Mutations in *THAP1* (*DYT6*) and generalised dystonia with prominent spasmodic dysphonia: a genetic screening study

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Summary

Background *DYT6* is a primary, early-onset torsion dystonia; however, unlike in *DYT1* dystonia, the symptoms of *DYT6* dystonia frequently involve the craniocervical region. Recently, two mutations in *THAP1*, the gene that encodes THAP (thanatos-associated protein) domain-containing apoptosis-associated protein 1 (THAP1), have been identified as a cause of *DYT6* dystonia.

Methods We screened *THAP1* by sequence analysis and quantitative real-time polymerase chain reaction (PCR) in 160 white patients of European ancestry who had dystonia with an early age at onset (n=64), generalised dystonia (n=35), a positive family history of dystonia (n=56), or facial or laryngeal dystonia. Another 160 patients with dystonia were screened for reported and novel variants in *THAP1*. 280 neurologically healthy controls were screened for the newly identified and previously reported changes in *THAP1* and these and an additional 75 controls were screened for a rare non-coding mutation.

Findings We identified two mutations in *THAP1* (388_389delTC and 474delA), respectively, in two (1%) German patients from the 160 patients with dystonia. Both mutation carriers had laryngeal dystonia that started in childhood and both went on to develop generalised dystonia. Thus, two of three patients with early-onset generalised dystonia with orobulbar involvement had mutations in *THAP1*. One of the identified patients with *DYT6* dystonia had two family members with subtle motor signs who also carried the same mutation. A rare substitution in the 5′untranslated region (–236_235GA→TT) was found in 20 of 320 patients and in seven of 355 controls (p=0·0054).

Interpretation Although mutations in *THAP1* might have only a minor role in patients with different, but mainly focal, forms of dystonia, they do seem to be associated with early-onset generalised dystonia with spasmodic dysphonia. This combination of symptoms might be a characteristic feature of *DYT6* dystonia and could be useful in the differential diagnosis of *DYT1*, *DYT4*, *DYT12*, and *DYT17* dystonia. In addition to the identified mutations, a rare non-coding substitution in *THAP1* might increase the risk of dystonia.

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Introduction

The dystonias are a group of movement disorders that are characterised clinically by involuntary twisting, repetitive movements, and abnormal postures.[1] Mutations in *THAP1*, the gene that encodes THAP1 (thanatos-associated protein [THAP] domain-containing apoptosis-associated protein 1), underlie *DYT6* dystonia.[2] *DYT6* dystonia is inherited in an autosomal dominant manner with reduced penetrance and was first described in Amish–Mennonite families. *DYT6* dystonia is characterised clinically by an early age of onset, with symptoms that frequently start in craniocervical muscles and tend to spread to adjacent body regions;[3] laryngeal dystonia that causes speech difficulties is particularly common.

On the basis of the original linkage[4] but within a narrower candidate region,[3] a heterozygous frameshift mutation (134_135insGGGTT; 137_139delAAC) in *THAP1* has been identified in four families with Amish–Mennonite origins.[2] This mutation is inherited from a common founder. In addition, a missense mutation (241T→C [Phe81Leu]) has been found in a German family.[2] Both mutations are located in exon 2 of *THAP1*.

THAP1 belongs to a family of sequence-specific, DNA-binding, cellular factors that function as nuclear pro-apoptotic proteins[5] and regulate proliferation in endothelial cells.[6] In addition to the thanatos-associated protein domain at the N terminus, which binds DNA, THAP1 also contains a proline-rich region and a nuclear-localisation signal (NLS) at the C-terminal end (figure).[5,7]

To clarify the frequency and spectrum of mutations in *THAP1* and the associated phenotypic spectrum in patients with dystonia, we analysed the *THAP1* coding region with qualitative and quantitative screening methods in a large group of neurologically well-characterised patients with various forms of dystonia.

