Published in final edited form as:

Curr Neurol Neurosci Rep. 2014 August ; 14(8): 462. doi:10.1007/s11910-014-0462-8.

Recent Advances in the Genetics of Dystonia

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Abstract

Dystonia, a common and genetically heterogeneous neurological disorder, was recently defined as “a movement disorder characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements, postures, or both.” Via the application of whole-exome sequencing, the genetic landscape of dystonia and closely related movement disorders is becoming exposed. In particular, several “novel” genetic causes have been causally associated with dystonia or dystonia-related disorders over the past 2 years. These genes include *PRRT2* (DYT10), *CIZ1* (DYT23), *ANO3* (DYT24), *GNAL* (DYT25), and *TUBB4A* (DYT4). Despite these advances, major gaps remain in identifying the genetic origins for most cases of adult-onset isolated dystonia. Furthermore, model systems are needed to study the biology of *PRRT2*, *CIZ1*, *ANO3*, $G\alpha_{olf}$, and *TUBB4A* in the context of dystonia. This review focuses on these recent additions to the family of dystonia genes, genotype-phenotype correlations, and possible cellular contributions of the encoded proteins to the development of dystonia.

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Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of Interest Jianfeng Xiao and Satya R. Vemula declare that they have no conflict of interest.

Keywords

Dystonia; *PRRT2*; *CIZ1*; *GNAL*; *ANO3*; *TUBB4A*; Whole-exome sequencing

Introduction

Along with essential tremor, restless legs syndrome, and Parkinson disease, dystonia is a movement disorder commonly seen by neurologists in outpatient clinics. Genetic factors play an important role in the development of primary or isolated dystonia since more than 10 % of affected individuals report a positive family history [1•]. In 2013, dystonia was newly defined by an international consensus committee consisting of investigators with years of experience in this field as a “movement disorder characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements, postures, or both.” Dystonic movements are typically patterned with twisting and may be tremulous. Dystonia is often initiated or worsened by voluntary actions and associated with overflow muscle activation [2, 3]. The group classified dystonia along two axes: clinical characteristics, including age at onset, body distribution, temporal pattern, and associated features (additional movement disorders or neurological features); and causes, which include inherited (dystonia forms of proven genetic origin), acquired, and idiopathic (unknown causes) [2]. Future improvements in phenotypic (e.g., functional neuroimaging and postmortem pathology) and genetic resolution will serve to challenge the new definition and classification of dystonia. In fact, the new classification scheme was largely driven by recent developments in the genetics of dystonia and related neurological disorders [1•, 2, 4].

To date, more than 20 monogenic inherited dystonias and dystonia-related disorders (DYT1-DYT25) have been reported in Online Mendelian Inheritance in Man (OMIM), and many of the casual genes have been identified (Table 1). These include autosomal dominant forms due to mutations in *TOR1A* (DYT1), *TUBB4A* (DYT4), *GCHI* (DYT5a/ DYT14), *THAP1* (DYT6), *PNKD* (DYT8), *SLC2A1* (DYT9/DYT18), *PRRT2* (DYT10), *SGCE* (DYT11), *ATPIA3* (DYT12), *CIZ1* (DYT23), *ANO3* (DYT24), and *GNAL* (DYT25); autosomal recessive forms due to mutations in *TH* and *SPR* (DYT5) and *PRKRA* (DYT16); and an X-linked recessive form due to mutations affecting expression of *TAF1* (DYT3) (Table 1) [5, 6•, 7–12, 13•, 14•, 15•, 16–19, 20•]. Until the advent of whole-exome sequencing, the genetics of dystonia had progressed slowly after identification of dopa-responsive dystonia being caused by mutations in *GCHI* in 1994 [7], and isolation of the classic *TOR1A* GAG mutation as the first sequence variant (SV) associated with isolated dystonia in 1997 [5]. In less than 2 years, five additional genes associated with isolated dystonia and dystonia-related disorders have been reported. These include *PRRT2*, *CIZ1*, *ANO3*, *GNAL*, and *TUBB4A* [6•, 11, 13•, 14•, 15•, 20•, 21•]. In this article, we focus on these latest members of the dystonia family of genes. More extensive reviews of *TOR1A*, *THAP1* (DYT6), and other dystonia-associated genes are available elsewhere [1•, 22•, 23].

