cervical involvement that sometimes starts in childhood or young adulthood comparable to patients with DYT27 dystonia. Up to 10% of these patients show generalization, and brachial manifestation has not yet been described in DYT25 dystonia.[11-13] Finally, dominant DYT24 dystonia (*ANO3* mutation) can also cause cranio-cervical dystonia with focal and segmental distribution, but more often with late onset.[7,13-15]

Considering the age of onset as another important diagnostic criterion, the DYT27 dystonia extends the spectrum of early-onset dystonia, which so far mainly consisted of DYT1, DYT2, DYT6, DYT16, and in some cases DYT24 and DYT25.

In conclusion, recessive mutations in the *COL6A3* gene cause early-onset isolated dystonia with focal, segmental, or generalized distribution in the cranio-cervical region, upper limbs, and trunk with interindividual heterogeneity. Therefore, even if the pathogenic role of *COL6A3* mutations in autosomal-recessive dystonia needs to be confirmed by other groups, this newly described entity should be considered when treating patients with similar phenotypes and suspected recessively inherited dystonia. ■

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

GNAL Mutation in Isolated Laryngeal Dystonia

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ABSTRACT

Background: Up to 12% of patients with laryngeal dystonia report a familial history of dystonia, pointing to involvement of genetic factors. However, its genetic causes remain unknown.

Method: Using Sanger sequencing, we screened 57 patients with isolated laryngeal dystonia for mutations in known dystonia genes *TOR1A* (DYT1), *THAP1* (DYT6), *TUBB4A* (DYT4), and *GNAL* (DYT25). Using functional MRI, we explored the influence of the identified mutation on brain activation during symptomatic task production.

Results: We identified 1 patient with laryngeal dystonia who was a *GNAL* mutation carrier. When compared with 26 patients without known mutations, the *GNAL*

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carrier had increased activity in the fronto-parietal cortex and decreased activity in the cerebellum.
Conclusions: Our data show that *GNAL* mutation may represent one of the rare causative genetic factors of isolated laryngeal dystonia. Exploratory evidence of distinct neural abnormalities in the *GNAL* carrier may suggest the presence of divergent pathophysiological cascades underlying this disorder. © 2016 International Parkinson and Movement Disorder Society
Key Words: Dystonia, spasmodic dysphonia, genetic factors, neuroimaging

Introduction

Isolated laryngeal dystonia (LD), or spasmodic dysphonia, is a focal adult-onset dystonia primarily affecting speech production. LD is characterized by involuntary spasm-inducing voice breaks with strained and strangled voice quality in the adductor form (ADLD) and excessive breathiness in the abductor form (ABLD). Despite well-described clinical symptoms, the underlying causes of this disorder remain unknown because challenges associated with traditional genetic studies have hindered the identification of LD-specific causative genes. On the other hand, familial history of dystonia in about 12% of LD patients[1,2] points to the contribution of genetic risk factors. Laryngeal involvement has also been reported in the cohorts of patients with generalized or segmental dystonias who are carriers of DYT1, DYT4, DYT6, and most recently, DYT25 mutations.[3-5] However, it is rare that any of these known gene mutations result in isolated LD.

In this study, we investigated the contribution of DYT1, DYT4, DYT6, and DYT25 mutations as possible genetic causes of isolated sporadic and hereditary LD using Sanger sequencing of the corresponding coding regions. Because genes have a direct influence on brain organization,[6] we conducted an exploratory study to examine brain activation in an identified mutation carrier when compared with healthy controls and sporadic and familial LD cases using functional MRI during symptomatic speech and syllable production.

Materials and Methods

In this study, 57 patients with isolated LD were recruited for genetic screening of known dystonia genes *TOR1A* (DYT1), *THAP1* (DYT6), *TUBB4A* (DYT4), and *GNAL* (DYT25), which were previously found in dystonias with laryngeal involvement. A total of 41 patients (23 ADLD/18 ABLD) were sporadic, and 16 patients (12 ADLD/4 ABLD) had a family history of LD and/or other primary dystonias (see demographic details in Table 1). All patients except 1 ABLD (African American/Jewish) and 1 ADLD (African American) were Caucasian. None of the patients had any present or past history of other neurological, psychiatric, or laryngeal problems. All patients had isolated LD, which was confirmed by fiberoptic nasolaryngoscopy, without involvement of any other body regions. The average age of onset was 40.9 ± 12.3 (mean ± standard deviation) in all sporadic LD patients, and 40.9 ± 13.1 in all familial LD patients (see further demographic details per clinical phenotype in Table 1).

