

TABLE 1. Comparison of AHC, DYT12, and patients with the *ATP1A3* p.D923N mutation

	AHC	Present case	Case 2 ⁷	Cases 3, 4, and 5 ⁸	Case 6 ⁸	DYT12
Onset age	<18 mo	2 y	4 y	2 mo, 2 y, 3 y	8 mo	4 y<
Dystonia	Episodic	Episodic, long-lasting, ~2 y	Fixed, ~4 y	Episodic, 2 mo, ~8 y	Episodic, ~8 mo	Fixed
Hemiplegia	Episodic	Episodic, ~2 y	Flaccidity	Episodic ~2 y, 2 y, 3 y	Episodic ~2 y	No
Seizure	Frequent	No	No	No	No	No
Fluctuation	Frequent	Positive	Positive	Positive	Positive	No
Triggers	Fever, stress	Fever, stress	Fever	Exercise	Fever, exercise	Fever, stress
Muscle tone	Hypotonia	Mild hypotonia	Hypotonia	NA	NA	Normal
Motor delay	Mild to severe	Mild delay	Mild delay	NA	NA	No
Ataxia	Frequent	Positive	Positive	Positive	NA	Frequent
Dysarthria, dysphagia	Frequent	Positive	Positive	Positive	NA	Frequent
Cognitive deficit	Frequent	No	Lost words	Borderline to mild mental deficiency	Mild mental deficiency	No
Causative gene	<i>ATP1A3</i> (D923Y in 1 case)	<i>ATP1A3</i> (D923N)	<i>ATP1A3</i> (D923N)	<i>ATP1A3</i> (D923N)	<i>ATP1A3</i> (D923N)	<i>ATP1A3</i> (D923N in case 1)
Familial	Rare	No	No	Positive	Positive	Positive

Case 2 is a patient from Anselm's article.⁷

Cases 3, 4, and 5 are patients II-2, III-2, and III-3 from Roubergue's article.⁸

Case 6 is patient IV-1 from Roubergue's article.⁸

AHC, alternating hemiplegia of childhood; DYT12, rapid-onset dystonia-parkinsonism; NA, not available.

References

1. Heinzen EL, Swoboda KJ, Hitomi Y, et al. De novo mutations in *ATP1A3* cause alternating hemiplegia of childhood. *Nat Genet.* 2012;44:1030-1034.
2. Rosewich H, Thiele H, Ohlenbusch A, et al. Heterozygous de-novo mutations in *ATP1A3* in patients with alternating hemiplegia of childhood: a whole-exome sequencing gene-identification study. *Lancet Neurol.* 2012;11:764-773.
3. Ishii A, Saito Y, Mitsui J, et al. Identification of *ATP1A3* mutations by exome sequencing as the cause of alternating hemiplegia of childhood in Japanese patients. *PLoS One.* 2013; 8:e56120.
4. Brashear A, Dobyns WB, de Carvalho Aguiar P, et al. The phenotypic spectrum of rapid-onset dystonia-parkinsonism (RDP) and mutations in the *ATP1A3* gene. *Brain.* 2007;130: 828-835.
5. Ozelius LJ. Clinical spectrum of disease associated with *ATP1A3* mutations. *Lancet Neurol.* 2012;9:741-743.
6. Zanotti-Fregonara P, Vidailhet M, Kas A, et al. [¹²³I]-FP-CIT and [^{99m}Tc]-HMPAO single photon emission computed tomography in a new sporadic case of rapid-onset dystonia-parkinsonism. *J Neurol Sci.* 2008;273:148-151.
7. Anselm IA, Sweadner KJ, Gollamudi S, Ozelius LJ, Darras BT. Rapid-onset dystonia-parkinsonism in a child with a novel *ATP1A3* gene mutation. *Neurology.* 2009;73:400-401.
8. Roubergue A, Roze E, Vuillaumier-Barrot S, et al. The multiple faces of the *ATP1A3*-related dystonic movement disorder. *Mov Disord.* 2013;28:1457-1459.

mary dystonia by one group¹ and subsequently confirmed by another.² Mutations in this gene appear to cause autosomal dominant, primary dystonia with a cervical predilection and evidence of incomplete penetrance.¹

The initial discovery paper by Fuchs et al reported *GNAL* mutations in 6 out of 39 families screened (~15%). However, in the subsequent study by Vemula et al, only 3 *GNAL* mutations were detected in 760 subjects with familial or sporadic primary dystonia (<0.5%). An accurate estimate of the prevalence of *GNAL* mutations is important as a mutation frequency of ~15% would justify early and widespread genetic testing of *GNAL* in familial dystonia, whereas a frequency of <0.5% would not.

We screened *GNAL* by Sanger sequencing using the DNA samples from 192 probands (136 female and 56 male) with either familial or sporadic cervical dystonia, selected from a library of research samples on the basis of a clinical description of focal or segmental dystonia that included the cervical region. Local ethics committee approval was obtained for the study. A family history, defined as one or more first or second-degree relatives with dystonia, was recorded in 84 cases. Tremor was recorded in 53 cases. All familial cases had been screened for TOR1A, THAP1 and ANO3 mutations and were negative.