TUBB4A/DYT4

DYT4 (OMIM 128101) was assigned to the unique Australian-English “whispering dysphonia” kindred, which was first described by Parker [24] in 1985. This pedigree exhibits an autosomal dominant inheritance pattern of a neurological syndrome that includes dysphonia, a gait disorder, increased prevalence of psychiatric disorders, dysmorphic facies, and variable cognitive impairment. The dystonia may become generalized [6•]. Some patients exhibit a striking “hobbyhorse” ataxic gait which is unlike anything seen in primary or isolated dystonia. Despite the presence of Wilson disease in several family members, the two *ATP7B* mutations do not segregate with dystonia [25]. Despite the highly penetrant dysphonic phenotype in the large multigenerational pedigree, successful linkage analysis was not reported until 2012, when two independent groups simultaneously identified a pathogenic variant in *TUBB4A* using different samples from the same kindred [6•, 21•]. Hersheson et al. [6•] performed genome-wide linkage analysis in 19 family members with SNP chips and mapped DYT4 to chromosome locus 19p13.2-3. Then, with whole-exome sequencing in two definitely affected patients, a single missense mutation (c.4C>G, p.R2G) was localized to exon 1 of *TUBB4A*, which encodes tubulin, beta 4A class IVa (TUBB4A) [6•]. These findings were confirmed by an independent team using a similar strategy in 14 family members from the same kindred [21•]. Lohmann et al. [21•] also reported a missense variant (c.2723 G>A, p.R908H) in *DOT1L* (DOT1-like histone H3K79 methyltransferase) which co-segregated with dystonia but was also present in four of nine unaffected family members. Sequencing of *TUBB4A* in 394 unrelated subjects with dystonia revealed a missense variant (c.811G>A, p. A271T) in a subject with late-onset segmental dystonia that included spasmodic dysphonia [21•]. However, cosegregation in one other putatively affected family member was not established for the A271T variant.

TUBB4A encodes TUBB4A, a member of the β -tubulin family. *TUBB4A* is spliced into multiple transcripts. The major transcript encodes a 495-amino acid protein that is expressed in brain. Tubulins are globular proteins and include six family members: α -tubulin, β -tubulin, γ -tubulin, δ -tubulin, ϵ -tubulin, and ζ -tubulin. The most prevalent members are α -tubulin and β -tubulin, which associate with each other as heterodimers to form microtubules, an essential component of the cytoskeleton. Microtubules are critical for a variety of cellular processes, such as mitosis, intracellular transport, neuron morphology and ciliary and flagellar motility [26]. In humans, six β -tubulins with high sequence similarity (93–98 % to TUBB4A) have been characterized and associated with a variety of neurodevelopmental disorders, including microcephaly with structural brain abnormalities (TUBB), lissencephaly (TUBA1A), polymicrogyria (TUBA1A, TUBB2B, TUBB3), and ocular motility disorders (TUBB3) [27].

Consistent with a vital role for TUBB4A in the structure of the central nervous system, a recurrent de novo missense mutation (p.Asp249Asn) was identified in patients with hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) [28]. This mutation affects a highly conserved asparagine that interfaces with α -tubulin and could disrupt dimerization, microtubule polymerization, or microtubule stability [28]. H-ABC is a rare leukodystrophy characterized by onset in infancy or childhood, developmental delay, movement disorders, and spasticity. H-ABC movement disorders may include dystonia,

ataxia, and choreoathetosis. Magnetic resonance imaging shows evidence of hypomyelination, cerebellar atrophy, and volume loss in the putamen. A similar phenotype with slowly progressive spastic paraparesis, segmental dystonia, intellectual disability, behavioral problems, incomplete myelination, and progressive cerebellar atrophy has been linked to a p.E410K variant in *TUBB4A* [29].

The similar and overlapping phenotypes seen with R2G (DYT4), D249N (H-ABC), and E410K missense mutations in *TUBB4A* suggest that DYT4 is a forme fruste of H-ABC [30]. In comparison with the DYT4 pedigree, the more severe and complex clinical phenotypes seen with the D249N and E410K mutations limit reproductive capacity [31]. Given the fundamental role of *TUBB4A* in neuronal biology and its highly conserved amino acid sequences, it is not surprising that only eight missense variants appear in the Exome Variant Server database and that only three of these eight are predicted to be deleterious (Table 2).

To determine the contribution of *TUBB4A* to the pathogenesis of primary or isolated dystonia, in general, high-resolution melting and Sanger sequencing were used to interrogate *TUBB4A* in a cohort of 575 subjects with laryngeal, segmental, or generalized dystonia [30]. No pathogenic variants in *TUBB4A* were identified in any of the subjects. Therefore, given the allelic association of DYT4 with H-ABC, we concluded that clinical testing for *TUBB4A* mutations is not justified in spasmodic dysphonia or other forms of primary dystonia [30].