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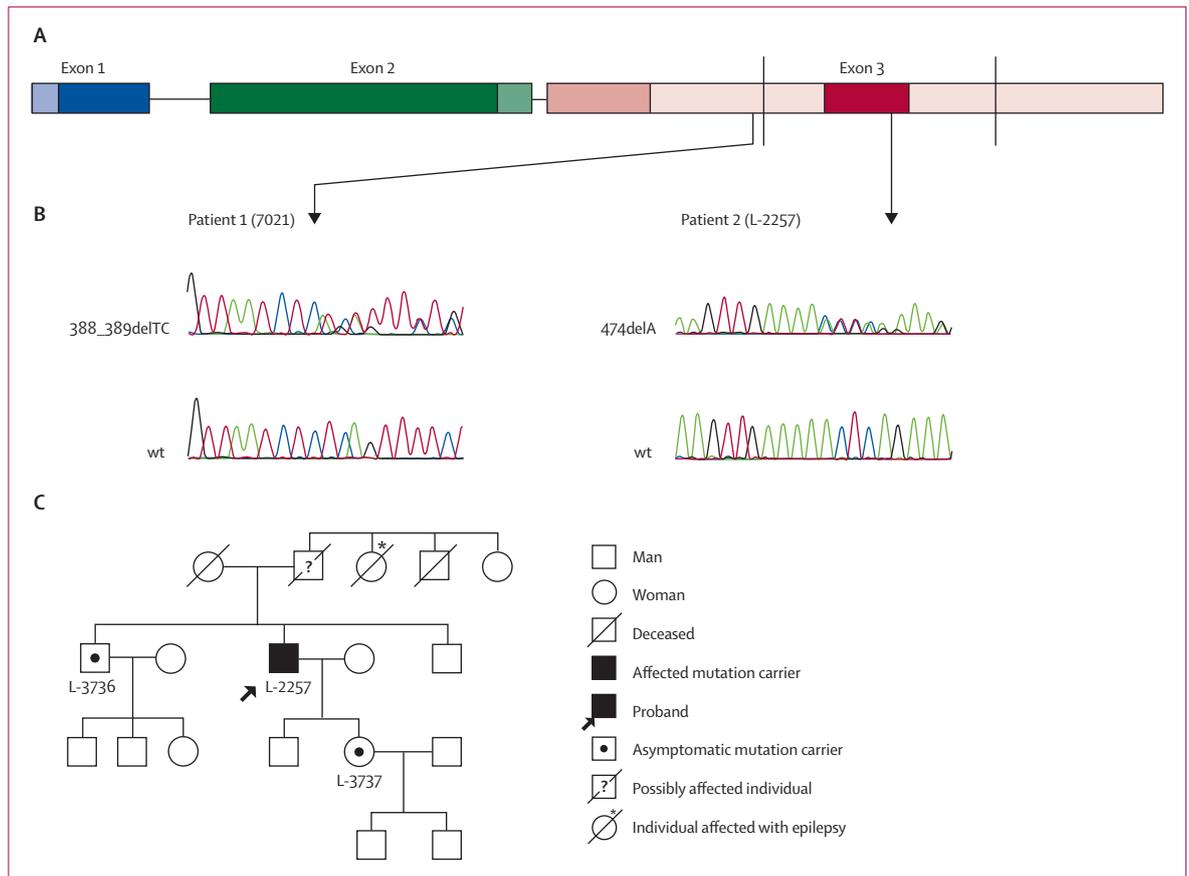23538 Lübeck, Germany
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Figure: Two mutations in *THAP1*

(A) Schematic representation of the coding region of *THAP1* and the position of the mutations in the exon 3 identified in this study. Vertical bars show the positions of the predicted novel, premature, stop codons. Dark blue and dark green=THAP domain-encoding region. Pink=proline-rich encoding region. Dark red=nuclear localisation sequence encoding region. (B) The exact position and electropherograms of the deletions identified in our patients; the corresponding wild-type (wt) sequences are shown below. (C) Pedigree of patient 2: family members included in the clinical and molecular genetic studies are denoted by their code number. The proband is the index patient (L-2257) who carries the 474delA mutation. THAP=thanatos-associated protein.

Methods

Participants

This study was approved by the local ethics committee of the University of Lübeck. After giving informed consent, all patients underwent a standardised neurological examination by a specialist in movement disorders (SAS, JH, NB, SZ, AS, H-CJ, RW, VSK, HS, EA, AM, and CK). Unrelated patients with dystonia and controls were recruited in northern Germany. The diagnosis of dystonia was based on the accepted clinical criteria, and disease severity was rated with the Burke–Fahn–Marsden (BFM) dystonia rating scale.[8] Secondary causes, such as structural lesions or exposure to toxic or neuroleptic substances, were excluded by neuroimaging and patient history, respectively. Patients with the GAG deletion in *DYT1* were also excluded. Patients were analysed in two groups (A and B). Table 1 shows the demographic and clinical data for the two patient groups.

