

Analysis of DYT1 and DYT6 in Thai patients with primary dystonia

Pichet Termsarasab MD, Chutima Papsing MSc, Pirada Witoonpanich MD MSc, Teeratom Pulkes MD PhD

Division of Neurology, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Abstract

Background: DYT1 and DYT6 dystonias are the two most common genetic primary dystonias. However, they are rare in the Asian population and have never been reported in Thailand. DYT6 dystonia typically presents with craniosegmental dystonia with speech involvement, whereas DYT1 dystonia typically presents with lower limb dystonia, which tends to become generalized over time. **Methods:** Blood samples were collected from 14 patients with primary dystonia evaluated in five tertiary hospitals in Thailand. Genotyping of the *TOR1A* and *THAP1* gene was performed. **Results:** Two patients were found to have a missense mutation, p.M143V (c.427A>G), in exon 3 of the *THAP1* gene confirming the diagnosis of DYT6 dystonia. One patient was a woman who developed blepharospasm and lower cranial dystonia at the age of 38 years. Her dystonia spread to the neck and arm six months later. The other patient developed focal hand dystonia at the age of 34 years. The *TOR1A* mutation was not identified in any of these 14 patients.

Conclusion: This is the first report of DYT6 dystonia in Thai patients, and the third reported case in the literature of DYT6 dystonia patients with the p.M143V variant. Our study supports the pathogenicity of the p.M143V variant in this disorder. In Thailand, DYT6 dystonia, while rare, may be more common than DYT1 dystonia. In addition, the p.M143V mutation may be common among DYT6 dystonia in Thais. Further studies with a larger number of patients may elucidate the phenotypic spectrum and reveal the true prevalence of DYT6 dystonia in Thai patients.

Keywords: Primary dystonia, DYT1 dystonia, DYT6 dystonia, isolated dystonia, DYT-TOR1A, DYT-THAP1, TOR1A, THAP1

INTRODUCTION

DYT1 and DYT6 dystonias (or DYT-TOR1A and DYT-THAP1) are the two most common genetic forms of primary or isolated dystonia. DYT6 dystonia typically presents in late childhood or adolescence, which is older than the typical age at onset of DYT1 dystonia. The classic clinical features of DYT6 dystonia are craniosegmental dystonia involving neck, bilateral arms, larynx (spasmodic dysphonia), and/or face, but the phenotypes can widely vary.¹ In contrast to DYT1 dystonia, DYT6 dystonia has less tendency of becoming generalized during its clinical course.²

TOR1A and *THAP1* were identified as the gene which causes DYT1 and DYT6 dystonias.³ DYT1 dystonia was described not only in the Ashkenazi Jew, but also in non-Jewish populations including French, Italian, Russian and other European Caucasians.⁴ It was accounted for 80% of early-onset primary dystonia in the Ashkenazi Jew and

16-53% in the non-Jews. DYT6 dystonia were originally reported in the Amish-Mennonites, but later also described in European Caucasians and Brazilian.⁴ Screening of the *THAP1* gene found DYT6 dystonia in 7/610 (1.1%) German patients with various forms of dystonia⁵, and 1/158 (0.6%) DYT1-negative cases in Italy.⁶ In Asian populations, DYT1 dystonia is less common than in Western populations, accounted for only 2.7% and 3% of primary dystonia in Chinese and Korean^{7,8} respectively, and 3.4% of various forms of dystonia in Japanese.⁹ Data about DYT6 in Asian populations are more limited. In one Chinese study, DYT6 was found in 1.8% of primary dystonia.⁷ In Thailand, DYT1 and DYT6 dystonia have never been reported. This study analyzed *TOR1A* and *THAP1* mutations in 14 Thai patients with primary dystonia and reported the first two Thai cases with the *THAP1* mutation.

METHODS

This study was approved by the Ethical Committee, Ramathibodi Hospital, Mahidol University (ID 05-59-39). Appropriate genetic counseling was provided, and informed consent was obtained from all patients. The blood samples of 14 patients from five tertiary neurological centers were collected during 2009-2017 for genetic analysis at Neurogenetic Laboratory, Ramathibodi Hospital. Among eight patients recruited from Ramathibodi Hospital, two were clinically examined by the corresponding author T.P. Medical records of the six remaining patients were reviewed. Regarding the referred patients from other institutions, only blood samples with a brief medical history were available. Thus, clinical information was limited in those patients. DNA analysis of the *TOR1A* and *THAP1* genes was carried out as shown in the appendix.

