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DYT2 screening in early-onset isolated dystonia

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ABSTRACT

Background: Mutations in *HPCA*, a gene implicated in calcium signaling in the striatum, have been recently described in recessive dystonia cases previously grouped under the term "DYT2 dystonia". Positive patients reported so far show focal onset during childhood with subsequent generalization and a slowly progressive course to adulthood.
Methods: 73 patients with isolated dystonia of various distribution, manifesting within 21 years of age, were enrolled in this Italian study and underwent a mutational screening of *HPCA* gene by means of Sanger sequencing.
Results/conclusions: Mean age at onset was 10.2 (±5.1) years and mean age at the time of genetic testing was 33 (±14.2) years. Mean disease duration at the time of enrollment was 22.7 (±12.8) years. None of the patients enrolled was found to carry *HPCA* mutations, rising suspicion that these probably represent a very rare cause of dystonia in childhood-adolescence. Larger studies will help determining the real mutational frequency of this gene also in different ethnic groups.

1. Introduction

Dystonia is a hyperkinetic movement disorder characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements, postures, or both. Dystonia is often initiated or worsened by voluntary action and associated with overflow muscle activation. Dystonia is defined "isolated" if no additional neurological abnormalities

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with the exception of tremor are detectable on examination. In the current classification by Albanese et al.,[1] the age to discriminate between childhood- and adult-onset dystonia has been set at 21 years and a detailed categorization has been adopted, subdividing the age at onset as follows: infancy (birth to 2 years); childhood (3–12 years); adolescence (13–20 years); early adulthood (21–40 years); late adulthood (>40 years).

Isolated dystonia in children has a wide differential diagnosis, and early-onset cases generally differ from late-onset ones in terms of anatomical sites affected and rate of generalization; in fact, children and adolescents often show an initial lower limb involvement with a high tendency to spread to other body sites, while adult-onset dystonia commonly remains focal (e.g. blepharospasm) or segmental.

Most genetically inherited dystonias show an autosomal dominant mode of inheritance with reduced penetrance and variable expressivity.[2] However, a few families of different ethnical background have been described in which dystonia is recessively inherited. Some of them were collectively grouped under the umbrella term "DYT2" or "DYT2-like" dystonia,[3,4] whereas "DYT17" refers to a locus mapped on chromosome 20p11.2-q13.12 in a single Lebanese family.[5]

Thanks to Next Generation Sequencing (NGS) techniques, the identification of dystonia-related genes has significantly improved in recent years, making it possible to formulate a definite genetic diagnosis in an increasing proportion of patients. In 2015, using a combination of homozygosity mapping and whole exome sequencing, Charlesworth et al.[6] identified mutations in Hippocalcin (*HPCA*) as the cause of DYT2 early-onset recessive dystonia in a previously-published Sephardic Jewish kindred from Iran[3] and in an additional unrelated case from Sri Lanka. *HPCA* encodes a neuronal calcium sensor protein expressed mainly in the striatum which exerts its Ca^{2+}-dependent activity by interacting with downstream proteins still under investigation; perturbation of calcium signaling and neuronal excitability has thus been proposed as an important mechanism in the pathogenesis of this kind of genetic dystonia.

The frequency of *HPCA* mutations in isolated dystonia is unknown and the only available genetic screening investigating it,[7] including a heterogeneous population of patients with dystonia has been recently published, failing to identify new positive cases.

However, no studies focusing only on pediatric-onset dystonia are available. In this paper, we screened a cohort of genetically undefined isolated dystonia cases for *HPCA* mutations, focusing on patients with onset from infancy to adolescence, namely within 21 years of age.

2. Methods

Patients previously referred either to the Carlo Besta Neurological Institute, Milan, or the Mendel Institute, Rome for clinical assessment of dystonia were included in this study. Subjects with isolated dystonia with various distribution and onset before 21 years of age, lacking a definite genetic diagnosis were enrolled. All patients tested negative for the recurrent GAG deletion of the DYT1/*TOR1A* gene. Moreover, in all patients Dopa-Responsive Dystonia (DRD) had been previously ruled out either genetically, on the basis of cerebrospinal fluid neurotransmitter profile or on clinical grounds after an appropriate Levodopa trial. Among patients enrolled, 34 also tested negative for mutations in the *PRKRA* gene (DYT16), a rare cause of early-onset, recessive dystonia-parkinsonism described in few families so far, which can be characterized only by dystonia at onset and for several years over the disease course.[8]

After obtaining informed consent, the subjects included in this study were blood sampled and DNA was extracted from peripheral blood lymphocytes according to standard procedures. In some cases genetic analysis was performed after a long disease history, thanks to the availability of patients' DNA in our biobank. Also in these cases, patients' consent was retrieved. All exons and flanking intronic regions of *HPCA* were Sanger sequenced (primer sequences and conditions available upon request – disturbimovimento@istituto-besta.it). Clinical and demographic information were obtained by direct interview and by reviewing patients' clinical records and videos.

3. Results

A total of 73 patients (28 females, 45 males) were enrolled. All but three patients (1 from Albania, 1 from China and 1 from India) were of Italian origins. The mean age of onset of dystonia was 10.2 (±5.1) years and mean age at the time of genetic testing was 33 (±14.2) years. Mean disease duration at the time of enrollment was 22.7 (±12.8) years.

Parental consanguinity was documented in 4 (5.5%) patients, possible in 1 (1.4%) and absent in 68 (93.1%) enrolled subjects. Eight patients (11%) had at least one sibling affected by dystonia, indicating a possible recessive pattern of inheritance, but in none of these cases parental consanguinity was documented.

Clinical features of enrolled subjects are shown in Table 1.

