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Screening of *GNAL* variants in Brazilian patients with isolated dystonia reveals a novel mutation with partial loss of function

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Abstract

GNAL was identified as a cause of dystonia in patients from North America, Europe and Asia. In this study, we aimed to investigate the prevalence of *GNAL* variants in Brazilian patients with dystonia. Ninety-one patients with isolated idiopathic dystonia, negative for *THAPI* and *TORIA* mutations, were screened for *GNAL* variants by Sanger sequencing. Functional characterization of the G α_{olf} protein variant was performed using the bioluminescence resonance energy transfer assay. A novel heterozygous nonsynonymous variant (p.F133L) was identified in a patient with cervical and laryngeal dystonia since the third decade of life, with no family history. This variant was not identified in healthy Brazilian controls and was not described in 63000 exomes of the ExAC database. The F133L mutant exhibited significantly elevated levels of basal BRET and severely diminished amplitude of response elicited by dopamine, that both indicate substantial functional impairment of G α_{olf} in transducing receptor signals, which could be involved in dystonia pathophysiology. *GNAL* mutations are not a common cause of dystonia in the Brazilian population and have a lower prevalence than *THAPI* and *TORIA* mutations. We present a novel variant that results in partial G α_{olf} loss of function.

Keywords

dystonia; genetics; *GNAL*; DYT25; G α_{olf}

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CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

INTRODUCTION

Dystonia is a hyperkinetic movement disorder characterized by sustained or intermittent muscle contractions leading to abnormal movements and/or postures [1]. According to the latest consensus, the term “isolated dystonia” refers to a phenomenological characteristic where dystonia is the only motor feature, with the exception of tremor, and has no implications for the etiology [1]. In the etiology axis, the consensus defined three categories: inherited (dystonia forms of proven genetic origin), acquired (dystonia due to a known specific cause, except for genetic causes) and idiopathic (dystonia of unknown cause, including sporadic and familial cases). Dystonias initially classified as idiopathic can be reclassified as inherited, once causal genes are discovered. To date, several genes have been associated with isolated dystonia [2]. Mutations in *THAPI* and *TORIA* are well-known causes of this disorder and have been characterized in Brazilian patients [3]. The progress of next generation sequencing (NGS) technologies has led to the identification of new genes associated with this disorder. *GNAL* was recently identified as a causative gene for dystonia [4].

GNAL encodes α subunit of the stimulatory G protein ($G\alpha_{olf}$) with prominent expression in the striatum where it forms heterotrimeric complex with G protein $\beta\gamma$ subunits [5]. This complex couples dopamine D1 receptors in the region to stimulation of adenylyl cyclase type 5 that produces second messenger cAMP. Disruption of $G\alpha_{olf}$ can lead to impaired dopaminergic signal transduction in the striatum [5–7].

The prevalence of *GNAL* mutations in dystonia was initially reported as 15% in a study that analyzed specific families [4]. However, studies conducted in European and Asian populations reported frequencies below 2.6% [8–10]. Therefore, it is necessary to collect more data in order to determine the prevalence of *GNAL* variants, as well as to report novel variants with pathogenic potential in different populations.

In this study, we aimed to investigate the prevalence of *GNAL* variants as the cause of dystonia in a group of Brazilian patients. We identified a novel variant of the gene in a patient with dystonia and showed that it alters $G\alpha_{olf}$ function.

METHODS

Patients

Ninety-one patients with idiopathic isolated dystonia were recruited by movement disorders specialists participating in the project “Brazilian Network for the study of dystonia” approved by the institutional review board. Informed written consent was obtained from all participants. They have been screened in a previous study and were negative for mutations in *TORIA* and *THAPI* [3]. Table 1 lists the patients’ clinical characteristics.

Molecular Analysis

DNA was extracted from peripheral blood using the Gentra Puregene® Blood DNA Kit (Qiagen) according to the manufacturer’s instructions. *GNAL* variants were screened in the 12 exons and splice sites of the isoforms NM_182978 and NM_001142339 by Sanger

sequencing on an ABI 3500 XL Genetic Analyzer (Applied Biosystems) following standard protocols. Primer sequences and PCR conditions were similar to those described by Fuchs et al. [4], except for exon 1 of NM_001142339 and for exon 2 (1F-gaaacaattctcgtgtaaaaag/1R-tagccgggtctgagcaatc and 2F-attgctcagaccggctag/2R-gaatgtctactccattatc).

