Mutations in GNAL cause primary torsion dystonia

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Dystonia is a movement disorder characterized by repetitive twisting muscle contractions and postures1–2. Its molecular pathophysiology is poorly understood, in part owing to limited knowledge of the genetic basis of the disorder. Only three genes for primary torsion dystonia (PTD), TOR1A (DYT1)3, THAP1 (DYT6)4 and CIZ1 (ref. 5), have been identified. Using exome sequencing in two families with PTD, we identified a new causative gene, GNAL, with a nonsense mutation encoding p.Ser293* resulting in a premature stop codon in one family and a missense mutation encoding p.Val137Met in the other. Screening of GNAL in 39 families with PTD identified 6 additional new mutations in this gene. Impaired function of several of the mutants was shown by bioluminescence resonance energy transfer (BRET) assays.

PTD is a subgroup of dystonia that was originally considered idiopathic. It is defined by the presence of dystonia (with or without tremor) as the only neurological sign, as well as the absence of historical, imaging or laboratory findings, suggesting an acquired cause or non-primary form of dystonia (for example, Wilson disease)6. PTD is clinically and causally heterogeneous, with a substantial genetic component7. Analyses of clinical subgroups, for example, focal adult and early-onset generalized, are consistent with autosomal dominant inheritance8–10 but with reduced penetrance, which ranges from 15% for focal PTD to 30–60% for generalized PTD10–12. Incomplete penetrance, together with genetic and clinical heterogeneity, complicates the identification of genes involved in PTD by traditional linkage analysis. As an alternative, we employed exome sequencing to systematically search for causative genes for PTD.

We performed exome sequencing in individuals from two families with PTD (Supplementary Fig. 1, asterisks). The clinical features of the affected individuals in these families are summarized in Table 1 and Supplementary Table 1 (families D1 and D)13–15. Exome sequencing produced approximately 50 million paired reads per sample, more than 97% of which were mapped to the genome. The average coverage was 52×, with more than 80% of targeted bases covered at 20×. Reads were mapped to the human reference genome sequence (assembly GRCh37/hg19), and allelic variants were detected. About 60,000 variants were called per individual (Supplementary Table 2). Because dystonia is rare and inherited in an autosomal dominant manner in these pedigrees, the causative mutation is expected to be an extremely rare heterozygous variant shared by all affected family members. Comparing sequenced family members, we found 11,124 heterozygous shared variants in family P and 4,578 in family D1. These variants were further compared to dbSNP release 132, with 458 and 208 previously unknown variants identified in each family, respectively. After annotation by both the SIFT16 and SeattleSeq Annotation servers, 68 missense and 1 nonsense variants remained in family P, and 20 missense variants were defined in family D1 (Supplementary Table 2). Identification of insertion and/or deletion (indel) variants was performed in a similar fashion for family P only (Online Methods and Supplementary Table 3).

Family P was previously subjected to a whole-genome scan that identified 3 regions of potential linkage, with logarithm of odds (LOD) scores ranging from 1.5 to 2.1 (data not shown). Within these regions, we found seven new coding variants: four single-nucleotide substitutions and three indels that were shared by all three affected individuals in family P.

Among these variants, the mutation encoding a p.Ser293* alteration in the GNAL gene cosegregated with dystonia in the remaining members of family P (Supplementary Fig. 1). Furthermore, an additional variant in this gene, encoding a p.Val137Met alteration, was found among the 20 missense variants shared by all 4 members in family D1; it segregated with the disease in this family (Supplementary Fig. 1) and was not observed in the 572 control chromosomes we tested. In addition, neither the p.Ser293* nor p.Val137Met alteration was present in any of the 274 control chromosomes we tested.

In summary, GNAL encodes an S-adenosylmethionine (SAM)-dependent methyltransferase, with biological activity of the enzyme shown by bioluminescence resonance energy transfer (BRET) assays. The molecular mechanism of this new functional gene remains to be determined.

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found in ~3,500 European exomes in the National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project database.