As an exploratory study to investigate possible neural correlates of gene mutations in LD, 27 ADLD patients from the same cohort, including 13 sporadic ADLD, 13 familial ADLD, and 1 mutation carrier ADLD (18 women/9 men; 58.9 ± 9.6 years of age), as well as 27 age- and gender-matched healthy controls (18 women/9 men; 53.9 ± 9.4 years of age) underwent

TABLE 1. Demographics of participants

	Type of LD	Gender	Age at exam (mean ± SD)	Age at onset (mean ± SD)	Ethnicity
Sporadic LD	All (N = 41)	33 F/8 M	57.6 ± 10.6	40.9 ± 12.3	39W/1AA/1AA-J
	ADLD (n = 23)	16 F/7 M	57.9 ± 10.4/59.7 ± 9.2	41.2 ± 12.6/41.9 ± 13.1	22W/1AA
	ABLD (n = 18)	17 F/1 M	58.8 ± 9.4/70	41.5 ± 12.4/58	17W/1AA-J
Familial LD	All (N = 16)	13 F/3 M	61.2 ± 11.1	40.9 ± 13.1	16W
	ADLD (n = 12)	10 F/2 M	56.9 ± 10.7/58.6 ± 10.4	40.6/13.3 ± 16.8	12W
	ABLD (n = 4)	3 F/1 M	58.7 ± 10.9/63	40.9 ± 15.6/31	4W
Controls	All (N = 12)	18 F/9 M	53.9 ± 9.4	NA	12W
Handedness			All: right on Edinburgh Inventory		
Language			All: monolingual English speakers		
Cognitive status			All: Mini-Mental State Examination ≥ 27 points		

The average age (± standard deviation). Healthy controls, no history of neurological, psychiatric, or laryngeal problems; LD, laryngeal dystonia; ADLD, adductor laryngeal dystonia; ABLD, abductor laryngeal dystonia; W, white; AA, African American: J, Jewish; F, female; M, male; NA, not applicable; SD, standard deviation.

