Mutation Screening of the DYT6/*THAP1* Gene in Italy

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Video 

Abstract: **Mutations in the *THAP1* gene on chromosome 8p21-p22 (DYT6 locus) have been recently reported as causative of autosomal dominant primary torsion dystonia (PTD) in four Amish–Mennonite families and in 12 additional probands of different ancestry. We sequenced the *THAP1* gene in 158 patients with *DYT1*-negative PTD who had onset of symptoms below 30 years and/or positive family history. One sporadic Greek male patient, aged 57 years, was found to carry a novel heterozygous missense variant in *THAP1* exon 3 (p.Cys170Arg), of likely pathogenic significance. This subject first presented with right writer's cramp at age of 10 years and, subsequently, developed left arm dystonia and an extremely severe left laterocollis, without further spreading to other body districts. Our findings expand the genotypic spectrum of *THAP1* and strengthen the association with upper body involvement, including the cranial and cervical districts that are usually spared in DYT1-PTD.** © 2009 Movement Disorder Society

Key words: primary torsion dystonia; DYT6; THAP1; torticollis

Primary torsion dystonia (PTD) is characterized by sustained muscle contractions, causing twisting and repetitive movements and abnormal postures, and can be inherited as an autosomal dominant or recessive trait.[1] Only two genes (*TOR1A*/DYT1 and *THAP1*/DYT6) have been identified so far that cause autosomal dominant PTD with reduced penetrance. A 3-base pair (GAG) deletion in the *TOR1A* gene represents a common cause of early-onset dystonia, especially among Ashkenazi Jews.[2] The DYT1 phenotype usually presents with onset in a limb and rapid generalization, with sparing of the cranial-cervical district.[2–4] Recently, mutations in the *THAP1* (thanatos-associated protein domain-containing apoptosis-associated protein 1) gene have been identified in DYT6-linked families, including a founder Amish-Mennonite mutation.[5] Two large screenings of PTD patients have subsequently identified *THAP1* mutations in 9 of 36 (25%) DYT1-negative families with early-onset nonfocal PTD and in 2 of 160 (1%) patients with PTD that was early-onset and/or generalized and/or familial and/or involving the face and laryngeal districts.[6,7] In the 50 patients with *THAP1* mutations so far reported, the DYT6 phenotype appears to be characterized by onset mostly in the first two decades, with generalization in about half cases and frequent involvement of the upper body, including arms, cranial, and cervical districts. In particular, up to 78% patients presented cranial dystonia, of whom a large proportion experienced speech problems related to dysarthria and/or spasmodic dysphonia.[6,7]

Aim of this study was to investigate the frequency and phenotypic spectrum of *THAP1* mutations in a large cohort of PTD patients ascertained in Italy.

PATIENTS AND METHODS

The *THAP1* gene was first tested in 130 patients with familial or sporadic early-onset PTD, recruited from the movement disorders centers of the Catholic University in Rome and the C. Besta Institute in Milan. An arbitrary cut-off has been set at 30 years, as >90% of known patients with *THAP1* mutations had an onset below this age.[5–7] We subsequently tested a second group of 28 PTD probands with onset above 30 years

The first two authors contributed equally to this work.

Additional Supporting Information may be found in the online version of this article.

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TABLE 1. *Demographic and clinical data of tested patients*

	Early-onset PTD	Late-onset PTD
Number (sex M/F)	130 (75M/55F)	28 (6M/22F)
Origin	128 Italian[a]	All Italian
Age at onset	13.3 ± 8.6 (1–29)	53.1 ± 12.0 (32–73)
Age at examination	34.0 ± 17.0 (1–75)	67.6 ± 10.9 (45–80)
Family history		
Positive	26	28
Negative	104	0
Distribution of dystonia		
Focal	34	10
Segmental	31	18
Multifocal	3	0
Generalized	54	0
unknown[b]	8	0

Ages at onset and at examination are presented as mean ± standard deviation (range).

[a]One Greek, one Albanian.

[b]Latest examination <1 year from onset.

and positive family history. All 158 patients tested negative for the *DYT1* deletion, fulfilled a diagnostic flow-chart for dystonia[8] and had no signs of secondary dystonia. Inclusion criteria did not consider either the site of onset or the distribution of dystonia at latest examination (Table 1). The project was approved by the local ethics committees and written informed consent was obtained by all patients or legal representatives.

