THAP1 mutations (DYT6) are an additional cause of early-onset dystonia

H. Houlden, MD, PhD
S.A. Schneider, MD, PhD
R. Paudel, MSc
A. Melchers, MD
P. Schwingenschuh, MD
M. Edwards, MD, PhD
J. Hardy, PhD
K.P. Bhatia, MD

Address correspondence and reprint requests to Dr. Henry Houlden, University College London Institute of Neurology, Queen Square, London WC1N 3BG, England
h.houlden@ion.ucl.ac.uk
or Prof. Bhatia at
k.bhatia@ion.ucl.ac.uk

ABSTRACT

Background: The clinical phenotype of DYT6 consists mainly of primary craniocervical dystonia. Recently, the *THAP1* gene was identified as the cause of DYT6, where a total of 13 mutations have been identified in Amish-Mennonite and European families.

Methods: We sequenced the *THAP1* gene in a series of 362 British, genetically undetermined, primary dystonia patients (78 with focal, 186 with segmental, and 98 with generalized dystonia) and in 28 dystonia-manifesting DYT1 patients and 176 normal control individuals.

Results: Nine coding mutations were identified in the *THAP1* gene. Two were small deletions, 2 were nonsense, and 5 were missense. Eight mutations were heterozygous, and 1 was homozygous. The main clinical presentation of cases with *THAP1* mutations was early-onset (<30 years) dystonia in the craniocervical region or the limbs (8 of 9 patients). There was phenotypic variability with laryngeal or oromandibular dystonia present in 3 cases. Four of 9 *THAP1* cases developed generalized dystonia.

Conclusions: The number of *THAP1* mutations has been significantly expanded, indicating an uncommon but important cause of dystonia. Coding mutations account for 9 of 362 dystonia cases, indicating a mutation frequency of 2.5% of dystonia cases in the population that we have screened. The majority of cases reported here with *THAP1* mutations had craniocervical- or limb-onset segmental dystonia, but we also identified 1 homozygous *THAP1* mutation, associated initially with writer's dystonia and then developing segmental dystonia. Three of our patients had a nonsense or frameshift *THAP1* mutation and the clinical features of laryngeal or oromandibular dystonia. These data suggest that early-onset dystonia that includes the involvement of the larynx or face is frequently associated with *THAP1* mutations. **Neurology® 2010;74:846–850**

GLOSSARY

mRNA = messenger RNA; **SNP** = single nucleotide polymorphism; ***THAP1*** = Thanatos-associated–domain containing, apoptosis-associated protein 1.

Dystonia is defined by the presence of sustained involuntary muscle contractions, often leading to abnormal postures and movements.[1-3] The underlying cause of dystonia may be acquired, developmental, or genetic.[4] To date, 16 genetic loci have been associated with dystonia, most of which are inherited as an autosomal dominant trait with reduced penetrance. The DYT1, DYT6, DYT7, and DYT13 genes are associated with primary or pure dystonia.[5,6]

DYT6 is a dominantly inherited dystonia that causes an early-onset primary torsion dystonia with a sex independent penetrance of 60%.[7] Originally defined in 2 Amish-Mennonite families, DYT6 is equally likely to start in the craniocervical region as in the legs, and in the majority of cases spreads to involve both body parts. The age at onset in DYT6 is later than in DYT1 families (18.9 vs 13.6 years).[8] Recently, the DYT6 gene was identified as the Thanatos-associated–domain containing, apoptosis-associated protein 1 (*THAP1*) gene.[9] In total, 12 mutations have so far been identified in American Amish-Mennonite and European families.[9-11] The *THAP1* gene is part of a family of THAP proteins that bind specific DNA

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From the Department of Molecular Neuroscience (H.H., R.P., A.M., J.H.), Sobell Department of Motor Neuroscience and Movement Disorders (S.A.S., P.S., M.E., K.P.B.), University College London Institute of Neurology, England; and Schilling Department of Clinical and Molecular Neurogenetics at the Department of Neurology (S.A.S.), University of Luebeck, Germany.