PRRT2/DYT10

DYT10 (OMIM 128200) was assigned to paroxysmal dystonia with kinesigenic choreoathetosis in 1998 [32], and later to paroxysmal kinesigenic dyskinesia (PKD) or episodic kinesigenic dyskinesia. The paroxysmal dyskinesias are an important category of movement disorders characterized by the sudden onset of involuntary movements that may include one or more of the following: dystonia, chorea, athetosis, and ballism. The paroxysmal dyskinesias are divided into four major types: PKD, paroxysmal nonkinesigenic dyskinesias (PNKD), paroxysmal exertion-induced dyskinesias (PED), and paroxysmal hypnogenic dyskinesias. Precipitating factors are included within the name of each type: kinesigenic (sudden movements), nonkinesigenic (triggered by factors such as alcohol, caffeine, fatigue, and anxiety), exertion- or exercise-induced (running or prolonged walking), and hypnogenic (non-rapid-eye-movement sleep). The paroxysmal dyskinesias may be familial (hereditary), sporadic, or secondary to other neurological or metabolic disorders (e.g., head trauma, multiple sclerosis, hypoparathyroidism). Typically, dystonia is the foremost involuntary movement manifested by patients with PKD, PNKD, PED, and paroxysmal hypnogenic dyskinesia. Compared with primary dystonia, PKD is relatively rare. In general, familial cases of PKD follow an autosomal dominant inheritance mode with incomplete penetrance. In patients with PKD, recurrent and brief attacks of involuntary movement are usually triggered by sudden voluntary movement. These attacks usually begin in childhood or early adulthood and may include various combinations of dystonia, chorea, and athetosis affecting the face, trunk, arms, and legs. In many patients, paroxysmal dystonia is the sole or predominant clinical manifestation [1]. PKD often improves with age, and the

vast majority of patients show a favorable response to relatively low doses of anticonvulsant medications, particularly carbamazepine and phenytoin [1•, 33].

Most patients with PKD harbor loss-of-function mutations in *PRRT2*, which encodes proline-rich transmembrane protein 2 (PRRT2). A *PRRT2* hotspot mutation (c.649dupC, p.R217Pfs*8) likely causes haploinsufficiency via nonsense-mediated decay of mutant transcripts [33]. PKD, infantile convulsions and choreoathetosis (ICCA), and benign familial infantile epilepsy (BFIE) may appear in the same family. Moreover, PKD, ICCA, and BFIE had been linked to chromosome locus 16p11.2 for over 10 years [34]. With the advent of whole-exome sequencing, this was confirmed in 2011 after the causal gene, *PRRT2*, was first identified in both familial and sporadic PKD, ICCA, and BFIE in Han Chinese, Japanese, Caucasians, and African-Americans [11, 33, 35]. *PRRT2* mutations also cause rare cases of PED, PNKD-like syndromes, hemiplegic migraine, episodic ataxia, febrile seizures, and childhood-absence epilepsy. Over the past 3 years, more than 60 *PRRT2* mutations have been found in PKD, ICCA, and BFIE. Some of the mutations, including the hot spot mutation (c.649dupC), were shared among PKD, ICCA, and BFIE phenotypes [35]. *PRRT2* is a four-exon gene that encodes PRRT2, a 340 amino acid protein with two putative transmembrane domains near its C-terminal end. PRRT2 is highly expressed in the developing nervous system, particularly the cerebellum [11]. PRRT2 may interact with SNAP-25, a component of the molecular machinery involved in the release of neurotransmitters at the presynaptic membrane [36]. Most *PRRT2* mutations are frameshift or nonsense mutations which result in truncated proteins and lead to haploinsufficiency and, possibly, dysregulation of neurotransmitter release. An intensive review by Heron and Dibbens [35] indicated that mutations in *PRRT2* account for 62–100 % of familial cases of PKD, 40–100 % of familial cases of BFIE, and 33–100 % of familial cases of ICCA; although the percentages are lower in the sporadic cases, with mutations found in 27–50 % of sporadic PKD patients and 29–100 % of patients with sporadic benign infantile seizures. Genetic testing for mutations in *PRRT2* is straightforward and should be pursued in patients with typical PKD, ICCA, and BFIE phenotypes, particularly when supported by a positive family history.

CIZ1/DYT23

Cervical dystonia, also known as spasmodic torticollis, is the commonest type of focal dystonia, affecting over one million people worldwide, with most cases being of the late-onset type (more than 20 years of age). Genetic factors play a major role in late-onset cervical dystonia given that 10 % of probands have one or more affected family members [13••]. However, the rarity of large kindreds suggests that causal genetic variants are incompletely penetrant. In 2012, using linkage and haplotype analyses in combination with whole-exome sequencing, Xiao et al. [13••] mapped the causal SV in an American autosomal dominant cervical dystonia pedigree [37] to microsatellite marker D9S159 on chromosome locus 9q34.11. Whole-exome sequencing exposed an SV (c.790A>G, p.S264G) in exon 7 of *CIZ1* which encodes cyclin-dependent kinase inhibitor 1A interacting zinc finger protein 1 (CIZ1). This SV is predicted, “in silico,” to be pathogenic. Moreover, in vitro studies showed that this variant altered splicing of *CIZ1* and nuclear localization of CIZ1.