The three *THAP1* exons and exon-intron junctions were amplified from genomic DNA by polymerase chain reaction (primers and conditions available upon request), purified and sequenced bidirectionally using the Big Dye Terminator chemistry and an ABI PRISM 3130XL automated sequencer (Applied Biosystems). Multiple sequence alignments of the human THAP1 protein and its orthologues were generated by ClustalW software (http://www.ebi.ac.uk/clustalw/). Prediction of the possible impact of the identified variant on the protein function was obtained using PolyPhen (http://genetics.bwh.harvard.edu/pph/) and SIFT (http://sift.jcvi.org/) software. The structure of THAP1 in the amino acid range 148–185 has been modeled with the program MODELLER© 9v7, using as a template the crystallographic structure of General control protein GCN4 (PDB entry: 2efr).[9] Similarly, the putative dimerization between two THAP1 molecules has been simulated by reproducing the same topology of interactions observed for the dimer of GCN4.

RESULTS

Only 1 patient with early-onset PTD was found to carry a novel heterozygous missense variant in *THAP1* exon 3 (c.508T>C, p.Cys170Arg). This 57-year-old sporadic male patient was born in Xanthi (in the Northern Greek region of Thraki) from Greek ancestors; pregnancy, delivery, psychomotor development were uneventful. At age 10, he presented with right writer's cramp. Over the following 6 years, he developed abnormal posturing of the left arm and neck. The latest progressed in a few years to a severe left laterocollis associated with levoscoliosis, much more invalidating than the writer's cramp (see video). Dystonia failed to improve with anticholinergics, tetrabenazine, and myorelaxants. Botulinum toxin injections improved pain but failed to affect the dystonic posture. No other muscular districts have become affected over time, and dystonia remained segmental. In particular, no cranial or speech involvement could be observed. Brain magnetic resonance imaging and routine testing were normal. His parents died at 75 and 97 years without manifesting any dystonic feature; as far as the patient recalls, none of his family members (including his 60-year-old brother and 23-year-old daughter) is affected by any movement disorder.

The p.Cys170Arg variant affects an highly conserved residue in the coiled coil domain (CCD) (aa 139–190), was predicted as damaging both by PolyPhen (PSIC score: 3.168) and SIFT (score: 0.03), and it was not detected in 540 control chromosomes (Fig. 1). To gain insights into the effects of this mutation on the protein, we generated a structural model of THAP1 CCD (aa 148–185). We observed that all hydrophobic amino acids faced the same side of the CCD, while charged residues were regularly placed outwards. This pattern of alternating charged and hydrophobic residues in this specific region of THAP1 is consistent with potential intermolecular interactions between CCDs of two proteins. This is in line with the results of a previously reported large scale yeast-two-hybrid assay, that identified several putative THAP1 interactors among which the THAP1 protein itself, suggesting homodimerization.[10] We have therefore generated a possible model of self-interaction between two THAP1 molecules, although it is expected that THAP1 also heterodimerizes with different proteins through the same CCD. In our model, the Cys170 residue fell at the interface between the two molecules and replacement of this residue with an arginine produced a disruptive effect on the predicted intermolecular interaction (Fig. 1). In addition, the Cys170Arg change introduces a positively charged residue that likely interacts with the negatively charged Glu174 forming an intramolecular salt-bridge that perturbs THAP1 structure.