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Table 1 *THAP1* mutations and intronic variants identified in dystonia cases[a]

Family	Family history	Ethnic origin	Position	Mutation nucleotide	Mutation type	Amino acid change
F	No	English	Exon 1	c.7C>T	Heterozygous	Q3X
E	Yes	German	Exon 1	c.17C>T	Heterozygous	S6F
S	Yes	French	Exon 1	c.23A>G	Heterozygous	Y8C
M	Yes	English	1. Exon 1	1. c.77C>G	1. Heterozygous	1. P26R
			2. Intron 1	2. c.72-4T>C	2. Heterozygous	2. Intronic
G	No	Jewish	Exon 2	c.150T>G	Heterozygous	Y50X
K	No	Greek	Exon 2	c.174delT	Heterozygous	F58fs72X
LH	Yes	English	Exon 2	c.236delC	Heterozygous	T79fs119X
C	No	Mauritius/India	Exon 3	c.407A>G	Homozygous	N136S
J	Yes	English	Exon 3	c.506G>A	Heterozygous	R169Q

[a]*THAP1* reference sequence is NM_018105. Nucleotide 1 is the A of the ATG-translation initiation codon.[16] These changes were not seen in 176 United Kingdom and 40 Jewish control individuals. The N136S mutation was screened in 68 Indian controls, and the S6F, Y8C, and F58fs72X mutations were screened in 95 European control cases.

sequences and regulate cell proliferation through the pRB/E2F cell cycle target genes,[12,13] a pathway recently proposed to be involved in cell death in Parkinson disease.[14]

To assess the frequency and phenotype of mutations in the DYT6 gene, we screened a large series of 362 adult-onset dystonia cases. The *THAP1* gene was also examined in 28 DYT1 dystonia–manifesting patients to look for modifying factors.

METHODS

Standard protocol approvals, registrations, and patient consents. All patients gave informed consent, and ethics approval was obtained from the joint medical and ethics committee at The National Hospital for Neurology and Neurosurgery to perform this clinical and genetic study (University College London Hospital ethical approval 06/N076).

Patients. Patients were assessed and followed up by movement disorder specialists (K.P.B. and Professor N.P. Quinn, MD). All cases analyzed here were cases of primary dystonia where the genetic etiology was unknown.

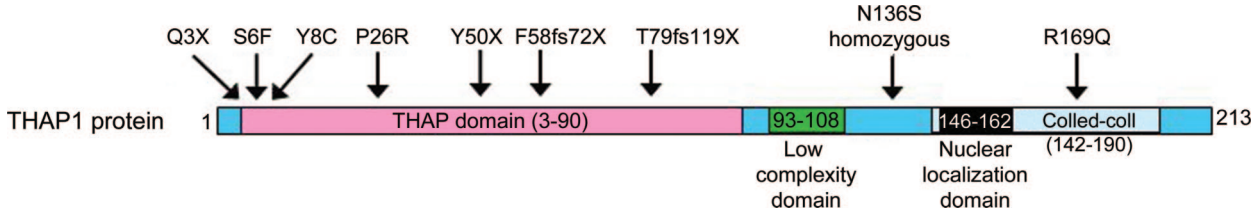
We sequenced the *THAP1* gene in a series of 362 British patients with clinically diagnosed dystonia who were genetically undetermined (for details, see appendix e-1 on the *Neurology*® Web site at www.neurology.org). Where there was a family history of dystonia, only the proband was analyzed. The proband in family J had previously been reported.[15] We also examined a series of 28 DYT1 dystonia–manifesting carriers, to look for possible modifying factors in these cases. The patients were all based in the United Kingdom, with a high frequency in and around London. We estimate that our British, mainly London population includes 15% of dystonia cases that were originally from other countries, mainly Europe or India. Overall, the dystonia population included 78 with primary focal, 186 with segmental, and 98 with a predominantly generalized dystonia. In this group, 272 had an age at onset less than 30 years. The control series that were sequenced consisted of 176 healthy United Kingdom white individuals who were older than 50 years and neurologically normal. We also sequenced the *THAP1* gene in 40 North London Jewish controls (entire gene) and analyzed 68 Indian and 95 Northern European control individuals for the specific mutations identified in patients with the respective ancestries.

Genetics. The DYT1 deltaGAG *TOR1A* gene had been screened in all cases. All 3 exons and flanking regions of the *THAP1* gene were sequenced. Mutations were confirmed by analyzing in a duplicate DNA sample from the proband. None of the coding mutations were identified in the control individuals described in the previous section. The c.-237_-236GA>TT and c.71+126bpT>C noncoding variants were seen in cases and controls, but the 3 other intronic variants that were identified in cases were not seen in controls. The difference in frequency of polymorphisms between cases and controls was analyzed using the χ^2 statistical analysis applying the Yates correction for continuity and also the χ^2 test for independence.