Whenever nonsynonymous variants were identified in the patients, allelic frequencies were investigated in 234 chromosomes of healthy Brazilian controls and in a population database of 63000 exomas (ExAC – Exome Aggregation Consortium). *In silico* analysis was performed using PolyPhen-2, SIFT, Mutation Taster, PROVEAN and CADD scores. The degree of amino acid conservation among species was accessed through the ClustalW2 program (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>).

Functional characterization was performed using the improved bioluminescence resonance energy transfer (BRET) assay [11] similar in design and concept to the assay previously used to evaluate the effect of mutations in $G\alpha_{olf}$ [4]. Briefly, constructs encoding dopamine D1 receptor, $G\alpha_{olf}$, Venus155–239– $G\beta_1$, Venus1–155– $G\gamma_2$, masGRK3ct-Nluc, PTX-S1 and Flag-tagged Ric-8B were transfected into HEK293/17 cells at a 1:6:1:1:1:1 ratio, with 7.5 μ g of total DNA delivered per 3.5×10^6 cells. After 16 h, cells were stimulated with 100 μ M dopamine followed by treatment with 100 μ M of D1 receptor antagonist SCH39166. One-way ANOVA followed by Tukey's post-hoc test was performed to determine statistically significant differences relative to wild-type control.

RESULTS

We identified four synonymous variants (c.66C>T, c.132C>T, c.1014C>T and c.1056C>T) in six probands and a missense variant c.399C>A (p.F133L) in a single proband (numbering is based on NM_001142339 and NP_001135811).

The variant p.F133L was identified in a 59 year-old female patient with cervical and laryngeal dystonia since the age of 23, with no family history. She was responsive to botulinum toxin. This variant was neither found in the 234 chromosomes of the normal controls nor among the 63000 exomas of the ExAC database (November, 2015). Protein alignment in ClustalW showed that phenylalanine at position 133 is well conserved throughout the species (Figure 1). The SIFT algorithm predicted that this variant is tolerated but *in silico* analyses using the Mutation Taster, PolyPhen-2 and PROVEAN algorithms predicted that it is detrimental to protein function. The scaled CADD score for this variant is 26.6, suggesting it is deleterious. To investigate the functional consequences we examined the impact of F133L mutation on the activation of $G\alpha_{olf}$ by D1R receptors using the BRET assays (Figure 2). In this system, application of dopamine triggers $G\alpha_{olf}$ dissociation from $G\beta\gamma$ subunits producing BRET signal which is quenched by subsequent antagonist application. The F133L mutant exhibited significantly elevated levels of basal BRET and severely diminished amplitude of response elicited by dopamine, that both indicate substantial functional impairment of $G\alpha_{olf}$ in transducing receptor signals. Despite significant reduction in functional activity, mutant $G\alpha_{olf}$ maintained considerable functional activity leading us to conclude that the mutation causes a partial loss of function.

DISCUSSION

In this study, we investigated *GNAL* variants in a group of Brazilian patients with idiopathic dystonia who were negative for *THAPI* and *TORIA* mutations and identified a novel putative pathogenic variant (p.F133L). The prevalence of disruptive *GNAL* mutations in this cohort of dystonia patients was 1.09%, which is consistent with those recently reported between 0.5% and 2.6% among individuals of European and Asian ancestry [8–11]. In Brazilian patients, idiopathic dystonia associated to *GNAL* mutations is less frequent than dystonia associated to *THAPI* (8.8%) or *TORIA* (2.6%) mutations [3].