The inclusion criteria for patients in group A (n=160) were: early age of onset (<26 years), and/or generalised dystonia, and/or a positive family history of dystonia.

Furthermore, group A also included patients with adult-onset sporadic focal or segmental dystonia with facial or laryngeal involvement (eg, musician's dystonia of the embouchure type, segmental dystonia with writer's cramp, or laryngeal dystonia) and the index patient of the Australian *DYT4* dystonia/whispering dysphonia family.[9] Group B (n=160) comprised patients who had sporadic dystonia but did not fulfil any of the criteria for inclusion in group A.

When we found that of our initially screened patients (group A) only those with early-onset generalised dystonia but none with late-onset with focal dystonia had mutations in *THAP1*, we decided to screen a further subsection of patients (late-onset sporadic [group B]) for the previously identified and newly found changes, to elaborate on the role of these genetic changes; therefore, group B was only tested for the two previously reported mutations (134_ 135insGGGTT; 137_139delAAC and 241T→C, both located in exon 2) and the newly identified changes seen in group A (388_389delTC and 474delA, both located in exon 3; and 236_235GA→TT in the 5′ untranslated region [UTR]).

	Group A (n=160)*†					Group B (n=160)‡				
	Generalised dystonia	Focal and segmental dystonias, including those with face or laryngeal involvement				Segmental dystonia	Focal dystonia			
		Cervical dystonia	Writer's cramp	Musician's dystonia (embouchure type)	Other segmental dystonias¶		Cervical dystonia	Musician's dystonia (hand)	Writer's cramp	Blepharospasm
Number with different diagnosis	35§	29	11	43	42	18	39	51	23	29
Sex										
Men	16	13	5	31	21	6	13	34	12	9
Women	19	16	6	12	21	12	26	17	11	20
Mean age at onset (years)	12·2 (9·5)	28·1 (6·8)	20·7 (9·6)	31·2 (8·0)	28·6 (16·2)	56·9 (6·5)	46·7 (10·0)	35·2 (11·6)	42·4 (10·2)	52·9 (8·7)
Ranges of age at onset (years)	1–33	10–37	3–34	15–53	3–54	46–70	30–76	20–66	29–68	30–66
Mean age at examination (years)	34 (14·8)	40·9 (11·07)	33·6 (6·8)	45·9 (10·5)	45·5 (15·3)	62·3 (8·2)	54·2 (10·1)	45·7 (13·1)	51·9 (10·3)	63·5 (8·7)
Mean duration of disease (years)	21·4 (19·9)‖	12·9 (12·7)	12·9 (10·7)	13·6 (10·8)‖	17·5 (11·3)	7·8 (9·6)	7·3 (6·7)	10·6 (10·1)	10·5 (0·5)	9·7 (8·4)
Family history										
Positive	11	9	3	27	6	0	0	0	0	0
Negative	10	19	6	13	23	15	32	51	13	23
Unknown	14	1	2	3	13	3	7	0	10	6

Data are number, mean (SD), or range. *64 patients had onset before age 26 years and 96 had onset after. †Comprehensive screening: sequence analysis and quantitative real-time PCR of the three exons. ‡Tested for: –236_235GA→TT in 5′ UTR (in exon 1); 134_135insGGGTT; 137_139delAAC and 241T→C (both in exon 2); and 388_389delTC and 474delA (both in exon 3). Group B was tested for these variants only, which is a limitation of our study. §Of 35 patients with generalised dystonia, 24 had onset before age 26 years and three patients had orobulbar involvement. ¶Of 42 patients with segmental dystonia, nine had orobulbar involvement. ‖Because data of the exact onset age were missing for a small number of patients, there are minor differences in the calculations. PCR=polymerase chain reaction.

Table 1: Demographic and clinical data of the two patient groups

280 neurologically healthy, white, sex-matched controls of European ancestry were screened for all the known mutations in *THAP1*. Furthermore, we tested a further 75 neurologically healthy German controls for the variation in the 5′ UTR of exon 1, to further investigate the role of this substitution.