RESULTS

A total of 9 coding mutations were identified (table 1, figure, and figure e-1). Eight mutations were heterozygous, and 1 homozygous mutation was identified. The mutations were mainly located in exons 1 (n = 5) and 2 (n = 3). The mutations were all private changes and not previously reported. The frameshift mutations in families K (F58fs72X) and LH (T79fs119X) led to a premature stop codon and a short messenger RNA (mRNA) species. Families F (Q3X) and G (Y50X) had nonsense mutations; by their nature, these also lead to a short mRNA species. Families E (S6F), S (Y8C), M (P26R), C (N136S), and J (R169Q) all had missense mutations (table 1 and figure). The mutation segregated with the disease in the 3 other affected cases, but not in 1 unaffected sibling in family S. In family E, the mutation was present in the affected mother and daughter.

Figure THAP1 protein with the mutation types and positions identified here and the THAP1 protein domains



The start ATG (codon 1) is indicated in the figure, and all mutations are labeled from this codon up to the stop codon TAA at amino acid 213. Reference sequence is NM_018105, and protein is NP_060575.

Table 2 Clinical details of the families with *THAP1* gene mutations and intronic changes[a]

Family	Age at onset, y	Current age, y	Sex	Onset feature	Disease course	Speech affected	Associated medical conditions
F	8	37	F	Laryngeal dystonia	Spread to generalized dystonia	Yes	Treated depression
E	20	50	F	Hand dystonia	Generalized with cervical dystonia	No	Treated depression
S	Teens	45	M	Foot dystonia	Generalized	No	Bipolar depression
M	17	56	F	Orofacial/oromandibular dystonia	Remained focal	Yes	Treated depression, cervical cancer
G	16	86	F	Arm and head tremor	Cervical and arm dystonia	Yes	
K	20s	38	F	Young-onset cranial and upper limb dystonia	Oromandibular with marked lingual dystonia	Yes	
LH	Teens	29	F	Arm tremor	Segmental bilateral arm and cervical dystonia	Yes	Cervical cancer and family history of psychosis
C	57	69	M	Writer's dystonia	Cervical dystonia and bilateral arm dystonia	No	Treated depression, alcohol abuse
J	3	30	F	Foot (limping) dystonia	Generalized dystonia (tremor of arms, legs, and head; writing dystonia, cervical dystonia)	Yes	

[a]In the associated medical conditions, patients who were treated for depression were not formally assessed with a rating scale. Two patients were treated for cervical cancer. Teens = onset between 15 and 20 years.

All coding mutations are highly conserved in species (figure e-1) except the R169Q mutation, which is conserved through mammals and fish but not in the 2 frog species analyzed. The mutation in family C was a homozygous N136S missense change; like the other mutations, this is conserved in species and not present in controls. In family M, a heterozygous missense coding mutation was identified, and an exon 2–4 bp intronic change was seen. This intronic change is unlikely to be pathogenic because it did not affect splicing using the SpliceView program (http://bioinfo.itb.cnr.it/oriel/splice-view.html), NNSPLICE 0.9 (http://www.fruitfly.org/seq_tools/splice.html), and FSPLICE 1.0 (http://linux1.softberry.com/berry.phtml). Two other intronic changes (c.71+87G>C and c.268-24A>G) that we identified were also not predicted to affect splicing. The clinical phenotype of the dystonia families with *THAP1* mutations is given in table 2 and discussed further in the Discussion.

Two *THAP1* gene polymorphisms were identified (c.-237_-236GA>TT and c.71+126T>C) and seen in dystonia cases and controls (table 1), but no other intronic or exonic changes were seen in the 176 control individuals (352 control chromosomes) sequenced (table 3). The *THAP1* gene was also sequenced in 40 Jewish control individuals (exon 2); the N136S mutation was screened in 68 Indian controls; and the S6F, Y8C, and F58fs72X mutations were screened in 95 European control cases. The c.-237_-236GA>TT polymorphism was not significantly different from controls: χ^2 analysis, $p = 0.18$ (Yeats) and $p = 0.11$ (Pearson). In the c.71+126T>C polymorphism, the frequency of the C allele was 13% (found in 95 of 362 patients with dystonia) as compared with 7% (found in 25 of 352 healthy controls): χ^2 analysis, $p < 0.01$ (Yeats and Pearson). The frequency of the c.71+126T>C polymorphism (86 of 618, 14%) in a white population gave a result similar to that of the diverse population when analyzed.