Dystonia caused by mutations in *THAPI*, *TORIA* and *GNAL* differ in terms of clinical characteristics, such as age of onset, site of onset and progression. Several studies indicate that phenotype is a major predictor for genotype, however, there is some degree of heterogeneity, and less typical cases affected by mutations in these genes may have overlapping characteristics [3,4,12]. The typical *TORIA* phenotype is characterized by early-onset (mean 13 years) limb dystonia (arms and legs equally affected) which frequently generalizes. Spread to cranial muscles is less common [3]. In *THAPI* mutations symptomatic carriers, the most frequent phenotype is early-onset dystonia (mean 16 years) initially in the arm or craniocervical region, legs are rarely the site of onset. Approximately half of the cases progress to multifocal/generalized forms. Dysphonia is also an important feature in more than two thirds of the cases [3]. The typical *GNAL* patient presents with adult-onset (mean 32 years) segmental dystonia (craniocervical), the neck is the most common site of onset, arm onset is rare, dystonia remains focal/segmental in most of the cases, speech is affected in approximately 44%, leg involvement is not common [4, 10, 12]. The patient carrying the p.F133L variant was of Italian and Portuguese ethnicity and a sporadic case with a typical phenotype of cervical dystonia developing the disease in the third decade of life. As we did not have access to the parents' DNA, we could not confirm whether the absence of a family history was due to a *de novo* mutation or incomplete gene penetrance. Other unaffected family members were not available to perform a segregation analysis, this information would have conclusively supported the pathogenic role of this variant. The functional assay revealed deficits in $G\alpha_{olf}$ coupling to D1R receptor. It has been demonstrated that the levels of $G\alpha_{olf}$ directly influence both basal and D1R-activated cAMP production in the striatum [5]. Thus, reduction in $G\alpha_{olf}$ function is expected to result in the reduction of adenylyl cyclase activation. Although we did not perform further functional studies, we can speculate that $G\alpha_{olf}$ variant F133L ultimately contributes to the disease phenotype by impairing coupling of neurotransmitter receptor signaling to cAMP production in the striatal neurons.

This is the first study to investigate *GNAL* variants in patients from Brazil. Our data confirm *GNAL* mutations as a cause of dystonia in this sample of patients with a lower prevalence than *THAPI* and *TORIA* mutations. The newly identified *GNAL* variant contributes to our understanding of this debilitating disorder. Future studies should be conducted to elucidate the mechanisms by which mutations in this gene cause dystonia in order to identify ideal therapeutic targets.

Acknowledgments

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References

1. Albanese A, Bhatia K, Bressman SB, et al. Phenomenology and classification of dystonia: a consensus update. *MovDisord*. 2013; 28(7):863–873.
2. Ozelius LJ, Lubarr N, Bressman SB. Milestones in dystonia. *Mov Disord*. 2011; 26(6):1106–26. [PubMed: 21626555]
3. da Silva FP Junior, dos Santos CO, Silva SM, et al. Novel THAP1 variants in Brazilian patients with idiopathic isolated dystonia. *J Neurol Sci*. 2014; 344(1–2):190–192. [PubMed: 24976531]
4. Fuchs T, Saunders-Pullman R, Masuho I, et al. Mutations in GNAL cause primary torsion dystonia. *Nat genet*. 2013; 45(1):88–92. [PubMed: 23222958]
5. Hervé D. Identification of a specific assembly of the g protein golf as a critical and regulated module of dopamine and adenosine-activated cAMP pathways in the striatum. *Front Neuroanat*. 2011; 5:48. [PubMed: 21886607]
6. Corvol JC, Studler JM, Schonn JS, Girault JA, Hervé D. Galpha(olf) is necessary for coupling D1 and A2a receptors to adenylyl cyclase in the striatum. *J Neurochem*. 2001; 76(5):1585–1588. [PubMed: 11238742]
7. Vemula SR, Puschmann A, Xiao J, et al. Role of Gα(olf) in familial and sporadic adult-onset primary dystonia. *Hum Mol Genet*. 2013; 22(12):2510–2519. [PubMed: 23449625]
8. Erro R, Bhatia KP, Hardy J. GNAL mutations and dystonia. *JAMA Neurol*. 2014; 71(8):1052–1053. [PubMed: 25111208]
9. Charlesworth G, Bhatia KP, Wood NW. No pathogenic GNAL mutations in 192 sporadic and familial cases of cervical dystonia. *MovDisord*. 2014; 29(1):154–155.
10. Kumar KR, Martemyanov KA, Lohmann K. GNAL mutations and dystonia--reply. *JAMA Neurol*. 2014; 71(8):1053–1054. [PubMed: 25111209]
11. Masuho I, Martemyanov KA, Lambert NA. Monitoring G Protein Activation in Cells with BRET. *Methods Mol Biol*. 2015; 1335:107–113. [PubMed: 26260597]
12. Saunders-Pullman R, Fuchs T, San Luciano M, et al. Heterogeneity in primary dystonia: lessons from THAP1, GNAL, and TOR1A in Amish-Mennonites. *MovDisord*. 2014; 29(6):812–818.