To confirm GNAL as a causative gene for dystonia, we performed Sanger sequencing of its entire coding region in 39 additional families with multiplex PTD of mixed European origin who were negative for mutations in TOR1A and THAP1. We detected new mutations in the GNAL gene in six additional families (19%), including one nonsense mutation (encoding p.Arg21*), two frameshift mutations (encoding p.Ser95fs*110 and p.Arg198fs*210), one missense mutation (encoding p.Glu155Lys), one in-frame deletion of three amino acids (p.Pro102_Leu104del) and one possible splice-site mutation at chromosome 18: 11,753,820 (hg19) (c.274–5T>C) upstream of exon 4 of GNAL (Fig. 1a, Table 1 and Supplementary Fig. 1). Bioinformatics analysis enumerating all possible splice sites in the human genome suggests that the wild-type sequence is ten times more likely to be found near an acceptor splice site than the mutant sequence 17. However, we were unable to prove this hypothesis in samples from affected individuals, as lymphoblasts were the only tissue available, and GNAL expression is undetectable by semiquantitative PCR and protein blot analysis in these cells (data not shown). None of the variants were detected in dbSNP135, ~3,500 European exomes or the 572 control chromosomes that we tested. Segregation analyses confirmed transmission of the GNAL mutations with the disease phenotype in all families for which DNA from multiple affected individuals was available (Supplementary Fig. 1). The Val137 and Glu155 codons were completely conserved in GNAL orthologs (Fig. 1b).

Among the 8 families with mutations, there were 28 individuals with complete clinical information who had definite dystonia (Table 1 and Supplementary Table 1). The average age of dystonia onset in the mutation carriers was 31.3 years and ranged from 7–54 years. Most carriers (82%) had onset in the neck, and 93% had neck involvement at final exam; however, most progressed to have dystonia at other sites, and only 46% had focal dystonia at last exam. Further, cranial involvement was present in 57% of carriers, and 44% had

### Table 1 Clinical characteristics of 28 subjects with GNAL mutations in 8 families

| Family | Gender | Age at onset (years) | Age at exam (years) | Dystonia distribution | Site of onset | Allele variant | Protein variant |
|--------|--------|----------------------|---------------------|-----------------------|--------------|---------------|---------------|
| D1a    | M      | 31                   | 75                  | S                     | Neck         | c.409G>A       | p.Val137Met   |
| 2      | M      | 44                   | 63                  | S                     | Neck, larynx, trunk | c.878C>A       | p.Ser293*     |
| 3      | F      | 26                   | 62                  | S                     | Neck         | c.463G>A       | p.Glu155Lys   |
| 4d     | M      | 7                    | 69                  | G                     | Legs         | c.283_284insT  | p.Ser95fs*110 |
| 5      | F      | 50                   | 60                  | S                     | Neck         | c.591_592insA  | p.Arg198fs*210|
| 6      | F      | 22                   | 49                  | S                     | Neck         | chr. 18: 11,753,820c |
| 7      | F      | 19                   | 51                  | S                     | Neck, face   | c.274–5T>C     | p.Arg198fs*210|
| D2     | F      | 18                   | 38                  | F                     | Neck         | c.182G>T       | p.Pro102.Val104del |
| 2d     | F      | 17                   | 38                  | S                     | Neck         | c.304_312delCTTCCAGTT | p.Pro102.Val104del |
| S      | d      | M                    | 18                  | F                     | Neck         | 1 M 31 75 S Neck | 1 M 44 63 S Neck | 1 M 47 72 F Neck | 1 M 54 59 F Neck | chr. 18: 11,753,820c |
| 2      | M      | 35                   | 38                  | G                     | Neck         | 2 M 47 48 F Neck | 3 M 47 48 F Neck | 4 F 11 19 F Tongue |
| B      | M      | 31                   | 41                  | F                     | Neck         | 3 F 31 41 F Neck | 2 d M 35 41 F Neck | 2d F 36 50 F Neck | c.274–5T>C |
| T      | M      | 31                   | 33                  | F                     | Neck         | 3d F 25 33 S Larynx | 1d F 25 33 S Larynx | 1 M 54 59 F Neck |
| D      | M      | 35                   | 41                  | F                     | Neck         | 2d F 36 50 S Neck | 2 d M 25 45 F Neck | 2 M 42 54 F Neck | c.61C>T |
| N      | M      | 20                   | 56                  | S                     | Neck         | d M 20 56 F Neck | 1 M 20 56 F Neck | c.304_312delCTTCCAGTT | p.Pro102.Val104del |

Chr., chromosome. Gender: F, female; M, male. Dystonia distribution: F, focal; S, segmental; M, multifocal; G, generalized.