RESULTS

There were eight males (male: female = 4:3). The mean age at the time of evaluation was 33.3 years (standard deviation = 12.1, range 18-51). *DYT1* was found in none of the patients. Two unrelated patients (Patients 1 and 2) had an identical missense mutation, p.M143V (c.427A>G), in exon 3 of the *THAP1* gene, confirming the diagnosis of *DYT6* dystonia. Clinical features in these two patients will be described below.

Patient 1 was a woman who developed blepharospasm at age 38 years. Six months later, she developed difficulty in moving her neck and tongue. Examination at age 39 years revealed craniosegmental dystonia. There was right head tilt and right shoulder elevation. In addition, she had dystonia of the perioral muscles and tongue, occasional blepharospasm, and mild left arm dystonia. There was no spasmodic dysphonia. Brain MRI was unremarkable. Dystonia was moderately improved with trihexyphenidyl, baclofen, and botulinum toxin injection. Upon a two-year follow-up, the distribution of her dystonia remained unchanged.

Patient 2 was a woman who gradually developed abnormal posturing of the right hand at age 34 years. In addition, she had right shoulder numbness and hand weakness, of which she was not aware of the onset and did not seek medical attention. Examination revealed dystonic posturing of the right hand, but no dystonia in any other body regions. There was also loss of pinprick sensation and light touch at the lateral aspect of the right proximal arm, and mild (grade

4/5) weakness of the right intrinsic hand muscles. Brain MRI demonstrated partial infarction in the left middle cerebral artery territory, mainly involving the precentral and postcentral gyri, as well as the left caudate nucleus. There was no involvement of the lentiform nucleus, internal capsule, thalamus and midbrain. Dystonia was mildly improved with clonazepam and remained focal during a one-year follow-up. Family history of dystonia was negative in both patients.

DISCUSSION

No *TOR1A* mutation in all 14 patients confirms the rarity of *DYT1* dystonia in Thailand. This is the first report of patients with *DYT6* dystonia in Thailand. Both patients carried the p.M143V mutation in the *THAP1* gene which was previously reported in only two cases: a German woman who developed cervical dystonia at age 46⁵, and an Indian boy who, at age 5, developed bibrachial dystonia which subsequently became generalized.¹⁰ Based on our data and the previous reports of dystonia patients with the p.M143V mutation, there seems to be no genotype-phenotype correlation. Negative family history in our patients may be due to reduced penetrance or *de novo* mutation. However, genetic testing of their parents and other family members was not available.

The p.M143V mutation, located in the coiled-coil region, upstream to the nuclear localization signal of the *THAP1* protein, has been predicted by *in silico* tools to be a benign variant. However, our two patients strongly support that this mutation is pathogenic.

Although the ages at onset generally vary from 3-62 years, the ages at onset of our patients (38 and 34 years) were considerably older than the mean age in previously reported cases of *DYT6* dystonia.¹¹ Craniosegmental dystonia in Patient 1 is the typical phenotype of *DYT6* dystonia. Focal hand dystonia in Patient 2, while confounded by the weakness and numbness from an ischemic stroke of unclear onset, fits with a previously reported, although less common, phenotype of *DYT6* dystonia.¹¹ Poststroke dystonia has been reported to be a consequence after lesions in the striatum especially putamen, lentiform nuclei, thalamus and midbrain.^{12,13} Caudate infarction leading to poststroke dystonia typically coexists with lesions in the lentiform nucleus, internal capsule or frontal region.¹⁴ While poststroke dystonia after isolated infarction of the head of caudate nucleus has been reported, this is

less common, and patients classically present with “starfish” hand which is not the case in our patient.¹⁵ While the possibility of poststroke dystonia cannot be completely excluded in our patient, focal hand dystonia has previously been reported and can represent the phenotype of DYT6 dystonia in Patient 2. It is also possible that stroke serves as a “second hit” in light of the underlying *THAP1* mutation, and render this patient more susceptible to focal hand dystonia after stroke. However, this speculation might not be feasible to prove, as it is based on a single case. Lack of generalization in both patients corresponds with the previous data, which shows that DYT6 dystonia has less of tendency of becoming generalized than DYT1 dystonia.²