Patients who had identified mutations were re-examined and their family members were contacted, examined, and blood samples collected whenever possible.

Procedures

Using previously published primer sequences,[2] we sequenced the three exons of *THAP1* and the exon–intron boundaries, including the entire 5′ UTR (group A), to detect changes in the sequence. To assess exon rearrangement (ie, deletions or multiplications of one or more exons), we used a quantitative duplex polymerase chain reaction (qPCR) assay with hybridisation probes (LightCycler 480, Roche Diagnostics, Mannheim, Germany). We individually co-amplified each of the three exons of *THAP1* and compared them with β globin as an internal standard. A relative ratio (concentration of each *THAP1* exon:β globin) between 0·8 and 1·2 was deemed a normal ratio; a ratio between 0·4 and 0·6 would be expected for a heterozygous deletion; a ratio between 1·3 and 1·7 indicates heterozygous duplications. See webappendix for the sequences of the primers and probes.

To assess the expression of both parental alleles at the cDNA level, we did reverse transcription PCR (RT-PCR) on samples of RNA from blood samples from our two mutation carriers and the available family members of patient 2. Amplified cDNAs from exon 2 and exon 3 were sequenced in both directions.

Statistical analysis

Comparisons were done with Fisher's exact test.

Role of the funding source

The sponsors had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

In the 160 patients with dystonia who were screened for mutations in *THAP1* (group A), we found three novel heterozygous changes: two in the coding region (388_389delTC [Val131fs133X] and 474delA [159fs180X]) and one in the 5′ UTR (–236_235GA→TT). We did not detect any exon rearrangement. The deletions in exon 3 were each found in only one patient and in none of the controls, which accounts for a mutation frequency of 1% in the tested and clinically preselected patients. The patients who had mutations in *THAP1* were two of

See **Online** for webappendix

	Patient 1 (7021)	Patient 2 (L-2257)	Daughter of patient 2 (L-3737)	Brother of patient 2 (L-3736)
Mutation	388_389delTC	474delA	474delA	474delA
Sex	Man	Man	Woman	Man
Age (years)	35 years	68 years	40 years	70 years
Age at onset (years)	9 years	10 years	NA	NA
Symptoms at onset	Speech problems followed by writer's dystonia	Writer's dystonia, followed by laryngeal dystonia	Asymptomatic	Asymptomatic
Symptoms on examination				
Dystonia of the face	Mild	Absent	Absent	Absent
Tongue dystonia	Mild	Absent	Absent	Absent
Dysarthria	Severe	Absent	Absent	Absent
Laryngeal dystonia	Severe	Moderate	Absent	Absent
Neck dystonia	Moderate	Moderate	Mild	Mild
Arm dystonia	Moderate	Moderate	Mild	Mild
Leg dystonia	Mild	Mild	Absent	Absent
Truncal dystonia	Mild	Absent	Absent	Absent
BFM score (points)	71	38	3	3
Investigations				
Routine blood tests*	Normal	Normal	..	..
Routine CSF analysis	..	Normal	..	..
Neuroimaging	Normal	Normal	..	..
EEG	Normal	..	..	..

Other symptoms were absent, including bradykinesia, pyramidal signs, cerebellar signs, eye movement abnormalities, cognitive features (eg, dementia and psychiatric features), sensory signs, autonomic features (eg, urinary or faecal incontinence). See webmaterial for detailed descriptions. *To exclude Wilson's disease, neuroacanthocytosis, and metabolic diseases. BFM=Burke–Fahn–Marsden dystonia rating scale. NA=not available. ..=not tested.

Table 2: Clinical characteristics of mutation carriers

35 with generalised dystonia, two of 24 with early-onset generalised dystonia, and two of three with early-onset generalised dystonia with orobulbar involvement.

In the two affected mutation carriers and in the brother and daughter of patient 2, the mutant and wild-type alleles were expressed and mRNA from both alleles was present in their blood. Table 2 summarises the clinical characteristics, and a detailed report can be found in the online supplementary material (webappendix). Segregation analysis was not possible in either family because no other affected family members were available for testing. However, the asymptomatic but clinically mildly affected daughter and brother of patient 2 both carried the mutation (figure).

