Parkinsonism and Related Disorders 17 (2011) 547–550

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

An African–American family with dystonia☆

Andreas Puschmann [a,b], Jianfeng Xiao [c], Robert W. Bastian [d], Jill A. Searcy [a], Mark S. LeDoux [c], Zbigniew K. Wszolek [a,*]

[a] Department of Neurology, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224, USA
[b] Department of Geriatric Psychiatry, Lund University, Lund, Sweden
[c] Department of Neurology, University of Tennessee Health Science Center, Memphis, TN, USA
[d] Bastian Voice Institute, Downers Grove, IL, USA

ARTICLE INFO

Article history:
Received 13 January 2011
Received in revised form
22 March 2011
Accepted 26 April 2011

Keywords:
Dystonia
Genetics
African–American
DYT6
THAP1
Adult-onset dystonias
Dystonia, Hereditary
Focal dystonia

ABSTRACT

The genetic cause of late-onset focal and segmental dystonia remains unknown in most individuals. Recently, mutations in Thanatos-associated protein domain containing, apoptosis associated protein 1 (*THAP1*) have been described in DYT6 dystonia and associated with some cases of familial and sporadic late-onset dystonia in Caucasians. We are not aware of any previous descriptions of familial dystonia in African–Americans or reports of *THAP1* mutations in African–Americans. Herein, we characterize an African–American (AA) kindred with late-onset primary dystonia, clinically and genetically. The clinical phenotype included cervical, laryngeal and hand–forearm dystonia. Symptoms were severe and disabling for several family members, whereas others only displayed mild signs. There were no accompanying motor or cognitive signs. In this kindred, age of onset ranged from 45 to 50 years and onset was frequently sudden, with symptoms developing within weeks or months. DYT1 was excluded as the cause of dystonia in this kindred. The entire genomic region of *THAP1*, including non-coding regions, was sequenced. We identified 13 sequence variants in *THAP1*, although none co-segregated with dystonia. A novel *THAP1* variant (c.-237-3G>T/A) was found in 3/84 AA dystonia patient alleles and 3/212 AA control alleles, but not in 5870 Caucasian alleles. In summary, although previously unreported, familial primary dystonia does occur in African–Americans. Genetic analysis of the entire genomic region of *THAP1* revealed a novel variant that was specific for African–Americans. Therefore, genetic testing for dystonia and future studies of candidate genes must take genetic background into consideration.

1. Introduction

Dystonia is a clinical diagnosis based on the presence of sustained involuntary muscle contractions causing twisting, repetitive movements, or abnormal postures. Focal dystonia affects a single body region and may manifest as blepharospasm, masticatory and/or lingual dystonia, spasmodic dysphonia, cervical dystonia, and hand–forearm dystonia (e.g., writer's cramp). Dystonia may spread to contiguous anatomical segments. Primary focal dystonia is typically late-onset (>26 years) and sporadic [1]. However, approximately 10% of subjects report a positive family history of dystonia [2]. Familial late-onset dystonia may be associated with sequence variants in the Thanatos-associated protein domain containing, apoptosis associated protein 1 (*THAP1*, DYT6) [2,3]. Clinical expression is variable and penetrance reduced. In many families with *THAP1* mutations identified to date, at least one member had early-onset dystonia [2,3]. Familial dystonia with exclusively late-onset has been associated with the DYT7 locus [4]. DYT13 dystonia may have a late onset, but the majority of cases described had early onset [5]. The genes implicated in DYT7 and DYT13 remain unknown. Additional families without the DYT designation have also been described [6]. The overwhelming majority of studies on dystonia have been conducted with Caucasian subjects. Scarce data suggest that dystonia may be less prevalent in populations of African descent than in Caucasians [7].

We describe an African–American (AA) family in which six members were affected by dystonia with late onset (mean, 47 years). Two additional individuals with milder clinical signs were classified as probably affected. To our knowledge, this is the first report of an AA family with hereditary dystonia.

2. Methods

Affected and unaffected family members were examined by A.P. or Z.K.W. at up to three time points. Blood samples were obtained and DNA was extracted from

☆ The review of this paper was entirely handled by an Associate Editor, V. Bonifati.

* Corresponding author. Tel.: +1 904 953 7228; fax: +1 904 953 0760.
E-mail address: Wszolek.Zbigniew@mayo.edu (Z.K. Wszolek).

1353-8020/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.parkreldis.2011.04.019

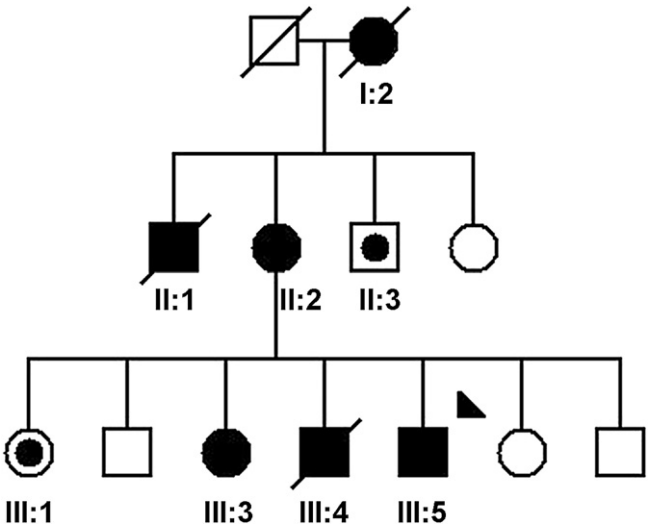

Fig. 1. Family Pedigree. Standard symbols are used. Round symbols indicate females, squares males, diagonal lines indicate the individual is deceased. Solid black symbols denote definitive dystonia; symbols with black points denote probable dystonia. The arrowhead indicates the proband.

