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


Summary

Background DYT6 is a primary, early-onset torsion dystonia; however, unlike in DYT1 dystonia, the symptoms of DYT6 dystonia frequently involve the cranio-cervical 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’exon 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. Mutations in THAP1, the gene that encodes THAP (thanatos-associated protein [THAP] domain-containing apoptosis-associated protein 1), underlie DYT6 dystonia. 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 cranio-cervical muscles and tend to spread to adjacent body regions; laryngeal dystonia that causes speech difficulties is particularly common.

On the basis of the original linkage but within a narrower candidate region, a heterozygous frameshift mutation (134_135insGGTT; 137_139delAAC) in THAP1 has been identified in four families with Amish–Mennonite origins. This mutation is inherited from a common founder. In addition, a missense mutation (24T→C [Phe81Leu]) has been found in a German family. 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 and regulate proliferation in endothelial cells. 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). 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.
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. 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. 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]).

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.
280 neurologically healthy, white, sex-matched controls of European ancestry were screened for all the known mutations in \textit{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,\textsuperscript{2} we sequenced the three exons of \textit{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 \textit{THAP1} and compared them with β globin as an internal standard. A relative ratio (concentration of each \textit{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 \textit{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 \textit{THAP1} were two of
Clinical characteristics of mutation carriers

<table>
<thead>
<tr>
<th>Patient 1 (7021)</th>
<th>Patient 2 (L-2257)</th>
<th>Daughter of patient 2 (L-3737)</th>
<th>Brother of patient 2 (L-3736)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mutation</strong></td>
<td>388_389delTC</td>
<td>474delA</td>
<td>474delA</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>Man</td>
<td>Man</td>
<td>Woman</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>35</td>
<td>68</td>
<td>40</td>
</tr>
<tr>
<td><strong>Age at onset (years)</strong></td>
<td>9</td>
<td>10</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Symptoms at onset</strong></td>
<td>Speech problems followed by writer’s dystonia</td>
<td>Writer’s dystonia, followed by laryngeal dystonia</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td><strong>Symptoms on examination</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dystonia of the face</td>
<td>Mild</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Tongue dystonia</td>
<td>Mild</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Dysarthria</td>
<td>Severe</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Laryngeal dystonia</td>
<td>Severe</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Neck dystonia</td>
<td>Moderate</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Arm dystonia</td>
<td>Moderate</td>
<td>Mild</td>
<td>Mild</td>
</tr>
<tr>
<td>Leg dystonia</td>
<td>Moderate</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Truncal dystonia</td>
<td>Mild</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>BFM score (points)</td>
<td>71</td>
<td>38</td>
<td>3</td>
</tr>
</tbody>
</table>

Investigations

- Routine blood tests*: Normal
- Routine CSF analysis: Normal
- Neuroimaging: 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 other 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, 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 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). 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, 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 craniofacial 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). 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 craniofacial involvement.

Other dystonia syndromes with prominent laryngeal involvement include whispering dystonia (DYT4), DYT17, dystonia linked to chromosome 20, and DYT12. DYT4 dystonia was first reported in 1985 by Parker 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. 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 spread from ocular to segmental and generalised dystonia, with severe dysphonia and dysarthria in all three. 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%. 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. In DYT11 dystonia associated with mutations in SGCE (which encodes sarcoglycan), reduced penetrance can be explained by genetic imprinting. 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, generalised 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 \textit{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 \textit{DYT6} dystonia.

\textbf{Contributors}

AD, SAS, KL, NB, HJ, RW, VSK, HS, EA, AM, LJO, and CK had the idea for the study. AD, KL, NB, SZ, AS, HJ, RW, VSK, AM, LJO, and CK organized the study. AD, SAS, KL, SW, HP, JH, NB, SZ, TF, AR, AS, HS, EA, AM, LJO, CK conducted the study, including sample collection, molecular analysis, interpretation of the data, and arranging patient revisits. AD designed the research project and did the statistical analysis. AD, SAS, and KL wrote and JH, SZ, TF, HJ, RW, VSK, HS, EA, AM, LJO, and CK reviewed the manuscript.

\textbf{Conflicts of interest}

We have no conflicts of interest.

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\textbf{References}