heterogeneity in the clinical spectrum of DYT6 dystonia is growing owing to the number of *THAP1* mutations reported in the literature since the gene discovery. This has made it difficult to establish a genotype-phenotype correlation in terms of clinical phenotypes reported for DYT6 cases. However, functional studies were more successful in demonstrating the mutation effect. Mutations in the THAP1 domain are thought to interrupt DNA binding, causing transcriptional dysregulation of THAP1 target genes [1]. A functional study in a THAP1 case with a frameshift mutation in the nuclear localizing signal region of *THAP1* demonstrated an impaired nuclear import of mutant THAP1 in vitro [18]; THAP1 is reported to interact with the promoters of *TOR1A*, mutations of which cause DYT1 dystonia [19], *TAF1*, implicated in DYT3 dystonia [20], and recently THAP1 has been reported to autoregulate its own expression [21]. Hence, studies of biochemical and cellular mechanisms may provide a link to abnormal motor control in DYT6 dystonia.

Our neuropathological investigation of these DYT6 cases utilized a similar IHC approach to that previously applied in DYT1-related dystonia cases [11, 12] and adult-onset dystonia cases [22] with the specific aim of determining whether brainstem neuronal cytoplasmic inclusions are a common hallmark of isolated dystonias [11]. We were unable to identify such inclusions in our study and, indeed, we found no neuropathological features that could be regarded as specific to DYT6 dystonia in either case. This study adds further evidence that morphological abnormalities detectable using current neuropathological techniques are not features of isolated dystonias. Further biochemical and molecular biology studies may provide an understanding of the pathogenesis of isolated dystonia.

Conclusion

This study provides the first ever detailed neuropathological study on two DYT6 brains. Our observations suggest that there were no detectable disease-specific morphological abnormalities in DYT6 dystonia using current neuropathological techniques. This study highlights the importance of cellular and biochemical studies in understanding the pathogenesis of isolated dystonia.

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