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Review

Genotype–phenotype correlations in THAP1 dystonia: Molecular foundations and description of new cases☆

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ABSTRACT

An extensive variety of *THAP1* sequence variants have been associated with focal, segmental and generalized dystonia with age of onset ranging from 3 to over 60 years. In previous work, we screened 1114 subjects with mainly adult-onset primary dystonia (Neurology 2010; 74:229-238) and identified 6 missense mutations in *THAP1*. For this report, we screened 750 additional subjects for mutations in coding regions of *THAP1* and interrogated all published descriptions of *THAP1* phenotypes (gender, age of onset, anatomical distribution of dystonia, family history and site of onset) to explore the possibility of *THAP1* genotype–phenotype correlations and facilitate a deeper understanding of THAP1 pathobiology. We identified 5 additional missense mutations in THAP1 (p.A7D, p.K16E, p.S21C, p.R29Q, and p.I80V). Three of these variants are associated with appendicular tremors, which were an isolated or presenting sign in some of the affected subjects. Abductor laryngeal dystonia and mild blepharospasm can be manifestations of *THAP1* mutations in some individuals. Overall, mean age of onset for THAP1 dystonia is 16.8 years and the most common sites of onset are the arm and neck, and the most frequently affected anatomical site is the neck. In addition, over half of patients exhibit either cranial or laryngeal involvement. Protein truncating mutations and missense mutations within the THAP domain of THAP1 tend to manifest at an earlier age and exhibit more extensive anatomical distributions than mutations localized to other regions of THAP1.

Contents

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1. Introduction

Screening studies from numerous groups covering several continents have shown that mutations in *THAP1* (THAP domain containing, apoptosis associated protein 1) are an important cause of both early- and late-onset primary dystonia. *THAP1* was the second gene to be associated with primary dystonia [1], the first being *TOR1A* [2]. A heterozygous GAG deletion in Exon 5 of *TOR1A* is responsible for DYT1 dystonia and an indel mutation in Exon 2 of *THAP1* causes DYT6 dystonia. Although inadequately studied to date, other causal mutations in *TOR1A* appear to be quite rare. In contrast, mutations in *THAP1* show greater diversity with missense mutations broadly distributed across its three exons [3,4]. Moreover, frameshift, non-coding and homozygous mutations in *THAP1* have also been associated with dystonia [4–6].

The phenotypic spectrum and anatomical patterns of clinical involvement differ among the two genes (*TOR1A* and *THAP1*) associated with primary dystonia. In the vast majority of cases, DYT1 dystonia begins in a limb [7]. In contrast, THAP1 dystonia is more heterogeneous with both craniocervical and limb onset described in various reports [3–5]. Herein, we present new cases of THAP1 dystonia and integrate clinical and genetic information derived from these subjects with the existing published literature to explore potential *THAP1* genotype–phenotype relationships and facilitate a deeper understanding of THAP1 biology. For the sake of clarity, the terms DYT1 and DYT6 will be limited to dystonia due to the seminal *TOR1A* ΔGAG and *THAP1* Exon 2 indel (c.135_139delinsGGGTTTA) mutations, respectively [1,2].

2. Newly identified missense mutations

2.1. Methods

2.1.1. Clinical subjects

All human studies were conducted in accordance with the Declaration of Helsinki with formal approval from the institutional review boards at each participating study site. All subjects gave written informed consent for genetic analyses, and use of their videos, audios and photographs. Recruitment of patients with primary dystonia and neurologically-normal controls has been described previously [4,8]. Clinical diagnoses were made by means of history and examination by one or more neurologists and/or neurolaryngologists at each site. In previous work, we screened 1114 subjects with mainly adult-onset primary dystonia and identified 6 missense mutations in conserved regions of THAP1 [4]. For this report, we screened 750 additional subjects for missense mutations in coding regions of *THAP1*.

2.1.2. Genetic analysis

High resolution melting (HRM) analyses were performed with the LightCycler® 480 Real-Time PCR system and High Resolution Master Mix (Roche) in accordance with manufacturer instructions and our laboratory protocol [4,9]. Melting curves and difference plots were analyzed using Gene Scanning Software. For samples with shifted melting curves, PCR products were cleaned using ExoSAP-IT® (United States Biochemical) and sequenced in the forward and reverse directions on the Applied Biosystems 3130XL Genetic Analyzer. Follow-up neurological examinations were performed on probands and select family members. Mutations in Exon 5 of *TOR1A* were excluded in all subjects. To predict the pathological character of single amino acid mutations, missense variants were analyzed with PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) [10], SIFT Human Protein (http://sift.jcvi.org/www/SIFT_enst_submit.html) [11] and MutationTaster (http://www.mutationtaster.org) [12].

2.2. Genotypes and phenotypes

As follow-up to previous work [4], we identified 5 additional missense mutations in THAP1 (Supplementary Table 1 and Fig. 2A). Three of these variants are associated with appendicular tremors, which were an isolated or presenting sign in some of the affected family members. Three of the 5 probands have a positive family history of dystonia (Fig. 3).

The p.A7D (c.20C>A) proband showed signs of subtle dysphonia at age 8 which became more overt during his teenage years. Right (dominant) hand-forearm dystonia developed during his freshman year in high school and he started to use his left hand to write. Bilateral, asymmetrical, upper-extremity tremors (dystonic) did not improve with levodopa. Trihexyphenidyl was mildly beneficial. This subject was last seen at 24 years of age. In addition to laryngeal dystonia (abductor subtype), right arm dystonia and bilateral upper extremity dystonic tremors, he also showed evidence of mild cervical, lower facial, lingual, and masticatory dystonia. His lower facial, masticatory and lingual dystonia showed moderate improvement with injections of botulinum toxin into the digastric, genioglossus, mentalis and lateral pterygoid muscles. This subject reported that none of his first or second degree relatives have obvious evidence of dystonia.

The p.K16E (c.46A>G) proband had evidence of spasmodic dysphonia (abductor subtype) and mild cervical dystonia at age 6 with significant disease progression at 14–16 years of age. Prior to bilateral globus pallidus interna (GPi) deep brain stimulation (DBS), generalized dystonia was manifest as spasmodic dysphonia (abductor subtype), blepharospasm, masticatory dystonia, lower facial dystonia, lingual dystonia, bilateral proximal arm dystonia (left > right), bilateral distal arm dystonia (right > left), truncal dystonia, and mild asymmetrical dystonia in the legs and feet. Gait was largely unimpaired. The laryngeal dystonia was severe, often rendering the patient's speech unintelligible. There was no significant improvement in any aspect of the generalized dystonia with trihexyphenidyl. The cervical dystonia was painful and treatment of cervical dystonia with injections of botulinum toxin was only mildly beneficial. The patient was able to alleviate his