The potential causality of *CIZ1* SVs as a cause of cervical dystonia has come under scrutiny. First of all, an SV in *SETX* (c.2385_2388delAAAG) also co-segregated with dystonia in the index family. It is possible that combined mutations in *CIZ1* and *SETX* were required for the development of dystonia in the index pedigree. Second, no other pedigrees with *CIZ1* mutations have been reported in the available literature. In our original report, only two of 308 additional cervical dystonia probands harbored a variant in *CIZ1* (Table 2). Moreover, these patients had sporadic dystonia, precluding cosegregation analysis. In addition, no *CIZ1* mutations were found in 12 Chinese pedigrees with autosomal dominant cervical dystonia [38]. Third, there are dozens of *CIZ1* missense variants in normal populations, and some of these are predicted to be pathogenic by one or more in silico programs.

Given concerns regarding the possibility of other pathogenic SVs in the American pedigree with linkage to chromosome locus 9q34.11 [13•, 39], whole-exome sequencing was repeated in one definitely affected subject. In brief, 98.44 % of exons were covered at a coverage of 20-fold or greater. On chromosome 9, 98.39 % of exons (3,371/3,426) were covered at a coverage of tenfold or greater, and 99 % or more were covered at a coverage of twofold or greater. Most importantly, no newly identified potentially pathogenic SVs were identified in repeated analyses, strongly supporting the causality of *CIZ1* c.790A>G (p.S264G). In particular, no pathogenic variants were identified in *TOR1A* [39]. Using high-resolution melting of *CIZ1* exon 2 (exon 1 is noncoding) in over 1,200 Caucasian subjects with dystonia followed by confirmatory Sanger sequencing, we have identified several novel and potentially pathogenic SVs within the QD1 domain of *CIZ1* in subjects with dystonia (Fig. 1). None of these SVs appear in existing variant databases (Exome Variant Server, dbSNP, 1000 Genomes).

CIZ1 is an 898 amino acid DNA replication factor with neural expression patterns similar to those of *THAP1* (DYT6) and *TOR1A* (DYT1). Robust expression of *CIZ1*, *THAP1*, and *TOR1A* is noted in fetal brain and adult cerebellum, particularly in Purkinje cells [13•, 40, 41]. *CIZ1* was first recognized through its interaction with p21Cip1/Waf1, a cyclin-dependent kinase inhibitor involved in G₁/S cell-cycle regulation and cellular differentiation. The cellular role and neural localization of *CIZ1* are compatible with current themes in dystonia research as four other dystonia-associated proteins [*THAP1* (DYT6), *TAF1* (DYT3), ataxia telangiectasia mutated, and guanine nucleotide binding protein, alpha activating activity polypeptide, olfactory type (G α_{olf})] are also involved in G₁/S cell-cycle regulation [42]. The role of wild-type and mutant *CIZ1* in terminally differentiated neurons requires further elucidation.

ANO3/DYT24

A British family with autosomal dominant adult-onset craniocervical dystonia was first reported in 2000 [43]. All five definitely affected family members exhibit tremulous cervical dystonia with a variable degree of associated upper-limb dystonic tremor, and most show spread to segmental dystonia over time. In addition, some subjects manifest spasmodic dysphonia and/or blepharospasm, but none exhibit generalized dystonia [14•, 44]. SNP-based linkage analysis on 15 samples identified five loci with identical logarithm of odds scores of 2.01 on chromosomes 4, 5, 6, 7, and 11. Whole-exome sequencing was performed

in two affected individuals and identified several candidate causal variants. The two exomes received 83 % and 90 % coverage with read depths of 2 or more. A heterozygous missense mutation (c.1480A>T, p.Arg494Trp) in exon 15 of *ANO3* on chromosome 11 co-segregated with dystonia in the index pedigree (family A) [14••]. Focused screening of exon 15 in 384 subjects with mainly cervical dystonia with or without upper-limb dystonic tremor exposed a second co-segregating SV (c.1470G>C, p.Trp490Cys) in family B. Targeted sequencing was used to identify additional variants in exons 2, 21, and 25 and the 5'-untranslated region of *ANO3*. The SVs in exons 2, 21, and 25 are predicted as tolerated and benign by SIFT and PolyPhen-2, respectively.