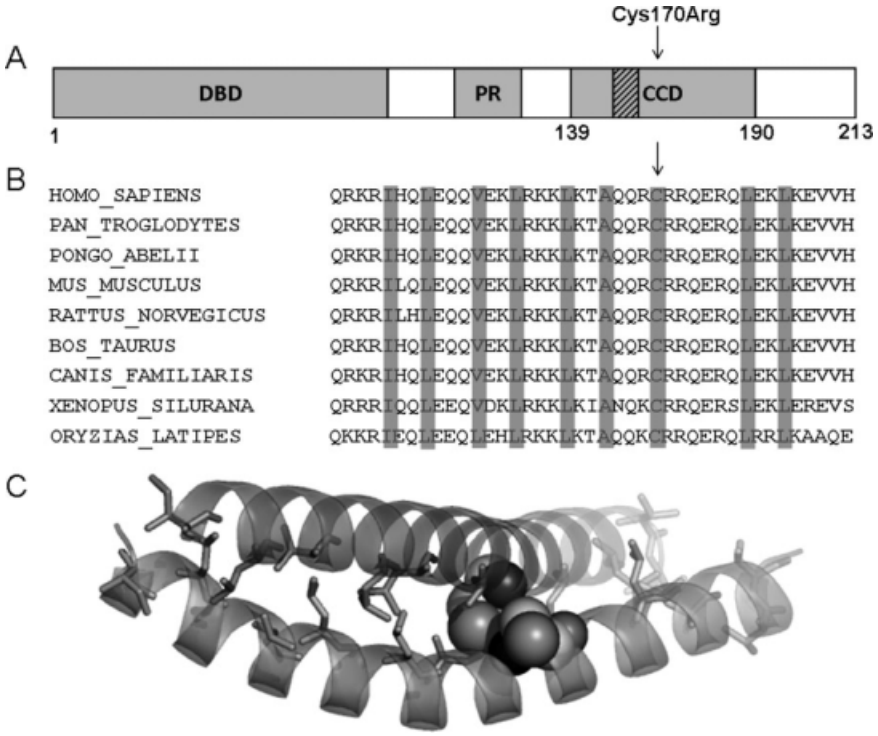

FIG. 1. Characterization of the p.Cys170Arg mutation. **A**: Schematic of the protein encoded by THAP1. DBD, THAP DNA-binding domain; PR, low-complexity proline-rich region; CCD, coiled-coil domain, including the nuclear localisation signal (dashed). **B**: Multiple alignment of THAP1 orthologues (aa 145–185). Hydrophobic residues are highlighted in grey, Cys170 is indicated by an arrow. **C** Model of homodimerization of THAP1. Each monomer (aa 148–185) is represented as a ribbon. Hydrophobic residues and Cys170 are shown respectively as sticks and spheres on each monomer.

DISCUSSION

We detected a *THAP1* missense variant of potential pathogenic significance in 1 of 158 PTD patients (0.6%), which is broadly in line with the 1% frequency reported in a recent study on European patients.[7] These figures are markedly lower than the 25% frequency reported by Bressman et al who included in the screening only familial cases in which at least 1 patient had nonfocal dystonia with onset below 22 years.[6] Yet, it must be noted that both our and the 2 patients carrying *THAP1* mutations reported by Djarmati et al. were sporadic, although one of these had two asymptomatic relatives with subtle signs of dystonia who also carried the mutation.[7] This lack of family history can be explained by the reduced penetrance of *THAP1* mutations, although we were not able to confirm this in our patient since both parents were dead and healthy relatives were not available for genetic testing. Indeed, we found no positive cases among our 17 probands who matched the inclusion criteria of the Bressman study,[6] and the mutation frequency in our cohort reached only 1.3% among patients with nonfocal dystonia and onset below 22 years (n = 76). Moreover, the only subject carrying a *THAP1* missense change in our cohort was from Northern Greece, whereas none of the 156 Italian patients carried pathogenic mutations. Thus, despite two families of Italian ancestry have been previously reported bearing *THAP1* mutations,[6] it appears that DYT6-dystonia represents a rare occurrence in Italy.

Our patient presented with segmental upper limb and neck dystonia in the absence of any facial, lingual, jaw or laryngeal involvement, supporting the conclusion that laryngeal dystonia or speech involvement, albeit frequently observed, are not mandatory features of the DYT6 phenotype.[6,7] However, the presence of cranial-cervical involvement may help differentiate this form from DYT1-PTD, that could present similar features but usually spares the cranial-cervical regions.[4,11]

The *THAP1* gene encodes a protein characterized by a conserved putative DNA-binding motif at the N-terminus, a proline-rich region, and a large coiled coil region at the C-terminus, which includes a nuclear-localization domain (Fig. 1).[5] Overall, eleven mutations including six missense, one nonsense, and four frameshift mutations have been described to date, with no obvious genotype-phenotype correlations. The novel

missense change identified in our study (p.Cys170Arg) is of likely pathogenic significance because it was not detected in over 500 Caucasian control chromosomes (although we did not have access to control samples from Northern Greece), and it was predicted to be deleterious by two distinct bioinformatic prediction software (PolyPhen and SIFT) and affected a highly conserved amino acid across species. Interestingly, the mutant residue represents the first variant to fall within the CCD of the protein, since all other missense changes clustered within the DNA binding domain. Although the function of the CCD is still largely unknown, it has been suggested that THAP1 may homo- or heterodimerize with another THAP1 protein or another member of the THAP family through this domain, and our molecular modeling results would be in favor of this hypothesis. These protein–protein interactions may be critical to enhance the DNA binding activity of the THAP1 zinc finger domain, which appears to be relatively weak, and in this light a mutation disrupting such intermolecular interactions may severely hamper the key function of DNA binding.[12]

In conclusion, our results expand the mutational spectrum of DYT6-PTD and implicate that this genetic form of dystonia is rare in Italy.

LEGEND TO THE VIDEO

The video illustrates the features of dystonia in the 57-year-old Greek patient carrier of the p.Cys170Arg mutation in the *THAP1* gene.

Segment 1. (Year 2004), Note the marked left laterocollis with subsequent limitation in neck movements, and the typical "geste antagoniste" (moving the right arm to the head) that completely abolishes the dystonic posturing of the neck. When the patient is sitting with his arms outstretched, there is dystonic posturing of both arms.

Segment 2. (Year 2009), The left laterocollis and the arm dystonic posturing have not changed over time. Note the writer's cramp when the patient is writing and the levoscoliosis associated with neck dystonia while the patient is walking.

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