	p.F133L
	↓
<i>H. sapiens</i> (NP_001135811.1)	YSQEFFD <u>H</u> V
<i>P. troglodytes</i> (XP_001172063.1)	YSQEFFD <u>H</u> V
<i>M. musculus</i> (NP_034437.1)	YSQEFFD <u>H</u> V
<i>R. norvegicus</i> (XP_002728601)	YSQEFFD <u>H</u> V
<i>C. lupus</i> (XP_849960.1)	YSQEFFD <u>H</u> V
<i>B. taurus</i> (NP_001096024.1)	YSQEFFD <u>H</u> V
<i>G. gallus</i> (NP_001008746.1)	YSQEFFD <u>H</u> V
<i>D. rerio</i> (NP_001007340.1)	YTQEFFE <u>H</u> A
<i>V. pacos</i> (XP_006213685.1)	YTEEFFE <u>H</u> A
<i>D. melanogaster</i> (FBgn0001123.1)	YPPEFYE <u>H</u> T
<i>C. elegans</i> (R06A10.2)	YPSEFYD <u>H</u> I
<i>Trichinella sp.</i> (KRX57741.1)	FLQDFFD <u>R</u> V

Figure 1.

Cross-species protein sequence alignment of Gaolf using ClustalW2.

Phenylalanine at position 133 is highly conserved.

Figure 2. Functional effects of F133L substitution in $G\alpha_{olf}$ assessed by the Bioluminescence resonance energy transfer (BRET) assay

A) Time course of G_{olf} activation in D1R-transfected cells. Responses to dopamine and SCH39166 (D1R selective antagonist) were monitored. Variations of p.F133L from wild-type $G\alpha_{olf}$ (wt) in the BRET signal indicate impaired function of the mutant. Representative traces are shown (n = 6). B) High basal BRET signal from p.F133L mutant before dopamine application suggests impairment of $G\alpha_{olf}/G\beta\gamma$ trimer formation. C) Unlike the wild-type protein (wt), the altered form (p. F133L) showed small response to dopamine application, which is consistent with impaired association with the $G\beta\gamma$ dimer. Values shown in B and C represent mean $\pm$ SEM from three independent experiments. One-way ANOVA followed by

Tukey's post-hoc test was performed to determine statistically significant differences relative to wild-type control. Triple asterisk (***) indicates $P < 0.001$, t-test.

Table 1

Clinical characteristics of the probands with dystonia.

Sex	Male: 30 (33%)
	Female: 61 (67%)
Age at exam	Mean: 48.7 years (SD± 17.5years)
	Range: 2 to 84 years
Dystonia age of onset	Mean: 32.5 years (SD± 18.1 years)
	Range: 3 months to 68 years
	Median: 29 years
Age of onset Distribution	Infancy (0 – 2 years): 2 (2.2%)
	Childhood (3 – 12 years): 15 (16.5%)
	Adolescence (13 – 20 years): 11 (12.1%)
	Early adulthood (21 – 40 years): 31(34%)
	Late adulthood (> 40 years): 32 (35.2%)
Family history	Negative: 67 (73.6%)
	Positive: 11 (12.1%)
	Possible *: 10 (11%)
	Not informed: 3 (3.3%)
Dystonia Distribution	Focal: 39 (42.8%)
	Segmental: 34 (37.4%)
	Generalized: 13 (14.3%)
	Multifocal: 5 (5.5%)

* Subjects reported family history of dystonia but family members were not examined for confirmation.