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Tremor and Other Hyperkinetic Movements

Brief Reports

DYT6 in Brazil: Genetic Assessment and Clinical Characteristics of Patients

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Abstract

Background: Several genes associated with dystonia have been identified. A mutation in one of these, *THAP1* (*DYT6*), is linked to isolated dystonia. The aim of this study was to assess the prevalence of *THAP1* gene mutations and the clinical characteristics of patients with these mutations in a clinical population in Brazil.

Methods: Seventy-four patients presenting with dystonia involving the cervical muscles and without mutations in the *TOR1A* (*DYT1*) gene or any other movement disorders were recruited at a movement disorders clinic between June 2008 and June 2009. All the patients underwent clinical examination and were screened for mutations of the *THAP1* gene.

Results: Three patients had the novel p.Gln97Ter *THAP1* nonsense mutation in heterozygosis. One of them had no family history of dystonia. Symptoms in this patient first appeared in his right arm, and the condition progressed to the generalized form. The other two patients belonged to the same family (cousins). Symptoms in the first patient started in her right arm at the age of 18 years and the condition progressed to the segmental form. The second patient, who carried the p.Arg169Gln missense mutation, developed dystonia in her left arm at the age of 6 years. The condition progressed to generalized dystonia.

Discussion: We conclude that *THAP1* mutations are also a cause, albeit uncommon, of segmental and generalized dystonia in the Brazilian population.

Keywords: Dystonia, cervical dystonia, *DYT1*, *DYT6*, movement disorders

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Introduction

Over the past 20 years, several mutant genes (from *DYT1* to *DYT25*) have been identified in patients with pure forms of dystonia, dystonia combined with other movement disorders, or sporadic dystonia. Until the recent description of an association of the *CIZ1* (*DYT23*), *ANO-3* (*DYT24*), and *GNAL* (*DYT25*) genes in families with dystonia, only the *TOR1A* (*DYT1*) and *THAP1* (thanatos-associated protein domain-containing, apoptosis-associated protein 1) (*DYT6*) genes had been linked to primary dystonia.[1] *THAP1* is inherited in an autosomal dominant fashion with penetrance ranging from 40% to 60%.[2] The onset of clinical features commonly occurs during childhood or adolescence.[2,3]

THAP1, a causative gene for DYT6 dystonia, contains three exons that encode a 213-amino acid THAP domain-containing protein that is considered to be involved in endothelial cell proliferation and proapoptotic processes and assumed to act as a transcription factor.[3–5] Extensive mutation analyses of the *THAP1* gene by several groups revealed more than 50 mutations, mainly concentrated in the THAP domain.[6] Reported clinical features of these mutations include a segmental or generalized progressive dystonia that involves mostly the upper limbs and craniocervical muscles. The phenotype is highly variable, even within a single family, ranging from patients who are asymptomatic to those with generalized dystonia.[3,7,8] In the present study, we report our findings of *THAP1* mutations in a

population of Brazilian patients with dystonia involving the cervical region.

Methods

Subject selection and clinical assessment

Subjects with dystonia who attended the Botulinum Toxin and Movement Disorders Outpatient Unit in the Neurology Service, Hospital de Clínicas, UFPR, from June 2008 to June 2009 were selected for the study. Two movement disorders specialists examined all the patients (C.H.C. and H.A.T.). All the patients underwent brain computed tomography (CT) and a cervical spine CT, and, depending on the results of their clinical assessment, cervical spine magnetic resonance imaging (MRI) and brain MRI as well as laboratory tests. Seventy-four consecutive patients (48 females) presenting with isolated dystonia affecting the cervical region (focal, segmental, multifocal, or generalized) were included in the study. None of the patients had the *TOR1A (DYT1)* mutation or any other movement disorder.

Mutation scanning of the THAP1 gene

DNA was extracted from peripheral blood leukocytes using the Puregene DNA Isolation Kit (Gentra Systems Inc., Minneapolis, MN). Amplifications were performed using GoTaq® Colorless Master Mix (Promega, Madison, WI) in a final volume of 2.5 μL containing 100 ng of genomic DNA and 10 pmol of each primer. Primers and polymerase chain reaction conditions were as previously described, and amplification was followed by sequencing.[8]

The study was approved by the local ethics committee (ref. 1676.093/2008-06).

Results

There were 19 familial cases (25.67%) in 13 families, and 55 cases were sporadic. Focal cervical dystonia was observed in 33 patients (44.59%), and 19 (25.67%) had segmental or multifocal dystonia. Generalized dystonia was observed in 23 patients (31.1%). Mean age at onset of the study population was 30.30 ± 20.97 years (range, 5 months to 72 years).