Study funding: Supported by a Bachman-Strauss Dystonia and Parkinson Foundation grant.

Relevant conflicts of interest/financial disclosures: Gavin Charlesworth reports no disclosures. Kailash P. Bhatia has received honoraria/financial support to speak/attend meetings from GSK, Boehringer-Ingelheim, Ipsen, Merz, and Orion pharma companies. He holds grants from the Bachmann-Strauss Dystonia Parkinson foundation, the Dystonia Society UK and the Halley Stewart Trust. Nicholas W. Wood holds grants from the Bachmann-Strauss Dystonia Parkinson foundation, the MRC and the Wellcome Trust.

Received: 1 July 2013; **Revised:** 11 August 2013;

Accepted: 19 August 2013

Published online 12 November 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.25713

No Pathogenic *GNAL* Mutations in 192 Sporadic and Familial Cases of Cervical Dystonia

Recently, using a whole exome sequencing approach, mutations in *GNAL* were identified as a novel cause of pri-

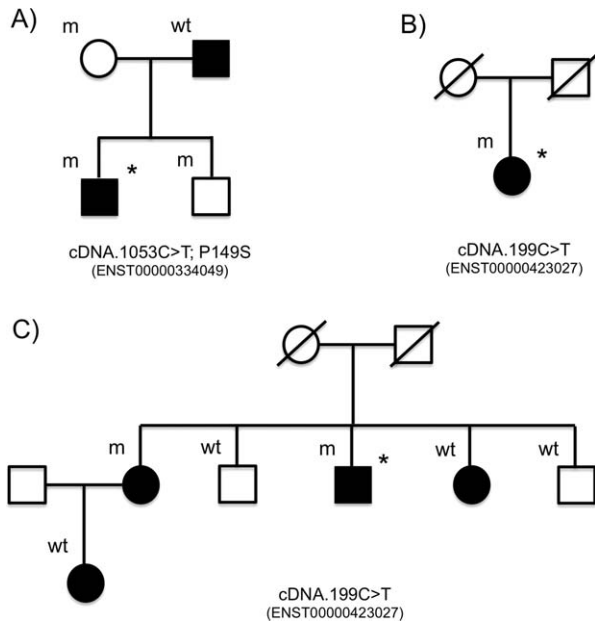


FIG. 1. Family trees for individuals with novel variants in *GNAL*. Genetic pedigrees for individuals with novel variants in *GNAL*. Affected family members are marked by shaded symbols. The variant found is indicated under the pedigree with transcript ID. Mutational status is indicated by 'm' for heterozygous mutation carriers and 'wt' for homozygous wildtype alleles. Index cases included in the initial screening are marked with an asterisk.

Primers were designed to amplify all exons, the exon/intron boundaries and the 5'UTRs of both major isoforms of *GNAL* (ENST00000334049 and ENST00000423027).

We identified only two novel single nucleotide variations in *GNAL* in three individuals in our case cohort. The first was a missense mutation in exon 2 of the gene (cDNA.1053C>T; P149S in ENST00000334049) that was detected in one individual with onset of cervical dystonia in the 4th decade, with a similarly affected father, suggesting autosomal dominant inheritance. However, segregation

analysis revealed the variant had in fact been inherited from his unaffected mother and was also present in his unaffected brother (see Fig. 1A), ruling it out as the cause of the dystonia in this family. The second variant, located in the 5'UTR of isoform 2 (cDNA.199C>T in ENST00000423027), was found in two individuals in our cohort exhibiting onset of cervical dystonia in the 6th decade. One individual was a sporadic case whereas the other was part of family with multiple affected members (see Fig. 1B-C). Despite affecting a highly conserved base (PhyloP score = 4.158), the variant failed to segregate with disease in the family, being absent in two affected individuals (see Fig. 1C).

In summary, we did not identify any mutations in *GNAL* that could be a cause of the dystonia in 192 cases drawn from the United Kingdom, including 84 familial cases. Our own data suggest that *GNAL* mutations do not represent a common cause of dystonia — in the U.K. population at least — and that the overall frequency of *GNAL* mutations may be closer to the figure obtained by Vemula et al² than the 15% initially reported by Fuchs et al.¹ This study also emphasises the importance of segregation analysis in establishing the pathogenicity or otherwise of novel variants and suggests that other novel genetic causes of dystonia remain to be identified. ■

Gavin Charlesworth, MRCP,¹ Kailash P. Bhatia, FRCP,²
Nicholas W. Wood, FRCP¹

¹Department of Molecular Neuroscience, UCL Institute of
Neurology, Queen Square, London, UK.

²Sobell Department of Motor Neuroscience and Movement
Disorders, UCL Institute of Neurology, Queen Square,
London, UK.

References

1. Fuchs T, Saunders-Pullman R, Masuho I, et al. Mutations in *GNAL* cause primary torsion dystonia. *Nature Genetics* 2012;45:88-92.
2. Vemula SR, Puschmann A, Xiao J, et al. Role of Galpha(olf) in familial and sporadic adult-onset primary dystonia. *Hum Mol Genet* 2013;22:2510-2519.