Journal of the Neurological Sciences 362 (2016) 299–303

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

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

Blepharospasm in a multiplex African-American pedigree

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ARTICLE INFO

Article history:
Received 25 October 2015
Received in revised form 19 January 2016
Accepted 1 February 2016
Available online 2 February 2016

Keywords:
Dystonia
Blepharospasm
THAP1
Sequence variant
African-American

ABSTRACT

Background: Isolated blepharospasm (BSP) is a late-onset focal dystonia characterized by involuntary contractions of the orbicularis oculi muscles. Genetic studies of BSP have been limited by the paucity of large multiplex pedigrees. Although sequence variants (SVs) in *THAP1* have been reported in rare cases of BSP, the genetic causes of this focal dystonia remain largely unknown. Moreover, in the absence of family history and strong *in silico* or *in vitro* evidence of deleteriousness, the pathogenicity of novel SVs in *THAP1* and other dystonia-associated genes can be indeterminate.

Methods: A large African-American pedigree with BSP was phenotypically characterized and screened for mutations in *THAP1*, *TOR1A* and *GNAL* with Sanger sequencing. Whole-exome sequencing of the proband was used to examine other dystonia-associated genes for potentially pathogenic SVs. *In silico* and co-segregation analyses were performed for a novel *THAP1* SV identified in the proband.

Results: Seven family members exhibited increased blinking and/or stereotyped bilateral and synchronous orbicularis oculi spasms with age of onset ranging from early childhood to late adult life (7 to 54 years). The proband was found to harbor a novel *THAP1* SV (c.314T>C, p.L105S). However, the p.L105S SV did not co-segregate with blepharospasm in the pedigree. Moreover, *in silico* analyses suggest that p.L105S is benign. No pathogenic or likely pathogenic SVs in other dystonia-associated genes were identified with whole-exome sequencing.

Conclusions: Blepharospasm can be familial and may be hereditary in African-Americans. A comprehensive array of *in silico* tools, and, if possible, co-segregation analysis should be used to classify SVs in dystonia-associated genes.

1. Introduction

Isolated blepharospasm (BSP) is a late-onset focal dystonia characterized by involuntary contractions of the orbicularis oculi muscles [1]. BSP is a relatively common form of focal dystonia, with prevalence estimates ranging from 1.6 to 13.3/100,000 worldwide [2]. Mean age of onset is from 50 to 60 years and women are more commonly affected than men [1,3]. Genetic factors are believed to play an important role in the etiopathogenesis of BSP given that 10 to 27% of affected individuals report a positive family history of dystonia [4,5]. BSP is within the phenotypic spectrum of several genetic forms of dystonia inherited in autosomal dominant fashion (DYT1, DYT6, and DYT25) [4,6]. Although rare cases of BSP as a singular dystonia manifestation have been linked to *THAP1* mutations, the genetic underpinnings of this important focal dystonia remain largely unknown and large pedigrees adequately powered for linkage analysis are quite uncommon [4,7]. Although one large Italian BSP family has been described in the literature [8], there have been no reports of large BSP pedigrees in other ethnic and racial groups.

2. Patients and methods

2.1. Human subjects

All human studies were conducted in accordance with the Declaration of Helsinki with formal approval from the University of Tennessee Health Science Center Institutional Review Board (01-07346-FB, 05-08331-XP, and 14-03320-XP). All subjects gave written informed consent for genetic analyses and disclosure of medical and demographic information. Subjects in this pedigree (Fig. 1) were examined by a neurologist (MSL) with subspecialty expertise in movement disorders. Subjects were asked to perform specific tasks including holding their eyes open, opening and closing their eyes gently, opening and closing their eyes forcefully, along with additional verbal and postural maneuvers design to capture masticatory, laryngeal or cervical involvement. Using motor severity classification from the Unified Dystonia Rating Scale, BSP was categorized as mild, moderate, severe or extreme. Medical histories were documented for each subject. A clinical diagnosis of definite BSP was given to subjects exhibiting increased blinking and stereotyped, bilateral and synchronous orbicularis oculi spasms inducing

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http://dx.doi.org/10.1016/j.jns.2016.02.003
0022-510X/

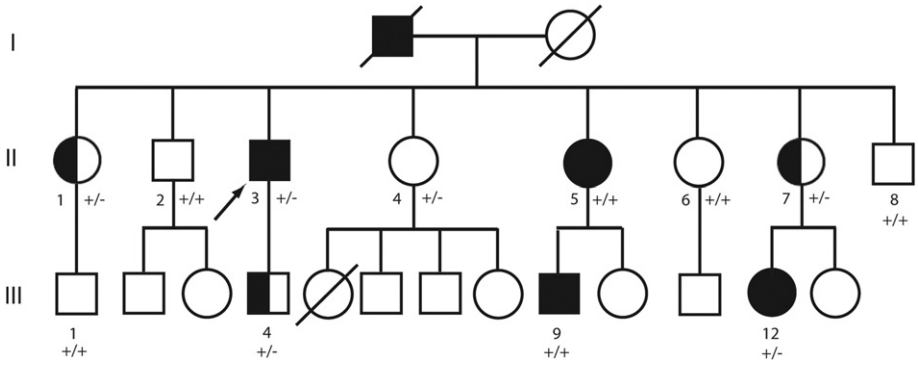

Fig. 1. Family pedigree. Arrow = proband. Filled symbols = affected. Half-filled symbols = possibly affected. +/+, homozygous wild-type allele. +/−, heterozygous for *THAP1* c.314T>C.

narrowing/closure of the eyelids [9]. Subjects with isolated episodes of increased eyelid blinking were given a diagnosis of possible BSP. Each affected or possibly affected family member was queried for the presence of sensory tricks.