ANO3, a 27-exon gene, encodes anoctamin 3 (ANO3), a 981 amino acid transmembrane protein that belongs to a family of Ca^{2+} -activated chloride channels with highest expression in the striatum. Some members of the ANO family (*ANO1-ANO10*) have been linked to disease (*ANO5*, *ANO6*, and *ANO10*), mainly via autosomal recessive mechanisms. For example, homozygous mutations in *ANO10* are found in a form of autosomal recessive spinocerebellar ataxia [45]. Functional studies using mutation-bearing fibroblasts from family A (p.Arg494Trp) showed defective endoplasmic-reticulum Ca^{2+} handling [14••]. *Ano3* knockout rats show diminished Slack expression, broadened action potentials, increased excitability and enhanced K_{Na} channel activity in dorsal root ganglia neurons [46]. These findings suggest that ANO3 plays a role in regulating the processing of pain in the peripheral nervous system. Although the *Ano3* knockout rats showed increased thermal and mechanical sensitivity, they did not manifest dystonia [46].

Results from knockout rats question the role of *ANO3* in dystonia. In the EVS database, more than 30 deleterious rare variants in *ANO3* are reported (0.48 % of available samples). Moreover, only two novel missense SVs (0.6 %) were found in a screening study of 342 German dystonia patients, and neither variant was predicted to be deleterious via in silico analyses. In addition, two novel missense variants were also found in 376 normal controls (0.5 %) [47]. Finally, rare variants in *ANO3* are not associated with essential tremor [48]. Taken together, these data suggest that the role of *ANO3* in dystonia and tremor is questionable and demands additional study.

GNAL/DYT25

Although without strong linkage data from whole-genome genotyping owing to relatively small pedigrees, two independent teams used whole-exome sequencing to identify 11 heterozygous *GNAL* mutations in 12 dystonia families with mainly adult-onset cervical or craniocervical dystonia [15••, 20••]. *GNAL* is located on chromosome locus 18p11.22-p11.21, within the 18p deletion syndrome (OMIM 146390) region. The 18p deletion syndrome is characterized by mental retardation, growth retardation, craniofacial dysmorphism, and in many patients, dystonia [49, 50]. This region also harbors loci for DYT7 (OMIM 602124, autosomal dominant adult-onset cervical dystonia) and DYT15 (OMIM 607488, autosomal dominant alcohol-responsive myoclonic dystonia) [51, 52]. At present, the potential relationships among DYT7, DYT15, and DYT25 are unclear. Moreover, the DYT7 gene locus for cervical dystonia on chromosome locus 18p is now questionable [53].

To date, more than 15 different *GNAL* mutations have been found to be associated with dystonia in a wide range of racial/ ethnic groups, including American Caucasians, Amish-Mennonites, European Caucasians, African-Americans, Chinese, and Japanese. These mutations include seven missense mutations, four nonsense mutations, two frameshift mutations, one small deletion, and one mutation (c.3G>A) which alters the start codon (Table 2). Data from a total of 43 patients from 17 families (16 males and 27 females) show that the average age of onset is 35 ± 13 (7–63) years. Over 83 % of the subjects (36 of 43) had onset in the neck as cervical dystonia, and in 42 % (18 of 43) the dystonia has remained focally distributed as cervical dystonia, with the remaining subjects showing spread to the cranial musculature or arms as segmental dystonia. Only 12 % of the subjects (five of 43) developed generalized dystonia. Ultimately, the neck is affected in over 95 % of subjects (41 of 43) [15•, 20•, 47, 54–59]. Although *GNAL* genotype–phenotype correlations are limited by sample size, currently available data appear to distinguish *GNAL* dystonia from dystonia associated with mutations in *TOR1A* and *THAPI*. DYT1 caused by mutations of *TOR1A* is usually characterized by early onset of dystonia in one limb with progression to a segmental or generalized distribution. DYT1 dystonia is rarely isolated to the cervical region [1•]. The mean age of onset for DYT6 dystonia caused by mutations of *THAPI* is 16.8 years. The arms and neck are the commonest sites of onset for *THAPI*-associated dystonia, with the neck and arms being ultimately affected in 77 % and 71 % of patients, respectively [22•]. In addition, the larynx is affected in 44 % of patients with pathogenic SVs in *THAPI*. Therefore, in comparison with *TOR1A* and *THAPI*, mutations in *GNAL* are more commonly associated with adult-onset cervical dystonia and are less likely to generalize. Similarly to other genes associated with isolated or primary dystonia, penetrance with *GNAL* mutations is incomplete.