We speculate that in the Thai population, DYT6 may be more common than DYT1 dystonia, and that the p.M143V mutation may be common, or even unique, in Thai patients with DYT6 dystonia. Determination of the true prevalence requires a larger number of patients. In the Chinese population, DYT6 was an uncommon primary dystonia, found in only two out of 111 patients (1.8%) with primary dystonia and seven out of 102 patients (6.9%) with non-DYT1 primary dystonia.^{7,16}

Our study may be limited by the retrospective nature and potential recruitment bias. The recruited patients from the tertiary centers may have phenotypes similar to cases of DYT1 and DYT6 dystonia previously described in the literature. Thus, there is a high pre-test probability. Despite the potential high pre-test probability, the tests were positive in only two patients with the *THAP1* mutation, and none with the *TOR1A* mutation. The number of patients in our study may appear low. However, in one larger study conducted in China of which the number of total population is approximately 20 folds compared to Thailand, there are 111 patients recruited which is only 7 folds of the number of recruited patients in our study.⁷ Therefore, compared with previous studies after adjusting for the number of total Thai population, the number of recruited patients in our study is not low. Despite recruitment of only 14 patients, our study provides a crucial preliminary information, since DYT1 and DYT6 dystonia have never been reported in the Thai population. Further studies with less-biased inclusion criteria and less selective pre-specified phenotypes will potentially recruit a larger number of patients, and may elucidate more insights about DYT1 and DYT6 dystonia in the Thai population including determination of the true prevalence.

In conclusion, our two patients with the identical p.M143V mutation in the *THAP1* gene represent the first two reported Thai patients with DYT6 dystonia and confirm pathogenicity of this variant.

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DISCLOSURE

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Conflict of interest: None

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Appendix

Primers and PCR conditions for PCR of DYT1

D1F: 5' CCTGGAATACAAACACCTAA 3'

D1R: 5' GGCTGCCAATCATGACTGTC 3'

PCR condition: Denaturing at 95°C for 10 minutes, stage 2 for 30 cycles of 95°C for 30 sec, annealing at 55°C for 30 sec, 72°C for 30 sec, and final stage at 72°C for 7 minutes

Fragment Analysis: To analyze the size of PCR fragment, the fragments was run on CEQTM 8000 Genetic Analysis Systems (Beckman Coulter, Fullerton, CA, USA).

Primers and PCR conditions for PCR of DYT6

Exon 1

THAP1_EX1F: 5' GCCAATAGTTAGCTTCCCAG 3'

THAP1_EX1R: 5' TGTTCCAGGAGCGCGAGAAA 3'

PCR condition: Denaturing at 95°C for 3 minutes, stage 2 for 30 cycles of 95°C for 10 sec, annealing at 60°C for 10 sec, 72°C for 10 sec, and final stage at 72°C for 10 minutes

Exon 2

THAP1_EX2F: 5' TAAGCTGGAAAGTTTGGGTGCC 3'

THAP1_EX2R: 5' GAGGGACGGTGCAGGAAAGAGA 3'

PCR condition: Denaturing at 95°C for 3 minutes, stage 2 for 30 cycles of 95°C for 10 sec, annealing at 65°C for 10 sec, 72°C for 10 sec, and final stage at 72°C for 10 minutes

Exon 3

THAP1_EX3F1: 5' CCTGGTCAGTCCACAGATTCT 3'

THAP1_EX3R2: 5' GAAACTCCTTTACAGGCTAGAGG 3'

PCR condition: Denaturing at 95°C for 3 minutes, stage 2 for 10 cycles of 95°C for 10 sec, annealing at 55°C for 10 sec, 72°C for 30 sec, and final stage at 72°C for 10 minutes

THAP1 analysis was determined by direct sequencing of all three exons and the exon-intron boundaries using Applied Biosystems 3730xl DNA Analyzer (Applied Biosystem, Foster City, CA, USA).