*Reported previously and updated here: family D1 (refs. 13,14,58), family P (refs. 14,15) and family S (ref. 59). The following are the corresponding identifiers from ref. 13 for the individuals in family D1: 1, individual 207; 2, individual 302; 3, individual 307; 4, individual 304; 5, individual 317; 6, individual 315; 7, individual 403. *Numbering is based on NM_001142339 and NP_001135811. *Splice-site mutation, genomic position from hg19 assembly. *Proband.
Brachial onset was not observed, and eventual arm involvement was seen in only 32% of carriers, distinguishing the phenotypic consequences of GNAL mutations from those of THAP1 mutation (Supplementary Table 4). All carriers were Caucasian of mixed European ancestry. Because it has been suggested that GNAL may be imprinted, we examined the parental origin of the mutations. Dystonia was inherited from the maternal and paternal sides equally, and there were no apparent phenotypic differences between maternally and paternalistically inherited cases. However, further study is required to fully determine whether the parental origin of the mutation affects penetrance or expression. In addition, no genotype-phenotype correlation could be discerned with regard to mutation type, and phenotypes varied within families with the same mutation (Table 1 and Supplementary Table 1).

GNAL is located on chromosome 18p centromeric to the DYT7 locus for focal dystonia and the DYT15 locus for myoclonus dystonia. Dystonia occurs in individuals with 18p deletion, and the absence of GNAL may contribute to dystonia in these cases. GNAL encodes the stimulatory α subunit, G\(\alpha_{olf}\) first identified as a G protein (guanine nucleotide–binding protein) that mediates odorant signaling in the olfactory epithelium. G proteins link seven-transmembrane-domain receptors to downstream effector molecules and function as heterotrimers composed of \(\alpha\), \(\beta\), and \(\gamma\) subunits. The predominant stimulatory G protein subunit in the brain is G\(\alpha_s\), but G\(\alpha_{olf}\) replaces G\(\alpha_s\) in striatal medium spiny neurons (MSNs). In MSNs, G\(\alpha_{olf}\) couples dopamine type 1 receptors (D1Rs) of the direct pathway and adenosine A2A receptors (A2ARs) of the indirect pathway to the activation of adenylyl cyclase type 5 (ref. 33–35). Relevant to dystonia, A2AR and G\(\alpha_{olf}\) are also expressed in striatal cholinergic interneurons (reviewed in ref. 36).

G\(\alpha_{olf}\) shares 80% amino-acid identity with G\(\alpha_s\) (ref. 29), and, on the basis of the crystal structure of G\(\alpha_s\) (ref. 37), G\(\alpha_{olf}\) is predicted to harbor a Ras-like domain that mediates GTP binding and hydrolysis and a helical domain that interfaces with the Ras-like domain and stabilizes nucleotide binding. We thus predicted that early truncating mutations (those encoding p.Arg21*, p.Ser95fs*110 and p.Arg198fs*210) ...
would remove essential functional regions of G\(\alpha_{olf}\), resulting in a loss-of-function phenotype. In addition, the Arg21* variant is likely degraded by nonsense-mediated decay, thus producing no protein. To investigate the impact of small deletions and more subtle missense mutations (Fig. 2), we used a cell-based BRET reporter system in which G\(\alpha_{olf}\) function is assessed by its ability to interact with G\(\beta\gamma\) subunits during the receptor-initiated cycle of nucleotide binding and hydrolysis. Introduction of wild-type G\(\alpha_{olf}\) resulted in significantly less G\(\beta\gamma\) interaction with the effector-based reporter, indicating efficient formation of the G\(\alpha_{olf}-G\beta\gamma\) heterotrimer. Stimulation of D1R with dopamine resulted in a rapid increase in the BRET signal, reflecting G\(\alpha_{olf}\) activation and resultant release of the G\(\beta\gamma\) subunits. Conversely, inactivation of D1R rapidly brought the signal to baseline, owing to GTP hydrolysis and heterotrimer reassociation. The Ser293* deletion mutant did not support any D1R-driven responses, whereas Val16Phex, a variant found in a single control sample, showed normal wild type–like behavior. Two mutants found in individuals with dystonia, Glu155Lys and Val137Met, showed intermediate phenotypes largely consistent with impaired association with the G\(\beta\gamma\) subunits (Fig. 2).