The initial report by Fuchs et al. [15•] suggested that almost 15 % of families (six of 39) with adult-onset cervical dystonia carried a *GNAL* mutation, but follow-up studies showed that the frequency is less than 0.5 % in mainly adult-onset dystonia patients [20•, 47, 55, 56]. The frequency of *GNAL* mutations in primary or isolated dystonia is in line with that in other genetic forms of dystonia such as DYT1 (0.2 %) and DYT6 (0.5 %) [22•, 23, 60].

GNAL encodes $G\alpha_{olf}$. The major variant (NP_001135811) contains 381 amino acids and is highly expressed in the olfactory bulb, striatum, and cerebellar Purkinje cells, and colocalizes with corticotropin-releasing hormone receptors in the latter [20•]. $G\alpha_{olf}$ plays a role in olfaction, coupling dopamine D₁ and adenosine A_{2A} receptors to adenylyl cyclase, and histone H3 phosphorylation. *Gnal* knockout mice and some patients with mutations in *GNAL* exhibit hyposmia [20•, 61].

Microarray data of lymphoblastoid cell lines from subjects with a p.V228F mutation in *GNAL* showed upregulation of genes involved in cell-cycle control and development, consistent with cellular pathways that have been implicated in dystonia [20•, 41, 62]. In vivo administration of the dopamine D₂ receptor blocker haloperidol to rodents induces a prolonged increase in the levels of Ser10 phosphorylated histone H3 in dopamine D₂ receptor expressing neurons of the dorsomedial and the dorsolateral striatum, and this effect is mediated through adenosine A_{2A} receptor mediated activation of $G\alpha_{olf}$ [63]. Histone H3 phosphorylation at Ser10 inhibits checkpoint kinase 1, a key component of the ataxia

telangiectasia mutated/ataxia telangiectasia and Rad3 related arm of the G₁/S checkpoint pathway [64]. Therefore, it is conceivable that loss of G_αolf function could manifest itself as dystonia via prolonged disruption of G₁/S cell-cycle control in striatal medium spiny neurons or cerebellar Purkinje cells.

Conclusions

GNAL is a bona fide primary or isolated dystonia gene, *DYT4* is not a primary or isolated dystonia gene but, instead, may be a forme fruste of H-ABC, and the suggested pathogenic roles of *CIZ1* and *ANO3* in dystonia will require confirmation by independent groups. At this time, clinical genetic testing for *GNAL* mutations is justified in subjects with familial adult-onset dystonia mainly manifested as cervical dystonia. However, clinicians must recognize that in silico analyses are imperfect and the pathogenicity of missense variants must be interpreted with caution [39]. For example, Fuchs et al. [15•] reported a normal control with a p.V16F variant in *GNAL*, whereas Charlesworth et al. [14•] reported a p.P149S variant in *ANO3* which did not segregate with dystonia. Dozens of putatively pathogenic missense variants in *CIZ1*, *ANO3*, and *GNAL* are present in public domain databases (Table 2). On the other hand, the mutation spectra of *DYT4*, *DYT23*, *DYT24*, and *DYT25* may be much broader than currently appreciated given that most screening studies largely ignored the promoter, untranslated, and intronic regions of the disease-associated genes.

Genetics opens doors for deeper understanding of dystonia and related movement and neurodegenerative disorders. Importantly, all five dystonia-associated genes discussed in this review were identified by whole-exome sequencing, which largely bypasses the limitations of traditional positional cloning. The next wave of genetics is upon us with relatively inexpensive whole-genome sequencing along with longer and more accurate reads that should expose increasingly larger portions of the dystonia genetic landscape.

With rapid advances come challenges. A recent report of an Amish pedigree described a proband with typical *DYT6* manifestations and an associated *THAPI* mutation, whereas her distant cousin harbored a de novo mutation in *TOR1A* and her aunt with blepharospasm was negative for *TOR1A*, *THAPI*, and *GNAL* mutations [59]. The reduced penetrance and variable expressivity of dystonia along with important phenotypic overlap among the different forms of dystonia should guide clinical judgment and genetic counseling.

The recently identified dystonia genes broaden our understanding of pathological cellular and systems networks. *CIZ1* and G_αolf are tied to the G₁/S cell-cycle checkpoint [62]. *PRRT2*, *ANO3*, and *TUBB4A* can be linked to other cellular mechanisms believed to play a role in dystonia and related disorders, such as control of synaptic function, the endoplasmic reticulum, and nuclear envelope function. G_αolf, *CIZ1*, *PRRT2*, and *TUBB4A* show high expression levels in brain structures, especially striatum and cerebellum, which have been implicated in the pathogenesis of dystonia [65–67].