We found *THAP1* mutations in two familial cases (A and B) and one sporadic patient (C). These three patients had arm-onset dystonia, which progressed to the segmental form in one patient and the generalized form in two (Table 1). A novel nonsense mutation c.289A>G (p.Gln97Ter) in exon 3 was found in heterozygosis in all three patients (Figure 1). One familial case (B) with an additional missense mutation (c.506G>A [p.Arg169Gln]) in exon 3 was a compound heterozygote. SIFT (Sorting Intolerant From Tolerant) analysis predicted this mutation to be tolerated and the other, p.GlnX, to be deleterious. Both this patient's parents were asymptomatic. The father was a simple heterozygote for a novel nonsense mutation c.289A>G (p.Gln97Ter). The mother, with no familial history, was a simple heterozygote for the mutation c.506G>A (p.Arg169Gln).

The proband had both mutations, and her parents had different *THAP1* mutations.

Table 1. Clinical Features of Patients with *THAP1* Mutations

Patient/ Family	Sex	Mutations	Type of Dystonia	Age of Onset (Years)	Evolution (Years)	Site of Onset	Speech Disorders	Familial History
A III-7, Family 3	F	p.Gln97Ter, exon 3	Segmental (neck and oromandibular region)	18	7	Right arm	No	Grandmother (I-2) with mild involuntary movements of the face. Possibly dysarthria and dysphonia in her old age
B III-11, Family 3	F	p.Gln97Ter and p.Arg169Gln, exon 3	Generalized (trunk, neck, larynges, oromandibular region, arms and legs)	6	10	Left arm	Dysarthria and dysphonia	
C Sporadic	M	p.Gln97Ter, exon 3	Generalized (trunk, neck, oromandibular region, arms and legs)	22	14	Right arm	Dysarthria	No familial history

M: Male; F: Female.