Although dysfunction of the basal ganglia is not the exclusive etiology of dystonia, abundant evidence supports the involvement of its many circuits, including dopamine pathways38–42. Further, in the basal ganglia, an imbalance between the indirect (dopamine type 2 receptor, D2R) and direct (D1R) pathways has been hypothesized, initiated by primary abnormalities in either the D2R43 or cholinergic43 systems. The identification of causative mutations in GNAL points to primary abnormalities in D1R and/or A2AR transmission as possibly leading to dystonia.

The amount of G\(\alpha_{olf}\) is rate limiting in the activation of adenylate cyclase type 5 after D1R stimulation36,44. Heterozygote G\(\alpha\)-null mice show a muted response to acute psychostimulant and caffeine exposure44. Homozygote null mice are anosmic and hyperactive at baseline and do not respond to acute psychostimulant exposure, but they do not manifest an overt movement disorder44–46. As a rate-limiting mediator of the D1R signal transduction system, G\(\alpha_{olf}\) has been physiologically linked to levodopa-induced dyskinesias (LID)37,48, a debilitating disorder arising from chronic administration of L-DOPA in individuals with dystonia and Parkinson’s disease (reviewed in ref. 49). We have previously posited the existence of compensatory alterations in the D1R signal transduction system in transgenic mice over-expressing mutant torsinA in dopaminergic neurons and suggested a shared molecular abnormality in dystonia and LID on the basis of the dopamine release deficit in mice encoding a Dyt1 mutation41,50. Within the striatum, G\(\alpha_{olf}\) is enriched in the striosomal compartment51, and it has been hypothesized that an imbalance in striosomal activity relative to the matrix compartment is an etiologic factor in the development of hyperkinetic movement disorders52. Genetically, G\(\alpha_{olf}\) has been associated with hyperactivity in attention deficit hyperactivity disorder and schizophrenia, although mutations have not been identified in individuals with these disorders53–55.

In conclusion, we have identified eight different mutations in GNAL, a new causative gene in primary dystonia. The phenotype in the eight multiplex families is predominately one of cervical dystonia, with a relatively broad range in the age of onset and spread to other muscles, especially the facial muscles, in over one-half of subjects. Refinement of this phenotype, as well as the role of GNAL in sporadic PTD, awaits further screening in both familial and sporadic cohorts. A functional assay testing several of the mutations is suggestive of loss of function. Along with mutations in the tyrosine hydroxylase biosynthetic pathway in dopamine-responsive dystonia (DRD)56, the identification of mutations in GNAL directly points to abnormalities in the dopamine and/or adenosine signal transduction pathways as the origin of dystonia pathophysiology. However, unlike the mutations in DRD, GNAL mutations place the primary causative abnormality postsynaptically in striatal dopaminergic neurons and/or cholinergic interneurons.

URLs. SeattleSeq Annotation, http://snp.gs.washington.edu/SeattleSeqAnnotation/; NHLBI Exome Variant Server, http://evs.gs.washington.edu/EVS/.

METHODS

Methods and any associated references are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

T.F. developed the computational analysis pipeline, analyzed the next-generation and Sanger sequencing data and wrote the manuscript. S.W. and E.A. performed molecular experiments. N.S. provided funding for the linkage studies. D.H. provided the antibody to GNAL. D.R. collected samples and provided clinical information for the subjects. M.S.L. and R.S.-P. performed the statistical analysis related to phenotype. S.F., A.E.L., T.-W.L., R.M.T., R.S.-P. and S.B.B. examined subjects. S.B.B. and R.S.-P. supervised the acquisition of clinical data and blood samples and assigned final clinical status. K.A.M. and M.E.E. formulated the functional assay. I.M. performed the assays of G\(\alpha_{olf}\) function. K.A.M. and I.M. analyzed and interpreted the functional data. L.J.O. designed and supervised the genetic studies. T.F., I.M., A.E.L., D.H., D.R., R.S.-P., M.S.L., N.S., K.A.M., M.E.E., S.B.B. and L.J.O. edited the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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V OLUME 45 | NUMBER 1 | JANUARY 2013

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ONLINE METHODS

Study subjects. Families were recruited through advertisement or referral from movement disorder physicians. We included only individuals from families having three or more affected individuals as determined by exam, medical record or reliable history and for whom the proband did not carry a TORIA or TTHAP mutation. One family (family P) with mixed Russian and Swiss Mennonite heritage was also screened for a founder mutation in ATM. Three families—families D1, P and S—have previously been reported. All study subjects gave informed consent before participation, and the study was approved by all institutional review boards. Videotaped exams and determination of affected status were undertaken as previously published (Supplementary Note). Human DNA control samples from European Caucasian blood donors (n = 376) were purchased from Sigma-Aldrich.