The −236_235GA→TT double substitution affects the 235th and 236th base pairs upstream of the ATG translation start codon of *THAP1* and is predicted to alter the first two nucleotides in *THAP1* mRNA. 20 of 320 patients (6%) were heterozygous for this substitution (8 patients from group A and 12 patients from group B) as were seven of 355 controls (2%; Fisher's exact test p=0·0054). One of the patients inherited the −236_235GA→TT substitution from his asymptomatic mother, who was homozygous for the double substitution. None of the previously reported mutations was detected in our sample.

Discussion

We screened 320 patients with dystonia for mutations in *THAP1*, which underlie *DYT6* dystonia, and found two novel mutations in two patients (1%) and in two asymptomatic family members, who, on examination, were found to have mild dystonia. Furthermore, after excluding the mutations in the index case of a family with *DYT4* dystonia, we provide evidence that *DYT4* dystonia/whispering dysphonia is caused by mutations in a gene other than *THAP1*.

We have also extended the mutational spectrum in *THAP1*; the previously described mutations were located in exon 2 of *THAP1*,[2] but we identified two novel frameshift mutations, which are both located in exon 3 (a two-nucleotide deletion [388_389delTC] and a one-nucleotide deletion [474delA]). Both mutations are predicted to cause a shift in the reading frame and thus lead to premature truncation in the C-terminal third of the protein (figure).

The 388_389delTC deletion is in a region of three TC repeats located before the region that encodes the nuclear localisation domain; thus, this mutant protein is predicted to lack the complete signal sequence required to enter the nucleus and bind to DNA. The 474delA deletion is also located in the region that encodes the nuclear localisation sequence and, to some extent, this mutation could also impair transport into the nucleus. Similar to the other deletion, 474delA occurs within a repeat of five adenosine nucleotides. 474delA was found in our index patient and also in his brother and daughter who, although asymptomatic, both showed subtle signs of dystonia.

Interestingly, the phenotype of patient 1 was substantially more severe than that of patient 2 (BFM score 71 points *vs* 38 points). This might be due to the effects of their respective mutations: THAP1 in patient 1 might lack a nuclear-localisation domain, whereas only the last three amino acids of this domain might be changed in patient 2. The mutated protein in patient 2 might retain a partial ability to be transported into the nucleus and bind to DNA, which possibly accounts for the milder phenotype seen in this patient.

The pathological significance of the −236_235GA→TT substitution is not clear. 20 of 320 patients (mean age at onset 38·5 (SD 18·6) years; range 4–76 years) and seven of 355 controls were heterozygous for this variant. This finding was significant (Fisher's exact test p=0·0054). The presence of this substitution in unaffected controls has several possible explanations: the −236_235GA→TT substitution might be a benign polymorphism and its higher frequency in our patient groups compared with the controls is a chance finding; it could be associated with *DYT6* through linkage disequilibrium with some other, as yet unidentified, genetic factor that causes dystonia; it could be a susceptibility factor; or it could present as a disease-causing mutation, per se, with reduced penetrance. The substitution is located outside of the coding region but its effects on the first

two nucleotides of the mRNA might indicate that it is either involved in the regulation of transcription or translation efficiency or that it affects the stability of *THAP1* mRNA. The mother of one of the patients who was screened was homozygous for this substitution but was asymptomatic. Consanguinity was excluded by history, and no other family members were affected.

We identified two men with *DYT6* dystonia, both of whom had onset in childhood: one had dysphonia and one had writing dystonia, followed by laryngeal involvement within 1 year. The course of their diseases was slowly progressive. Patient 1 developed marked jaw-opening dystonia, which is commonly prominent in pantothenate kinase-associated neurodegeneration (PKAN), neuroferritionopathy, neuroacanthocytosis, and rapid-onset dystonia (*DYT12*).[10] Our report adds *DYT6* dystonia to the list of differential diagnoses to consider when a patient presents with profound jaw dystonia.

Psychiatric features, which are not uncommon in other forms of dystonia,[11,12] were absent in our patients with *DYT6* dystonia. Cognitive and autonomic functions were also unaffected.