Table 3 *THAP1* common polymorphisms identified and their frequency in dystonia cases and controls[a]

Polymorphism	Position	Frequency in dystonia cases, alleles	Frequency in controls, alleles	Significance
c.-237_-236GA>TT	5′ UTR	TT = 12/724	11/352	None
		GA = 712/724	341/352	
c.71+126T>C	Intron 1	C = 95/724	25/352	Yes
		T = 629/724	327/352	$p < 0.01$

Abbreviation: UTR = untranslated region.
[a]The phenotype and video of family J have previously been reported.[15]

DISCUSSION The *THAP1* coding mutations identified here indicate an overall mutation frequency of 2.5% in the British population (table 1 and figure) as compared with the German dystonia population, where the mutation frequency was 1%.[11] In American (mainly German, Irish, or Italian ancestry) non-DYT1 multiplex dystonia families where at least 1 person had nonfacial involvement with an age at onset less than 22 years, the frequency was 25%.[10] This indicates that *THAP1* mutations are significantly less frequent than the most common genetic cause of primary torsion dystonia, DYT1.

The polymorphic c.-237_-236GA>TT change was not associated with dystonia in this study; the TT allele was previously seen in significant excess in

German patients with dystonia.[16] The C allele of the c.71+126T>C polymorphism was seen at a significantly higher frequency in dystonia cases. This suggests that this *THAP1* allele could be a risk factor for developing dystonia, but this association needs extending to include other single nucleotide polymorphisms (SNPs) in and around the *THAP1* gene. We have only analyzed this association in 1 population of cases and controls, and although the cases are predominantly from the United Kingdom, this group consists of patients with a number of ancestral backgrounds, even when only the white individuals are analyzed. It will be important for other groups to investigate these 2 *THAP1* polymorphisms and other SNPs in the *THAP1* region to confirm or not confirm this association.

All mutations identified here in the THAP domain are highly conserved (figure e-1) except the R169Q, which is partially conserved in species. The N136S and the R169Q mutations are located in the low-complexity proline-rich and the coiled-coil domains, respectively. The N136S mutation is a homozygous change indicating recessive inheritance; this has not previously been reported. This patient presented with writer's dystonia progressing to segmental dystonia in the neck and arms. The family contained only 1 affected case with dystonia; both parents were said to be unaffected, but we could not examine them because they were dead. The homozygous change is highly conserved; the mutation results in a change to a much less hydrophobic serine amino acid and will likely affect solubility and membrane interactions. Given the location of this change outside the THAP domain, to prove pathogenicity would require functional investigation, although perhaps a less severe mutation outside the *THAP1* domain would be expected in a recessive case.

The clinical presentation of our DYT6 patients was that of young-onset focal or segmental dystonia (table 2). This phenotype was similar to previous mutation reports.[9-11] Our series had an equal number of patients that presented with onset of dystonia in the craniocervical region or in the limbs. The ages at onset in our cases were less than 30 years except for 1 case (family C), who had an age at onset of 57 years. The age at onset of less than 30 years is consistent with previous reports, with the exception of 2 reported cases with onset at 38 and 49 years. Interestingly, 1 early-onset patient presented with laryngeal dystonia, which spread to become generalized (family F). In our series, a further patient had orofacial dystonia at onset (family M), and a third developed oromandibular dystonia with marked lingual dystonia (family K). These cases with laryngeal or oromandibular dystonia indicate that this is a frequent clinical feature in patients with *THAP1* mutations. Involvement of the larynx and oromandibular region was previously reported in patients with *THAP1* mutations.[11] Bressman et al.[10] found that 18% of cases presented with this form of dystonia, and in 68% of cases speech was affected.

The comparison between the clinical features of the cases with *THAP1* mutations and patients with DYT1 shows that in contrast to DYT1 dystonia, where speech involvement is rare, 6 of our patients had dysarthria or spasmodic dysphonia due to oromandibular, tongue, or laryngeal involvement. Tumors are common in the general population; however, it is interesting to note that 4 of our patients had tumors or a family history, in view of the role of *THAP1* in or links to leukemia, prostate cancer, and apoptosis.

In the previous 3 reports of *THAP1* mutations, no genotype phenotype effect was observed.[9-11] In our series, we did not observe an overall difference in *THAP1* mutation position and phenotype, but 2 of 3 of the cases with either laryngeal or oromandibular dystonia had a nonsense or a frameshift mutation (tables 1 and 2). This mutation type associated with laryngeal dystonia was also seen in 2 German families, suggesting a possible correlation.[11] The frequent involvement of the larynx, oromandibular region, and face and the common occurrence of speech problems in *THAP1*-associated dystonia suggest that this group of patients will be a particularly important group to target for genetic screening.

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