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Short communication

The prevalence of *PRKRA* mutations in idiopathic dystonia

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ABSTRACT

Introduction: DYT-*PRKRA* (DYT16) is considered a rare cause of dystonia-parkinsonism. The significance of this gene as a cause of dystonia and its phenotypical characterization must be determined in larger cohorts. We aimed to investigate the role of *PRKRA* in patients with dystonia.
Methods: We sequenced *PRKRA* in 153 unrelated Brazilian patients with idiopathic dystonia. The frequency of novel missense variants was investigated in healthy Brazilian controls and in public databases. Homozygosity in the *PRKRA* region was assessed through polymorphic markers.
Results: *PRKRA* variants were identified in seven probands with isolated dystonia, including a novel c.C795A variant in compound heterozygosity with the previously described c.C665T variant. Heterozygosity in the gene region was observed in two probands who were homozygous for c.C665T, indicating that this mutation originated from independent events, suggesting a hotspot.
Conclusion: *PRKRA* is not an unusual cause of idiopathic dystonia. In this cohort, it was responsible for 4.5% of the total of cases (4.9% of the isolated dystonia cases). The most common phenotype was early-onset isolated focal dystonia followed by generalization, parkinsonism was not observed. This is first report of *PRKRA* causing adulthood-onset dystonia. Screenings of large cohorts are recommended to investigate the role of this gene in isolated dystonia, as well as in dystonia-parkinsonism cases worldwide.

1. Introduction

Dystonia-parkinsonism related to DYT-*PRKRA* (DYT16) was first described by Camargos et al. in 2008, with the identification of a homozygous *PRKRA* variant in three Brazilian families with autosomal recessive inheritance, early-onset limb or cervical dystonia progressing to a generalized distribution, frequent association with parkinsonism and no response to anticholinergic or dopaminergic treatments [1].

PRKRA mutations are considered as a rare cause of dystonia but, till date, most of the descriptions have been limited to single family-based studies and only few screenings of larger cohorts have been conducted [1–6].

The significance of this gene as a cause of dystonia, as well as its phenotypical characterization, must be determined in larger cohorts. Here we described the screening of 153 unrelated dystonia patients of Brazilian origin, wherein we identified seven probands with *PRKRA* putative mutations.

2. Patients and methods

This study was approved by the institutional review board and informed written consent was obtained from all participants. A total of 153 unrelated Brazilian patients (56 males, 97 females) were recruited by movement disorders specialists in the participating centers, between 2012 and 2016. The inclusion criteria were

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based on the current consensus for the diagnosis of idiopathic dystonia [7]. Dystonia was assessed with the Fahn Marsden rating scale. Patients with focal, segmental, multifocal and generalized dystonia were included. Dystonia age of onset varied from 1 month-to 70 years (mean = 28.8 years). All patients had normal neuroimaging (brain computed tomography or magnetic resonance) and no evidence of metabolic or neurodegenerative diseases. The presence of parkinsonism was assessed with the Unified Parkinson's disease rating scale motor score (UDRS part III). Five had dystonia-parkinsonism, five had myoclonus-dystonia and one hundred and forty-three were isolated dystonia cases. Dystonia family history was positive for 16 patients, possible for 18 (history suggested dystonia, but they never received a medical diagnosis and were not available for clinical evaluation), 4 had no information and 115 denied dystonia in the family. Only patients without a known molecular diagnosis were included; part of this group was previously tested fot *TOR1A* and *THAP1* [8], *GNAL* [9], *SGCE* and *GCH1*, with negative results.Patients missing clinical information on possible causes of secondary dystonia, such as brain injury, birth conditions and use dopamine receptor blockers were excluded.

PRKRA (NM_003690.4) variants were assessed by targeted resequencing of peripheral blood DNA using a customized amplicon panel (TruSeq Custom Amplicon assay 1.5, Illumina) in the MiSeq system (Illumina, San Diego, CA), with an average coverage of 1250x. Paired-end sequences were analyzed using the TrueSeq Amplicon workflow available at Illumina Basespace. Briefly, quality control checks were conducted with FastQC (Phred $\geq$30), reads were aligned to the human reference genome (GRCh37/hg19) using Burrows-Wheeler Aligner (BWA) and variant calling was done with GATK v1.6 (Genome-Analysis-Tool-Kit). Mutect v1.1.5 and Pindel v0.2.5b8 were also used for calling SNPs and InDels. Variants were confirmed by Sanger sequencing. We verified the allele frequencies of novel nonsynonymous variants through the screening of 246 chromosomes from Brazilian healthy controls, in two Brazilian whole exome population databases (ABraOM [10] http://abraom.ib.usp.br and BIPMed-WES-db http://bipmed.org) and in the Genome Aggregation Database (gnomAD http://gnomad.broadinstitute.org/). *In silico* predictions of pathogenicity were performed using SIFT, MutationTaster, Polymorphism Phenotyping version 2 (PolyPhen-2), Protein Variation Effect Analyzer (PROVEAN), and Combined Annotation-Dependent Depletion (CADD) algorithms. The degree of amino acid conservation among species was assessed through protein multiple sequence alignment using Clustal Omega (http://www.clustal.org/omega/).