Onset of dystonia (defined by direct patients' observation or review of records) was in the lower limbs in 26% of cases; upper limbs, the cervical region and a multifocal involvement were present at the beginning in 15% of patients each, whereas in 20.5% of patients a generalized distribution was noted since the first clinical evaluation.

Sanger sequencing did not reveal exonic *HPCA* mutations in any subject enrolled. Exonic or genomic rearrangements involving the *HPCA* gene were not ruled out.

4. Discussion

Since the discovery of *TOR1A* gene in 1997,[9] 27 dystonia loci have been mapped, and 17 dystonia-related genes have been identified. DYT1 mutations remain the most common genetic cause of dystonia, especially in Ashkenazi Jews.[10] However, for some recently identified genes, a limited number of mutated patients have been reported, and the pathogenic role of some of them has been questioned.[11] In 2015 *HPCA* was discovered in a consanguineous Sephardic Jewish kindred including three affected siblings with childhood-onset

Table 1 – Clinical features of subjects enrolled.

Site of onset	
Cranial	2 (2.7%)
Oro-mandibular	2 (2.7%)
Cervical	11 (15.1%)
Trunk	2 (2.7%)
Upper limb	11 (15.1%)
Lower limb	19 (26%)
Multifocal	11 (15.1%)
Generalized	15 (20.5%)
Family history	
N	57 (78.1%)
Y	12 (16.4%)
P	4 (5.5%)
Consanguinity	
N	68 (93.1%)
Y	4 (5.5%)
P	1 (1.4%)

P: possible.

dystonia, with a slowly progressive course and generalization without major functional limitations in adulthood. The age of onset varied between 1 and 8 years and sites initially affected were lower limbs and the cranial-cervical region; the upper body resulted more markedly affected in adulthood.

Based on the observation that the only cases described so far were affected since childhood, we selected a population of dystonic patients with onset during childhood and adolescence according to the current classification of dystonia[1] to assess the mutational frequency of this gene in a specific age-selected population. We excluded all probands with clear autosomal dominant inheritance of dystonia, but purposely included in the study also sporadic cases, who could also have inherited recessive mutations from unaffected healthy parents, even in the absence of obvious consanguinity or of positive family history. The only *HPCA* mutational screening available in the literature has been recently published by Dobričić and colleagues[7] and included 435 patients with isolated dystonia, of which 107 were ≤20 years at the time of disease onset. None of the patients enrolled resulted positive for *HPCA* mutations.

Similarly, we failed to identify *HPCA* pathogenic variants in any of the 73 tested patients, indicating that mutations in this gene are a very uncommon in childhood-onset dystonia, as observed for other recently identified dystonia genes. For example, only 53 *GNAL*-positive patients have been reported since the original description of the gene in 2012,[12] and only 10 *ANO-3* positive patients have been fully characterized clinically.[13] Notably, four of them had childhood onset dystonia, ranging from 3 to 6 years, but no dedicated studies in children are available.

We acknowledge that multiplex ligation-dependent probe amplification (MLPA) detecting *HPCA* deletions or duplications was not performed in our patients, thus *HPCA* mutational frequency could have overall been underestimated; however, no *HPCA* exonic or genomic rearrangements have been reported in the literature so far.

At present, it is difficult to foresee whether *HPCA* genetic testing would be advisable in sporadic or familial recessive cases with childhood-onset dystonia, and more extensive studies are warranted to assess *HPCA* mutational frequency and related phenotypes in dystonic patients from different populations.

Conflict of interest

The authors declared that they have no conflict of interest.

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REFERENCES

1. Albanese A, Bhatia KP, Bressmann S, et al. Phenomenology and classification of dystonia: a consensus update. *Mov Disord* 2013;**7**:863–73.
2. Charlesworth G, Bhatia KP, Wood NW. The genetics of dystonia: new twists in an old tale. *Brain* 2013;**136**:2017–37.
3. Khan NL, Wood NW, Bhatia KP. Autosomal recessive, DYT2-like primary torsion dystonia: a new family. *Neurology* 2003;**61**(12):1801–3.
4. Moretti P, Hedera P, Wald J, et al. Autosomal recessive primary generalized dystonia in two siblings from a consanguineous family. *Mov Disord* 2005;**20**(2):245–7.
5. Chouery E, Kfoury J, Delague V, et al. A novel locus for autosomal recessive primary torsion dystonia (DYT17) maps to 20p11.22-q13.12. *Neurogenetics* 2008;**9**(4):287–93.
6. Charlesworth G, Angelova PR, Bartolomé-Robledo F, et al. Mutations in HPCA cause autosomal-recessive primary isolated dystonia. *Am J Hum Genet* 2015;**4**:657–65.
7. Dobričić V, Kresojević N, Marjanović A, et al. HPCA-related dystonia: too rare to be found? *Mov Disord* 2016;**31**(7):1071.
8. Camargos S, Scholz S, Simón-Sánchez J, et al. DYT16, a novel young-onset dystonia-parkinsonism disorder: identification of a segregating mutation in the stress-response protein PRKRA. *Lancet Neurol* 2008;**7**(3):207–15.
9. Ozelius LJ, Hewett JW, Page CE, et al. The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein. *Nat Genet* 1997;**17**(1):40–8.
10. Müller U. The monogenic primary dystonias. *Brain* 2009;**132**(8):2005–25.
11. Lohmann K, Schlicht F, Svetel M, et al. The role of mutations in COL6A3 in isolated dystonia. *J Neurol* 2016;**263**(4):730–4.
12. Fuchs T, Saunders-Pullman R, Masuho I, et al. Mutations in GNAL cause primary torsion dystonia. *Nat Genet* 2013;**45**:88–92.
13. Stamelou M, Charlesworth G, Cordivari C, et al. The phenotypic spectrum of DYT24 due to ANO3 mutations. *Mov Disord* 2014;**29**(7):928–34.