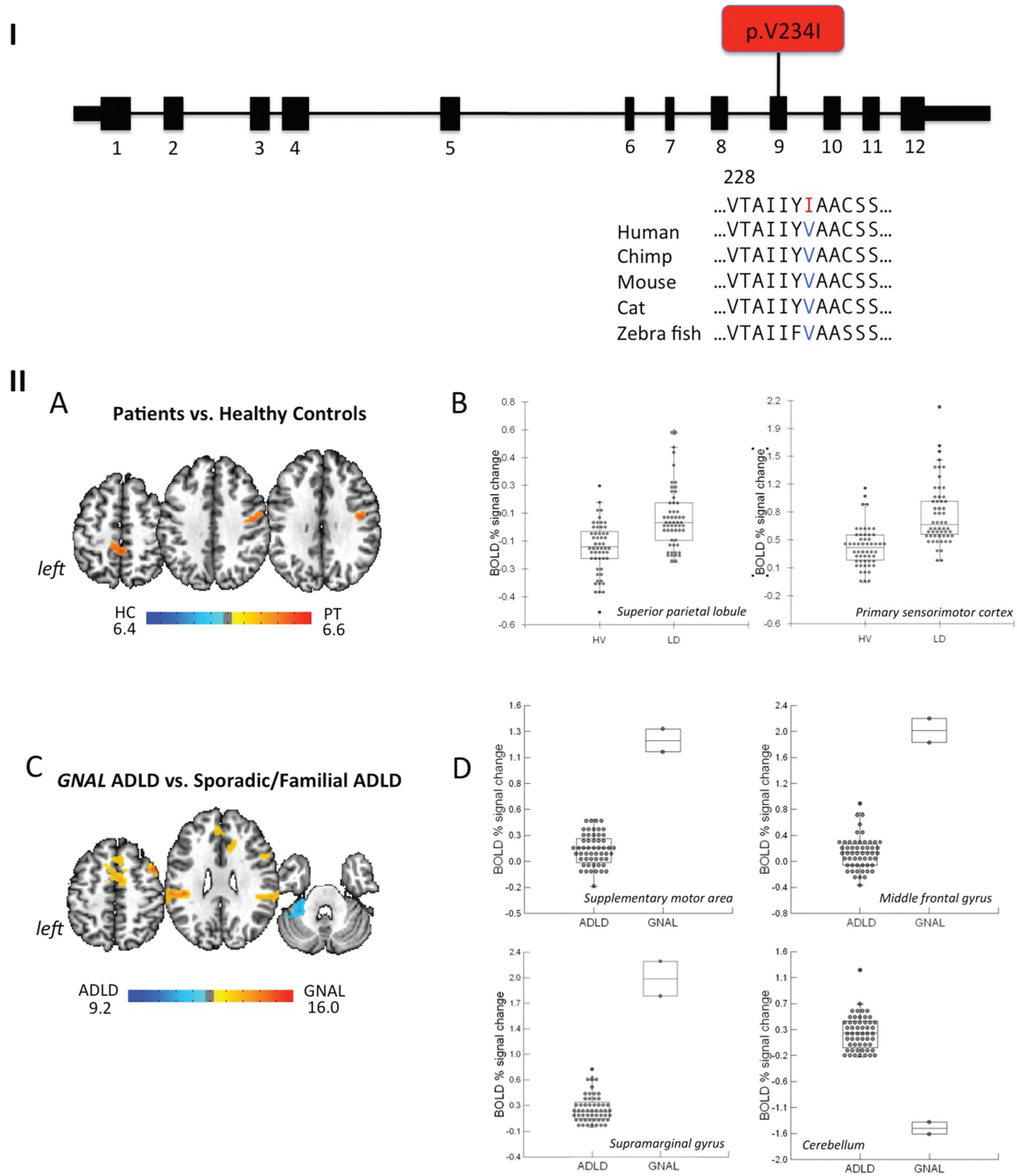

FIG. 1. (I) Mutation identified in the *GNAL* gene in an adductor laryngeal dystonia (ADLD) patient. Schematic of the exon-intron structure of the short isoform of *GNAL* with a mutation shown in red. Protein sequence alignment of $G_{\alpha olf}$ across species is obtained from RefSeq database and aligned using MutationTester. Altered residue is colored in red. **(II) Differences in brain activation during symptomatic voice production in the *GNAL* mutation carrier when compared with healthy controls and patients with sporadic and familial laryngeal dystonia.** (A) Statistically significant differences in brain activation during symptomatic sentence and syllable production between all 27 laryngeal dystonia patients, including the *GNAL* mutation carrier, sporadic and familial cases without known mutations, and 27 healthy controls are presented on a series of axial brains slices in the standard Talairach-Tournoux space (B) with the BOLD percent signal change in each individual shown on the bar charts at a family-wise error–correction of $P \leq .05$. (C) Significant differences between one *GNAL* mutation carrier and 26 sporadic and familial laryngeal dystonia patients are shown on a series of axial slices in the standard Talairach-Tournoux space with (D) the corresponding bar charts depicting the individual levels of BOLD percent signal change during the production of symptomatic sentences and syllables at a family-wise error–correction of $p \leq 0.05$. HC, healthy controls; PT, patients.

brain functional MRI during LD-symptomatic task production. The patients who received regular botulinum toxin injections for symptom management participated in the MRI study only if they were symptomatic at least 3 to 4 months after their last injection. All participants gave written, informed consent, which was approved by the institutional review board of the Icahn School of Medicine at Mount Sinai.

Genomic DNA was isolated from blood following the Purgene procedure (Gentra Systems, Minneapolis, Minnesota).

Polymerase chain reaction amplification across the GAG deletion region of the *TOR1A* gene was performed as previously described.[7] All exons and flanking regions of the *THAP1, TUBB4A*, and *GNAL* genes were sequenced as previously described.[5,8,9] Polymerase chain reaction products were enzymatically cleaned and sequenced by Sanger sequencing.[8]