Exome sequencing, variant calling and analysis. Genomic DNA was extracted from white blood cells using the Purgene procedure (Genta Systems). Both exome capture and sequencing were performed by PerkinElmer. Briefly, the Agilent SureSelect Human All Exon 50 MB library was used for exome capture as described by the manufacturer. The exome library was sequenced using the Illumina HiSeq 2000 paired-end module. Reads were mapped to the human reference genome sequence (assembly GRCh37/hg19) using the Burrows-Wheeler Alignment Tool (BWA) version 0.5.8c. Allelic variants were detected using the Genome Analysis Toolkit (GATK) . GATK was also used for base quality recalibration, local sequence realignment and variant filtering to minimize base calling and mapping errors. Allelic variant annotations were annotated using the SIFT and SeattleSeq Annotation tools. Comparison of allelic and indel variants between samples, comparison to the SNP data bases and variant selection were performed using in-house Perl scripts. NCBI dbSNP (versions 132 to 135) and the NHLBI exome variant database were used for the selection of new variants. The indels called in family P were compared in three affected individuals (Supplementary Table 3), and shared variants were selected. The shared indels residing in linkage regions were selected and annotated using SNPnexus . Because we found that a missense mutation in GNAL was the cause of dystonia in family D1, no analysis of indels was performed in this family.

PCR amplification and sequencing. Sanger sequencing was performed to confirm variants found by exome sequencing and to search for additional variants in the GNAL gene. Intron-based exon-specific primers were designed from the UCSC human genome assembly sequence (hg19) using the Integrated DNA Technologies Primer Quest online server, which is derived from Primer3 software (release 0.9) . Primers were designed to cover the GNAL transcripts NM_001142339, NM_001261443 and NM_182978. Standard PCR amplification was performed using the primers listed in Supplementary Table 5. Amplified fragments were cleaned and sequenced as described.

Analysis of the Goα-off cycle by BRET. Goα function in living cells was analyzed by monitoring the kinetics of association and dissociation with Gβγ subunits following activation of D1R by agonist. The assay measures agonist-dependent changes in bioluminescence resonance energy transfer between Gβγ tagged with Venus and its effector GRK3 tagged with RLuc8 and was conducted as previously described. Briefly, constructs expressing N-terminal 3x HA-tagged D1R, EE-tagged Goα, EE-tagged Goα, Venus155–239–Gβγ3, Venus155–155–Gβγ2, masGRKct-RLuc8 and Flag-tagged Ric-8B were transfected into HEK293 cells at a 1:6:1:1:1:2 ratio, with 5 μg of total DNA delivered per 4 x 106 cells. After 16 h, cells were stimulated with 100 μM dopamine followed by treatment with 100 μM haloperidol that has a reported 63 nM dissociation constant (Kd) for D1R. Vectors expressing EE-tagged Goα were constructed on the basis of the transcript NM_001142339.

Immunoblotting. Proteins resolved by SDS-PAGE were transferred to nitrocellulose membranes (GE Healthcare). Ponceau S staining of blots after transfection revealed equivalent loading of total protein, which was later shown by blotting with antibody to actin. Membranes were blocked with 5% nonfat dry milk diluted in 0.2% Tween-20 in TBS and incubated successively with primary antibody to GNAL (1:2,000 dilution), GFP (1:2,000 dilution) or actin (1:2,500 dilution) in blocking buffer overnight at 4 °C and horseradish peroxidase-conjugated secondary antibody (1:3,000 dilution in blocking buffer) for 1 h at room temperature. Proteins were visualized using ECL-Plus (GE Healthcare). Secondary antibodies were purchased from GE Healthcare. Rabbit polyclonal antibody to GNAL (146/49) was provide by D.H. and purified as described previously. Rabbit polyclonal antibodies to GFP (ab290) and β-actin (ab8227) were obtained from Abcam.

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