Acknowledgments

Our dystonia research has been supported by grants from the National Institutes of Health (K08NS001593, R01EY012232, R01NS048458, R01NS050185, R01NS069936, and U54NS065701), the Dystonia Medical Research Foundation, the Bachmann-Strauss Dystonia & Parkinson Foundation, Tyler's Hope for a Dystonia Cure, and the University of Tennessee Health Science Center Neuroscience Institute.

Mark S. LeDoux has received consultancy fees from Teva and Lundbeck. He has also received grants from the National Institutes of Health, the Cure Huntington Disease Initiative, and Prana. He has also received honorarium payments from Teva, Lundbeck, and UCB Pharma, as well as royalty payments from Elsevier.

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Fig. 1.

Dystonia-associated proteins and localization of disease-associated pathogenic sequence variants. *G1-G5* are GTP binding domains, and *ZF1-ZF3* are zinc-finger domains. AD acidic domain, *ANO3* anoctamin 3, *CIZ1* cyclin-dependent kinase inhibitor 1A interacting zinc finger protein 1, *Ga(olf)* guanine nucleotide binding protein, alpha activating activity polypeptide, olfactory type, *MH3* matrin 3 homologous domain 3, *NLS* nuclear localization signal, *QD1* glutamine-rich domain 1, *QD2* glutamine rich domain 2, *TUBB4A* tubulin, beta 4A class IVa

Table 1

Types of dystonia, modes of inheritance, and associated loci

HUGO/OMIM	Common name	Locus/gene	Inheritance	Protein affected
DYT1 (OMIM 128100)	Oppenheim dystonia	9q34.11/ <i>TOR1A</i>	AD	Torsin A [5]
DYT2 (OMIM 224500)	Autosomal recessive dystonia	Unknown	AR	Unknown
DYT3 (OMIM 314250)	Lubag (X-linked dystonia parkinsonism)	Xq13.1/ <i>TAF1</i>	XLR	TATA box binding protein associated factor 1 [19, 68]
DYT4 (OMIM 128101)	Australian whispering dysphonia family	19p13.3/ <i>TUBB4A</i>	AD	Tubulin, beta 4A class IVa [6•, 21•]
DYT5 (OMIM 128230)	Dopa-responsive dystonia	14q22.2/ <i>GCHI</i>	AD	GTP cyclohydrolase I [7]
(OMIM 605407)	Dopa-responsive dystonia	11p.15.5/ <i>TH</i>	AR	Tyrosine hydroxylase [16]
(OMIM 612716)	Dopa-responsive dystonia	2q13.2/ <i>SPR</i>	AR	Sepiapterin reductase [17]
DYT6 (OMIM 602629)	Mixed-type dystonia	8p11.21/ <i>THAP1</i>	AD	THAP domain containing, apoptosis associated protein 1 [8]
DYT7 (OMIM 602124)	Familial torticollis	18p	AD	Unknown
DYT8 (OMIM 118800)	Paroxysmal nonkinesigenic dyskinesia	2q35/ <i>PNKD</i>	AD	Paroxysmal nonkinesigenic protein [9]
DYT9 (OMIM 601042)	Paroxysmal choreoathetosis/spasticity	1p34.2/ <i>SLC2A1</i>	AD	Glucose transporter 1 [69]
DYT10 (OMIM 128200)	Paroxysmal kinesigenic dyskinesia	16p11.2/ <i>PRRT2</i>	AD	Proline-rich transmembrane protein 2 [11]
DYT11 (OMIM 159900)	Myoclonus-dystonia syndrome	7q21.3/ <i>SGCE</i>	AD	ϵ -Sarcoglycan [12]
DYT12 (OMIM 128235)	Rapid-onset dystonia parkinsonism	19q13.2/ <i>ATPIA3</i>	AD	Na ⁺ /K ⁺ -ATPase α_3 subunit [70]
DYT13 (OMIM 607671)	Italian family primary torsion dystonia	1p36.32-p36.13	AD	Unknown
DYT15 (OMIM 607488)	Myoclonus dystonia, Canadian family	18p11	AD	Unknown
DYT16 (OMIM 612067)	Young-onset dystonia parkinsonism	2q31.2/ <i>PRKRA</i>	AR	Protein kinase, interferon-inducible double-stranded RNA dependent activator [18]
DYT17 (OMIM 612406)	Lebanese family with generalized dystonia, dysarthria, and dysphonia	20p11.2-q13.12	AR	Unknown
DYT18 (OMIM 61216)	Paroxysmal exertional dyskinesia associated with hemolytic anemia	1p34.2/ <i>SLC2A1</i>	AR	Glucose transporter 1 [10]
DYT19 (OMIM 611031)	Paroxysmal kinesigenic dyskinesia	16q13-q22.1	AD	Unknown
DYT20 (OMIM 611147)	Paroxysmal nonkinesigenic dyskinesia 2	2q31	AD	Unknown
DYT21	Swedish family with adult-onset mixed dystonia	2q14.3-q21.3	AD	Unknown
DYT23 (OMIM 614860)	Adult-onset cervical dystonia	9q34.11/ <i>CTZ1</i>	AD	Cyclin-dependent kinase inhibitor 1A interacting zinc finger protein 1 [13•]
DYT24 (OMIM 615034)	Adult-onset cervical and segmental dystonia	11p14.2/ <i>ANO3</i>	AD	Anoctamin 3 [14•]
DYT25 (OMIM 615073)	Adult-onset cervical and segmental dystonia	18p11.21/ <i>GNAL</i>	AD	Guanine nucleotide binding protein, alpha activating activity polypeptide, olfactory type [15•, 20•]