2.2. Genetic analyses

Blood or saliva was acquired from 12 subjects, and DNA was extracted for variant screenings. Using previously defined primers, Sanger sequencing was employed to screen the proband for sequence variants (SVs) in *THAP1*, *GNAL* and Exon 5 of *TOR1A* [4,6,10]. For whole-exome sequencing, the proband's genomic DNA (3 μg) was sheared to yield 100–450 bp fragments, followed by processing with an Illumina (San Diego, CA) paired-end library preparation kit. Target enrichment was performed with the Agilent SureSelect Human All Exon V5 kit (Santa Clara, CA). Enriched DNA fragments were sequenced on Illumina's HiSeq 2000 platform as paired-end 100–125 base reads (Otogenetics Co., Norcross, GA USA). Sequence reads (FASTQ) were mapped to the human reference genome (NCBI build 37.1) with NextGENe® (SoftGenetics, State College, PA, USA). An integrated query of all NCBI databases with the search term 'blepharospasm' produced the following list of genes that have been associated with BSP as an isolated dystonia or part of a more complex neurogenetic syndrome: *THAP1*, *TOR1A*, *SGCE*, *ATCAY*, *CIZ1*, *GNAL*, *ANO3*, *PRRT2*, *ATM*, *PRKRA*, *GCH1*, *TAF1*, *PNKD*, *ATP1A3*, *SLC2A1*, *TH*, *SPR*, *TIMM8A*, *DRD5*, *CP*, *PANK2*, *FTL*, *FBX07*, and *DJ1*. SVs in reads mapped to these genes were analyzed *in silico* with the following programs which predict pathogenicity or deleteriousness: PolyPhen-2, MutationTaster, Sorting Intolerant From Tolerant (SIFT), Likelihood Ratio Test (LRT), and Combined Annotation Dependent Depletion (CADD). The allele frequency of identified SVs was compared to reported frequencies in dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), 1000 Genomes (1KG, www.1000genomes.org) and Exome Aggregation Consortium (ExAC, exac.broadinstitute.org).

A presumed SV in *DRD5*, detected with whole-exome sequencing, was examined with genomic DNA (gDNA) and complementary DNA (cDNA) from the proband using forward (DRD5_F, cagtccagcccgaaatgc, NM_000798.4: 383–400) and reverse primers (DRD5_R, cacgaaaaggtctgacacgg, NM_000798.4: 666–647) to generate a 284 bp amplicon. PCR was performed using 40 ng of peripheral blood gDNA or 2 μl of cDNA transcribed from 10 ng of total RNA along with 200 nM of each primer in a 10-μl reaction volume with HotStarTaq® Plus DNA polymerase from Qiagen (Valencia, CA). The following cycling conditions were employed: 95 °C for 15 min, 35 cycles at 95 °C for 10 s, 60 °C for 30 s, and 72 °C for 30 s.

3. Results

Herein, we report a large multiple African-American pedigree with BSP (Table 1, Fig. 1). Among the 12 subjects that were examined (Fig. 1), 7 were assigned a diagnosis of either definite or possible BSP (II-1, II-3, II-5, II-7, III-4, III-9, and III-12) with ages of onset ranging from 7 years to 54 years of age.

The proband (II-3) was most recently examined at 66 years of age and reported onset of BSP at 48 years of age. At age 62, he underwent an extensive dental extraction and noted the development of lower facial and jaw-opening masticatory dystonia several months later. Neurological examination showed evidence of severe BSP with increased blink frequency and prolonged spasms of the orbicularis oculi muscles. Lower facial involvement and involuntary jaw-opening were prominent. The proband has shown significant and consistent benefit from injections of incobotulinumtoxinA for treatment of BSP and lower facial dystonia. He has also received electromyographically-guided injections of incobotulinumtoxinA into the inferior head of the lateral pterygoid muscles with notable reductions in involuntary jaw opening.

Three other family members, II-5 (59 years old), III-9 (33 years old), and III-12 (27 years old) were given diagnoses of definite BSP. Subject II-5, a sister of the proband, reported episodes of increased blinking, possibly triggered by phacoemulsification of her cataracts. In agreement with our clinical examination, Subject II-5 noted that she was less severely affected than her son (Subject III-9). Subject III-12, a niece of the proband, first noticed increased eyelid blinking at the age of 7 years. Subject III-12 was diagnosed with Tourette syndrome as a child due to the presence of motor and phonic tics which largely resolved by 15 years of age. At her most recent evaluation, BSP was present with eyelid spasms and narrowing of the palpebral fissures but no motor or phonic tics were apparent. Unlike the proband, these three

Table 1
Phenotypes and *THAP1* genotypes.

Patient ID	Age (years)	Gender	Age of onset (years)	BSP severity	BSP diagnosis	*THAP1* c.314T>C	Other medical disorders
II-1	69	F	Unknown	Mild	Possible	Heterozygote	Diabetes
II-3	66	M	48	Severe	Definite	Heterozygote	Hypertension
II-5	59	F	54	Moderate	Definite	Normal	Phacoemulsification cataracts
II-7	50	F	Unknown	Mild	Possible	Heterozygote	None
III-4	30	M	Unknown	Mild	Possible	Heterozygote	None
III-9	33	M	30	Severe	Definite	Normal	None
III-12	27	F	7	Moderate	Definite	Heterozygote	None