MRI data were acquired on a 3 Tesla Philips scanner with an 8-channel head coil. Whole-brain functional brain images were obtained using a gradient-weighted echo planar imaging pulse sequence (repetition time (TR) = 2 seconds per volume and 10.6 seconds between volumes, echo time (TE) = 30 ms, flip angle (FA) = 90, field of view (FOV) = 240 mm, voxel size = 3.75 × 3.75 mm, 36 slices with 4-mm slice thickness) and an event-related sparse-sampling experimental design during the production of LD-symptomatic sentences (eg, "Are the olives large?"), syllables /iʔi/ and resting baseline, as described earlier.[10] A high-resolution T_1-weighted image was obtained for anatomical reference using magnetization prepared rapid gradient echo sequence (TR = 7.5 ms, TE = 3.4 ms, inversion time (TI) = 819 ms, FA = 8 degrees, FOV = 210 mm, 172 slices with 1-mm slice thickness). Following standard-image preprocessing and smoothing with 4-mm full-width at half-maximum Gaussian kernel, multiple linear regression was used to analyze task-related responses with a single regressor for the task convolved with a canonical hemodynamic response function and 6 motion parameters, including 3 translations along the XYZ axes and 3 rotations (pitch, roll, and yaw) as covariates of no interest. Statistical comparisons were performed using 2-sample *t* tests between 27 ADLD patients and 27 healthy controls to confirm the presence of brain abnormalities as reported earlier[10-12] and between 1 ADLD mutation carrier and 26 ADLD patients without known mutations to explore the pattern of abnormalities in the mutation carrier. The statistical significance was set at a family-wise error–corrected $P \leq .05$.

Results

Among 57 LD patients, none were carriers of *TOR1A* (DYT1), *THAP1* (DYT6), or *TUBB4A* (DYT4) mutations. However, 1 sporadic ADLD patient was a carrier of a novel coding variant in the *GNAL* (DYT25) gene. Clinically, this patient (Caucasian male, 37 years old at the time of initial evaluation with the onset of LD at the age of 36) presented with isolated ADLD without past or present familial history of dystonia or any other movement disorders. At the 4-year follow-up after the onset of disorder, the patient continued to exhibit isolated LD only, although a possibility of future spread of dystonia to other body regions cannot be ruled out. The mutation caused a G→A change in the coding region of the *GNAL* gene (at genomic position chr.18:11868562; hg19/GRCh37), resulting in an amino acid substitution, p.V234I (isoform NM_001142339) (Fig. 1-I). This variant is predicted by PolyPhen-2[13] to be probably damaging and by SIFT[14] to be deleterious. In addition, cross-species alignment using MutationTaster[15] showed that this variant gives rise to an amino acid substitution in a region of *GNAL* that is highly conserved throughout evolution. This mutation is not present in any of the variant databases including dbSNP 142 (http://www.ncbi.nlm.nih.gov/SNP/), the Exome Variant Server, NHLBI GO Exome Sequencing Project (http://evs.gs.washington.edu/EVS/), or the Exome Aggregation Consortium (http://exac.broadinstitute.org). This same variant was also present in the patient's unaffected mother, confirming the reduced penetrance of *GNAL* mutations as shown previously.[5,16]

At the neural level, comparisons between healthy controls and all ADLD patients, including both sporadic and familial patients as well as the *GNAL* carrier, showed typically increased brain activation during symptom production in the primary sensorimotor cortex and superior parietal lobule as reported previously[10,11] (Fig. 1-IIA,B). However, when comparing the *GNAL* mutation carrier with a group of sporadic and familial LD patients (both groups without known dystonia mutations), significant activation increases were identified in the supplementary motor area, middle frontal gyrus, and supramarginal gyrus that were distinctive of the *GNAL* mutation carrier, whereas the cerebellum was overactivated in the sporadic/familial ADLD patients (Fig. 1-IIC). Mean BOLD percentage signal change in the significant clusters showed that the *GNAL* carrier resided significantly outside the range of values of all sporadic and familial ADLD patients (Fig. 1-IID).

Discussion

Mutations in *GNAL* (DYT25), the first gene identified in adult-onset dystonia,[5] have been reported in approximately 0.4% to 1.7% of both sporadic and familial patients with predominantly cervical or cranio-cervical segmental dystonias.[5,16-23] Our finding of 1 *GNAL* carrier in 57 LD patients (1.8%) demonstrates that rare mutations in this gene do cause isolated LD, thus broadening the range of clinical

phenotypes of dystonia associated with *GNAL* mutations. Our findings further indicate that gene mutations may underlie even sporadic presentations of dystonia as a result of reduced penetrance, thus stratification of patients into sporadic and familial cases remains somewhat arbitrary pending the discovery of novel genetic factors contributing to this disorder. Because none of patients from our cohort had coding mutations in *TOR1A, THAP1*, or *TUBB4A* genes, these mutations are perhaps either absent or as rare as 0.4%[24] in patients with isolated LD.