Owing to the increasing number of genetically determined dystonia syndromes, clinicians might be puzzled as to how to proceed when it comes to genetic testing. However, patterns of age at onset and the distribution and presence of certain clinical features might point towards certain dystonia-causing genes. Unlike some other movement disorders (eg, spino-cerebellar ataxias or Parkinson's disease), in which the various genetic forms are often clinically indistinguishable from each other, dystonias that are associated with different *DYT* loci commonly have distinctive features. For example, *DYT1* dystonia typically begins in the lower limbs and only rarely involves cranial or bulbar muscles. *DYT6* dystonia frequently starts in the craniocervical region (15 of 29 previously described patients), and speech, due to involvement of the larynx, tongue, or jaw, was affected in two-thirds of patients (19 of 29).[2] Laryngeal involvement in particular might be suggestive of *DYT6* dystonia; our two patients also had profound speech involvement. Among the subgroup of 24 patients who had generalised dystonia with onset at younger than 26 years, three had orobulbar involvement (two of whom were positive for mutations in *THAP1*). The frequency of mutations was 8% (2 of 24). However, the true estimate of *DYT6* as the cause of primary dystonias needs further investigation.

Of the 20 patients who had the −236_235GA→TT substitution, eight had cervical dystonia, seven had musician's dystonia (four had embouchure dystonia and three had hand dystonia), and five had segmental dystonia (one had spasmodic dysphonia). Thus, two-thirds (13 of 20) of the affected carriers had dystonia with craniocervical involvement.

Other dystonia syndromes with prominent laryngeal involvement include whispering dystonia (*DYT4*),[9]

DYT17,[13] dystonia linked to chromosome 20, and *DYT12*.[14] *DYT4* dystonia was first reported in 1985 by Parker[9] in a large Australian kindred with hereditary whispering dysphonia (which is always the presenting sign) with an autosomal dominant mode of inheritance. The genetic cause has not yet been identified.[15] The phenotype of *DYT4* dystonia overlaps with that of *DYT6* dystonia, and it might be speculated that *DYT4* and *DYT6* dystonias are caused by the same gene; however, we were able to test the original *DYT4* index patient and exclude mutations in *THAP1*, which implies that *DYT4* and *DYT6* dystonias are genetically distinct. *DYT17* dystonia has recently been described in a consanguineous Lebanese family with three sisters who were affected by torticollis at ages 17, 19, and 14 years, respectively. Within a few years, their symptoms had spread to segmental and generalised dystonia, with severe dysphonia and dysarthria in all three.[13] However, in contrast with *DYT6* dystonia, the mode of inheritance of *DYT17* dystonia is autosomal recessive. Finally, *DYT12* dystonia is a rapid-onset dystonia–parkinsonism. Although *DYT6* and *DYT12* dystonias have some overlapping features (eg, autosomal-dominant inheritance and laryngeal involvement), other characteristics of *DYT12* dystonia were absent in our patients (eg, parkinsonism and rapid-onset), which argues against a clinical diagnosis of *DYT12* dystonia.

Both our patients with mutations belonged to group A and had early symptom onset and generalised dystonia with orobulbar involvement; however, neither had a clear family history of dystonia: there was a possible family history of Parkinson-like tremulous disorder in one and an unspecified muscular disorder in the other. Thus, patients with early-onset dystonia and speech involvement, with or without a family history, might be good candidates for *DYT6* genetic screening.

The reported penetrance of *DYT6* dystonia is about 60%.[3] Our findings are in keeping with this estimate. We identified two mutation carriers who were asymptomatic but showed mild clinical signs on careful neurological examination. Why some mutation carriers develop severe dystonia whereas others might only show mild or no signs is not clear. Similarly, a reduced penetrance has also been documented for other forms of dystonia, including *DYT1* dystonia and myoclonus dystonia (*DYT11*). In *DYT1* dystonia, the polymorphism Asp216His, which is encoded on the allele in trans with the GAG deletion, could be identified as a factor that modifies genetic penetrance.[16] In *DYT11* dystonia associated with mutations in *SGCE* (which encodes sarcoglycan), reduced penetrance can be explained by genetic imprinting.[17] However, the factors that affect the reduced penetrance of *DYT6* dystonia remain to be identified.

In summary, we have described two novel mutations in *THAP1* in two of three patients with early-onset, general-ised dystonia with orobulbar involvement and symptoms

that started in the upper body. No mutation was found among the other patients tested, which implies that mutations in *THAP1* are a rare finding in patients with various but mainly focal forms of dystonia. Therefore, laryngeal dystonia in patients with young-onset generalised dystonia might be a characteristic feature of *DYT6* dystonia.

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