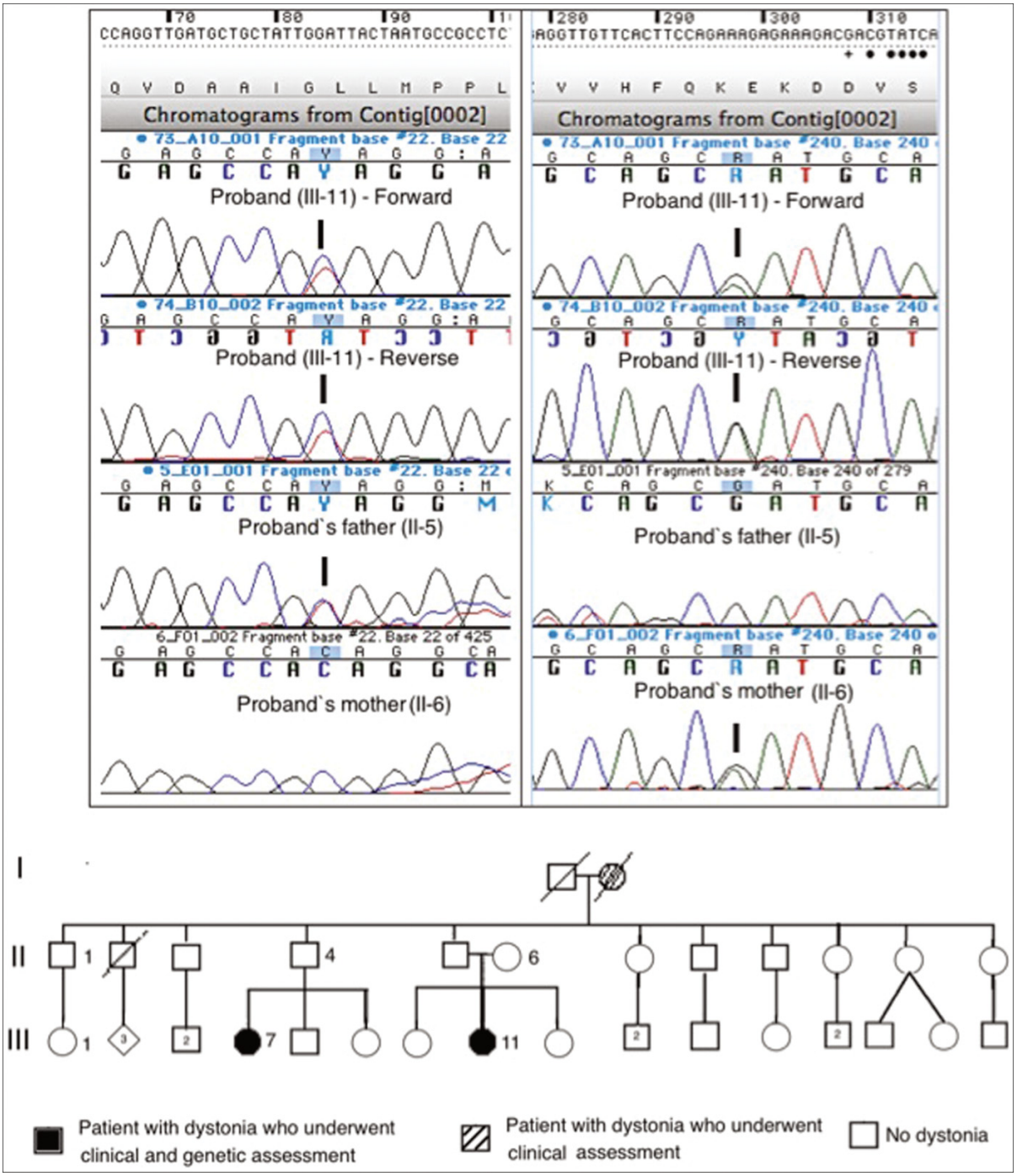

Figure 1. **Pedigrees of the Families with Patients with *THAP1* Mutations.** (A) Sequencing analysis of the *THAP1* gene showing the c.289A>G (p.Gln97Ter) mutation in proband III-11 and her father (II-9). (B) Sequencing analysis of the *THAP1* gene showing the c.506G>A (p.Arg169Gln) mutation in proband III-11 and her mother (II-10).

Discussion

The frequency of *THAP1* mutations in dystonia cases in various series in the literature varies from 0.6% to 4.7%, although it can reach 25% in a more specific population (onset before 22 years and a positive familial history).[9,10]

Among our patients with *THAP1* mutations, two were from the same family (A and B) and one was sporadic (C). All three had early-onset dystonia, which started in the arms and spread in a cranial–cervical direction, progressing to the generalized form in two of the three cases. In most DYT6 patients, dystonia starts in the arms or

cranial–cervical region and progresses to generalized or segmental dystonia.[3,6–10] When the focal form is observed in these patients, late onset is more common.[8,9] In the literature, approximately 40% of cases of dystonia do not have a familial history of the condition.[6]

We found a novel nonsense mutation (p.Gln97Ter) in a proline-rich region where few mutations have been described.[7–10] Despite this difference, the phenotypes of the patients in this study were very similar to those of most patients with mutations in other regions of the *THAP1* gene described in the literature.[6–10] We also found a missense mutation (p.Arg169Gln) in a familial compound heterozygote with the p.Gln97Ter mutation. Onset was earlier than in the other patient from the same family, and the dystonia progressed to a generalized form. We speculate that more severe manifestations may be explained by an additive effect of two compound heterozygous mutations. The p.Arg169Gln mutation has been described before in an early-onset familial case.[7] A prediction based on *in silico* analysis resulted in a possible deleterious effect (PolyPhen2), although using SIFT the amino acid substitution was predicted to be tolerated. While some homozygous mutations in the *THAP1* gene have been described, this is, to our knowledge, the first report of compound heterozygous mutations.[7,9–12] The novel nonsense mutation p.Gln97Ter is predicted to be deleterious because the premature termination codon is in a position that allows it to escape the nonsense-mediated mRNA decay pathway. Consequently, an aberrant truncated protein is expected to be generated.[13]

In the family with DYT6 dystonia (Figure 1), a significant variability in the phenotype can be observed. The grandmother possibly had a history of a late-onset speech disorder, an important feature of the DYT6 phenotype.[6–8] The parents of both probands were asymptomatic. The age of onset was different for both individuals, and in one of them the condition had not progressed beyond the segmental form at the time of writing, while in the other it progressed to the generalized form. Mutations in the THAP domain have been associated with an earlier age of onset of dystonia and with a more extensive anatomical distribution.[14] However, several studies indicate that there is little evidence to date of any correlation between phenotype and genotype.[15] Low penetrance may explain sporadic cases, including a case in our study (C). This heterogeneity of phenotypes and the asymptomatic patients could be explained by the low penetrance (about 60%) and variable expression of the *THAP1* gene.[2,6–8] Hence, additional genetic and environmental factors may affect disease manifestation and the severity of symptoms.

In agreement with previous studies, we suggest that analysis of the *DYT6* (*THAP1*) gene should be performed in patients with dystonia involving the cervical region, regardless of the anatomical distribution of the disease or the familial history. We conclude that *THAP1* mutations are also a cause of segmental and generalized dystonia in the Brazilian population.

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