AD autosomal dominant, *AR* autosomal recessive

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Table 2

Newly reported dystonia genes and associated variants along with in silico predictions of pathogenicity

Gene	Variants		SIFT	PolyPhen-2	Mutation/Taster	Publication	Missense ^d variants (pathogenic) ^b
	Coding	Protein					
GNAL	c.409G>A	V137M	Tolerated	Probably damaging	Polymorphism	Fuchs et al. [15**]	26 (16)
	c.878C>A	S293*	NP	NP	Disease causing		
	c.463G>A	E155K	Damaging	Probably damaging	Disease causing		
	c.283-284insT	S95Ffs*16	NP	NP	Disease causing		
	c.591-592insA ^c	R198Tfs*13	NP	NP	Disease causing		
	c.61C>T	R21*	NP	NP	Disease causing		
	c.304-312delCCTCCAGTT	P1 02 104del3	NP	NP	Polymorphism		
	c.682G>T	V228F	Damaging	Probably damaging	Disease causing	Vemula et al. [20**]	
	c.591dupA ^c	R198Tfs*13	NP	NP	Disease causing		
	c.733C>T	R245*	NP	NP	Disease causing		
CIZ1	c.3G>A	M1?	NP	NP	Disease causing		60 (45)
	c.284C>T	S95*	NP	NP	Disease causing	Miao et al. [55]	
	c.436G>A	V146M	Damaging	Probably damaging	Disease causing	Zech et al. [47]	
	c.733C>T	R245*	NP	NP	Disease causing	Dufke et al. [57]	
	c.514G>A	V172I	Tolerated	Benign	Disease causing	Saunders-Pullman et al. [59]	
	c.637G>A	G213S	Damaging	Probably damaging	Disease causing	Kumar et al. [58]	
	c.1057G>A	A353T	Damaging	Probably damaging	Disease causing		
TUBB4A	c.214C>T	P72S	Tolerated	Benign	Polymorphism	Charlesworth et al. [14**]	8 (3)
	c.790A>G	S264G	Tolerated	Probably damaging	Disease causing	Xiao et al. [13**]	
	c.2015G>T	R672M	Damaging	Probably damaging	Disease causing		
	c.139C>T	P47S	Damaging	Probably damaging	Polymorphism		8 (3)
	c.4C>G	R2G	Damaging	Polymorphism	Disease causing	Lohmann et al. [21**]	
	c.811G>A	A271T	Damaging	Benign	Disease causing		
	c.745G>A	D249N	Damaging	Damaging	Disease causing	Simons et al. [28]	

Gene	Variants	Protein	SIFT	PolyPhen-2	MutationTaster	Publication	Missense ^a variants (pathogenic) ^b
	Coding						
ANO3	c.1228G>A ^d	E410K	Damaging	Damaging	Disease causing	Blumkin et al. [29]	68 (31)
	c.161C>T	T54I	Tolerated	Benign	Polymorphism	Charlesworth et al. [56]	
	c.1470G>C	W490C	Damaging	Damaging	Disease causing		
	c.1480A>T	R494W	Damaging	Damaging	Disease causing		
	c.2053A>G	S685G	Tolerated	Benign	Disease causing		
	c.2586G>T	K862N	Tolerated	Benign	Disease causing		
	c.2497A>G	I833V	Tolerated	Benign	Disease causing	Zech et al. [47]	
	c.2917G>C	G973R	Tolerated	Probably damaging	Disease causing		

NP not predictable

^aExome Variant Server, NHLBI Exome Sequencing Project (<http://evs.gs.washington.edu/EVS/>)

^bExome Variant Server uses PolyPhen-2 to predict pathogenicity

^cSame variant

^dCorrected variant location (the variant in the original article was misspelled as c.1218G>A)