peripheral blood leucocytes as previously described [2,8]. In addition to freely structured notes, relevant information about the participants' history, symptoms, and signs was collected on structured questionnaires. The physical examination included tasks intended to visualize possible subclinical signs of dystonia, such as examining the head in various positions, varying phonatory tasks to enhance abductor or adductor dysphonias, and writing. Family members who were considered affected or possibly affected were videotaped. Written informed consent was obtained from all participants. Additionally, written authorization (consent-to-disclose) has been obtained from the family members on the video clip (Video Supplement), who had been given the opportunity to view the video and the manuscript. This study was approved by the institutional review board of Mayo Clinic.

Supplementary video related to this article can be found at doi:10.1016/j.parkreldis.2011.04.019.

Sequence variants in Exon 5 of *TOR1A* (DYT1) were excluded with high resolution melting as described by Xiao et al. [8]. Four samples (2 affected subjects, 2 unaffected subjects) from this family were used to comprehensively analyze the entire genomic region of *THAP1* (8757 bp, which includes the promoter region, 5′ untranslated region [UTR], Exons 1–3, Introns 1–2, and the 3′ UTR). This was done by performing two long-range polymerase chain reactions (PCR) with products of 4920 bp and 5092 bp, respectively, followed by Sanger sequencing. Long-range PCR was carried out using the SequalPrep™ Long PCR Kit from Invitrogen (Carlsbad, CA). Q-Solution from Qiagen (Valencia, CA) was added to the PCR reactions for improved specificity. Touch-down PCR was performed as follows: first, samples were denatured at 95 °C for 5 min; then, they were subjected to 35 cycles at 94 °C for 30 s, 62 °C for 30 s and 72 °C for 5 min; followed by a 0.2 °C decrease of the annealing temperature each cycle, and ending with a 10 min extension at 72 °C. PCR products were then examined on a 1% agarose gel. Specific bands of the appropriate size were cut from the gel and the DNA fragments were purified with QIAquick Gel Extraction Kit (QIAGEN). Purified DNA fragments were then sequenced using multiple primer pairs spaced approximately 600 bp apart (esupp Table 1) with an Applied Biosystems 3130XL Genetic Analyzer. The sequencing data were reviewed independently by two scientists (J.X. and M.S.L.).

A variant located 5′ to Exon 1 of *THAP1* (c.-237-3G>T) was detected in the proband which prompted screening of all available family members along with an additional 41 AA dystonia cases, 106 AA controls, 1569 Caucasian dystonia cases, and 1366 Caucasian unaffected and unrelated controls. To screen for this variant, high resolution melting of Exon 1 and its contiguous 5′ region was performed as previously described (2) using primers E1F and E1R (esupp Table 1). All variants were confirmed with Sanger sequencing in the forward and reverse directions. Fisher's exact test was used for statistical analyses.

3. Results

Eight family members were examined and blood samples were obtained from these individuals (Fig. 1). Three of the examined family members were definitely affected and two were probably affected. Several family members independently reported signs of dystonia in three deceased family members. These signs were substantiated on family photographs of two members **(II:3 and III:4)**, both showing marked cervical dystonia.

3.1. Patient descriptions

With the exception of one subject who possibly began to manifest cervical dystonia in his twenties, affected family members remained unaffected until they developed symptoms at age 45–63 years of age. Then, signs of dystonia became manifest within weeks to months, leading to marked impairment, and, in the proband's case, to an inability to continue working in his profession. Symptoms were varied and different forms of focal, segmental or generalized dystonia were found in the affected family members. The clinical data are summarized in Table 1. Representative videos of three affected family members can be viewed in the Video Supplement.

The **proband (III:5)** is an AA man whose head started to turn towards the right side at the age of 45 years. He also developed a head tremor. There were no precipitating events, and, in particular, no trauma. Significant worsening of his symptoms occurred within weeks, causing severe neck pain. Touching his cheek with his hand helped to ameliorate the involuntary movements. Similarly, warmth (from a heating pad), and lying down improved the situation. On examination, almost constant head turning towards the right side and an irregular "no–no" phasic head tremor were noted. There was palpable hypertrophy of the left sternocleidomastoid muscle. While

Table 1
Summary of clinical characteristics. See Methods section for detailed descriptions, and Video supplement for videos of family members as indicated. FHx, family history.

ID	Gender	Section in Video Supplement	DNA	Examined	Age at Examination (years)	Age at Initial Symptom	Blepharo-spasm	Oromandibular	Torticollis, Head Tremor	Dysphonia	Hand-Forearm	Lower Limb	Diagnosis
I:2	F				Deceased	50			X				Dystonia (acc to FHx)
II:1	M				Deceased	50			X				Dystonia (acc to FHx)
II:2	F	2	Y	Y	78	50	X	X	X	X			Segmental Dystonia
II:3	M		Y	Y	75	?				X	X		Probable Segmental Dystonia
III:1	F		Y	Y	64	63			X		X	X	Probable Generalized Dystonia
III:2	M		Y	Y	61								unaffected
III:3	F	3	Y	Y	53	50		X	X	X	X		Segmental Dystonia
III:4	M				Deceased	20's			X				Dystonia (acc to FHx)
III:5	M	1	Y	Y	46	45			X				Focal Dystonia
III:6	F		Y	Y	48								unaffected
III:7	M		Y	Y	43								unaffected