GNAL encodes the stimulatory α subunit of the G protein, G_{olf}, and has been linked to the mediation of odorant signaling in the olfactory epithelium,[25] striatal dopaminergic signaling via coupling with D_1 receptors of the direct pathway, and adenosine A2A receptors of the indirect pathway[26-31] as well as being colocalized with corticotropin-releasing hormone receptors in the cerebellar Purkinje cells.[16] Relevant to dystonia pathophysiology, abnormal dopaminergic function and altered structural and functional organization of the cerebellum were previously reported in isolated LD.[10-12,32-34] Because dopamine is one of the main modulators of brain function during cognitive and executive processes, the effects of striatal dopaminergic abnormalities may be reflected in aberrant fronto-parietal cortical activity, leading to additional alterations at the preparatory and sensorimotor integrative stages of motor sequence execution in the *GNAL* mutation carrier when compared with other LD patients. On the other hand, a similar level of abnormalities in the primary sensorimotor cortex appeared to be a shared feature of brain changes across all LD patients when compared with healthy controls. Greater cerebellar alterations in sporadic and familial cases without known genetic causes when compared with the *GNAL* mutation carrier are suggestive of the distinct contribution of this structure to the pathophysiology of different forms of dystonia. Although this fMRI study compared a single *GNAL* mutation carrier to a larger group of isolated LD cases with no known mutations, our results should be interpreted with caution because they offer only initial clues about the potential links between a particular pattern of brain activity and genetic status in LD and suggest that future studies of *GNAL* mutation carriers in LD and other forms of isolated dystonia are warranted. ■

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Altered Inhibitory Interaction Among Inferior Frontal and Motor Cortex in L-dopa-induced Dyskinesias

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ABSTRACT

Background: Levodopa-induced dyskinesias are associated with thalamo-cortical disinhibition and frontal area overactivation. Neuroimaging and transcranial magnetic stimulation studies have highlighted the involvement of the right inferior frontal cortex in levodopa-induced dyskinesias.

Methods: Using transcranial magnetic stimulation, we tested connectivity between the inferior frontal and contralateral motor cortex in Parkinson's disease patients with and without levodopa-induced dyskinesias compared with age-matched controls. Furthermore, in dyskinetic patients, connectivity between the inferior frontal and contralateral motor cortex was assessed before and after a single session of continuous theta-burst stimulation applied over the inferior frontal cortex.

Results: Dyskinetic patients showed abnormal facilitatory connectivity between the inferior frontal and motor cortex when compared with the nondyskinetic group. Continuous theta-burst stimulation over the inferior frontal cortex eliminated such facilitatory connectivity and decreased the levodopa-induced dyskinesias that was induced by a supramaximal dose of levodopa.

Conclusion: In dyskinetic patients, a weaker inhibitory cortico-cortical interaction between the inferior frontal and contralateral motor cortex could be involved in levodopa-induced dyskinesias and restored by continuous theta-burst stimulation over the inferior frontal cortex. © 2016 *Movement* Disorder Society

Key Words: Parkinson's disease; connectivity, transcranial magnetic stimulation, inferior frontal cortex, levodopa-induced dyskinesia

Introduction

Levodopa-induced dyskinesias (LIDs) are a common complication of Parkinson's disease (PD) as a result of long-term levodopa therapy.[1,2] LIDs are considered the consequences of a pathological imbalance between "direct" and "indirect pathways."[3-5] Alternative models suggest an abnormal glutamatergic transmission in motor areas[6] and maladaptive striatal plasticity[7] as possible explanations for LIDs. Transcranial magnetic stimulation (TMS) studies have highlighted an overactivation of frontal areas, mainly the motor cortex and supplementary motor area (SMA).[8-10] Neuroimaging studies suggest that LIDs could depend on dysfunctional coupling between the right inferior frontal cortex (IFC) and the SMA[11] or an abnormal increase of gray matter in the IFC,[12] identifying a right-lateralized network involved in inhibitory control.[13-16] In particular, dyskinetic patients have been characterized by altered patterns of functional connectivity between the right IFC, contralateral M1, and the ipsilateral putamen. This could reflect a pathological inhibitory control of the IFC over the primary motor cortex. We recently demonstrated that continuous theta-burst stimulation (cTBS) applied on the right IFC, a protocol that induces long-term depression-like effects, is partially able to counteract the overactivity of motor

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