3. Results

PRKRA variants were identified in seven probands: Six probands had the c.C665T (p.P222L)/rs121434410 mutation in homozygosity, which has been previously described in DYT-*PRKRA* cases [1,3,4,6]. One proband was a compound heterozygous, presenting the c.C665T and a novel exon 8 variant, c.C795A (p.S265R). This variant changes a serine to an arginine in a conserved region of PACT domain 3 (supplementary fig. S1). It is not described in the population databases GnomAD (123136 exomes), ABraOM (609 Brazilian exomes), and BIPMed (106 Brazilian exomes) and it was also not found in 246 chromosomes from Brazilian controls. Except for Mutation Taster (score 110), all the other four algorithms indicated that this variant is deleterious: Polyphen-2 (score 1.000), SIFT (score 0.001), PROVEAN (score −3.491), and CADD score (28.1). The clinical characteristics of the probands are described in Table 1. Most of the cases had early-onset dystonia with generalization, but two had adulthood-onset (at 25 and 53 years). Parkinsonism was not observed. Except for two probands, none of them reported consanguinity in the family. To verify whether the homozygous c.C665T variant was originated by independent events, we screened four polymorphic SNVs covered by the panel (rs3997879, rs3997878, rs3997877, and rs150679361) and one frequent insertion (rs150523431), spanning a 15,930bp region of *PRKRA* containing this variant. Parents and other family members were not available for evaluation and genetic testing, therefore haplotypes could not be phased. Four of these markers were identified in the 3′UTR region and with a distance of less than 4.5 kb from c.C665. Heterozygous markers were identified in two patients who carried the homozygous c.C665T mutation, indicating that it was originated by, at least, two independent events in this group (Table 2).

4. Discussion

Until now, DYT-*PRKRA* has been considered as a rare cause of autosomal recessive familial dystonia-parkinsonism. In this study, we aimed to investigate its role in idiopathic dystonia and identified seven unrelated patients (4.5%) carrying putative pathological variants, all of them with pure dystonia (4.9% of the isolated dystonia cases). The most frequent variant associated with this condition is c.C665T. Affected members of Polish, Italian and the original Brazilian families carrying c.C665T share a stretch of homozygous markers identical by state surrounding the *PRKRA* region [1,4,6].

In our group of patients, we observed heterozygosity in this region, indicating that c.C665T originated by, at least, two independent events. The Brazilian population is highly admixed between Europeans, Amerindians and Africans. Except for one proband of Japanese ethnicity (patient 6), all others were admixed and could not precisely inform the ethnical background of their ancestors. We were able to trace back the birthplace of their grandparents, and most of them were born in Brazil; four families were from different cities of the Northeast, at a maximum distance of 1000 Km apart. In this case, we cannot rule out the possibility of a founder effect, but two of these probands had heterozygous markers in the gene region, suggesting independent events (Table 2). The original families carrying the c.C665T mutation [1] came from a different region of the country and had no relatives in the Northeast of Brazil (Dr. Sarah Camargos, personal communication). Considering that a third proband carrying this variant (patient 6) had Japanese ethnicity and was not admixed, we can speculate that a third independent event might have generated it, and that this site is a mutation hotspot.

Of note is that the majority of the patients were sporadic cases and lacked consanguinity, which is not surprising, considering this gene has mutation hotspot. Curiously, patient 2 who had consanguineous parents, showed heterozygosity for the markers in the gene region, indicating that each allele came from a different ancestor.

Except for the lack of parkinsonism, which has been reported in other studies [1,5], most of the patients had a phenotype similar to others previously described [1,4,6] with early-onset focal dystonia followed by generalization, and no response to anticholinergic or dopaminergic drugs. In DYT-*PRKRA*, the presence or absence of parkinsonism does not correlate with dystonia age of onset or its corporal distribution [1–6]. Other as yet unknown genetic factors can be contributing for the phenotypical variability. Although most patients carried the same variant, a wide range in age of onset was observed (8–53 years). Morover, this is the first report of *PRKRA* causing early and late-adulthood-onset dystonia. Upper or lower limbs are the preferential sites of onset [1–6]; in teenagers and adult patients, the cervical region may be affected from the beginning and the disease course can be milder, without generalization. Two cases (patients 4 and 6) had a short follow-up, therefore it was not possible to determine whether dystonia still

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