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Genetics in Dystonia: An Update

Tania Fuchs, PhD¹ and Laurie J. Ozelius, PhD²

Tania Fuchs: Tania.fuchs@mssm.edu; Laurie J. Ozelius: laurie.ozelius@mssm.edu

¹Department of Genetics and Genomic Sciences, Ichan School of Medicine at Mount Sinai, One Gustave L. Levy Place Box 1498, New York, NY 10029, Phone: 212 659-1337, Fax: 212 849-2508

²Department of Genetics and Genomic Sciences and Neurology, Ichan School of Medicine at Mount Sinai, One Gustave L. Levy Place Box 1498, New York, NY 10029, Phone: 212 659-6753, Fax: 212 849-2508

Abstract

The past year has been extremely successful with regards to the genetics of dystonia with the identification of four new dystonia genes (*CIZ1*, *ANO3*, *GNAL* and *TUBB4A*). This progress was primarily achieved because of the application of a new technology, next generation DNA sequencing, which allows rapid and comprehensive assessment of patient's genomes. In addition, a combination of next generation and traditional Sanger sequencing has expanded the phenotypic spectrum associated with some of the dystonia plus (*ATPIA3*) and paroxysmal loci (*PRRT2*). This article reviews the newly identified genes and phenotypes and discusses the future applications of next generation sequencing to dystonia research.

Keywords

Dystonia; genetics; next generation sequencing; exome; *CIZ1*; *ANO3*; *GNAL*; *TUBB4A*; phenotypic spectrum

Introduction

Dystonia is a movement disorder characterized by sustained muscle contractions, causing twisting, repetitive movements and abnormal postures [1]. A revised definition has recently been put forth that expands on this definition and contains other aspects that are essential and unique elements of dystonia including that "dystonic movements are typically patterned, twisting and may be tremulous and are often initiated or worsened by voluntary action and associated with overflow muscle activation" [2]. This paper also proposes a new classification system based on the consideration of two axes: clinical characteristics (including age at onset, body distribution, temporal pattern, coexistence of other movement disorder and other neurological manifestations) and etiology (including nervous system pathology and inheritance). In this new scheme, primary dystonia would be referred to as isolated dystonia and include other clinical descriptors such as early onset generalized or segmental with onset in adulthood while dystonia plus forms would be considered combined

Compliance with Ethics Guidelines

Conflict of Interest

Tania Fuchs declares that she has no conflict of interest.

Laurie J. Ozelius has received royalties from Athena Diagnostics for patents related to DYT1 and DYT6 diagnostics.

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.

dystonia [2]. In this review we will use the current terminology but indicate the newer classification when possible.

Although most forms of monogenic dystonia are inherited as autosomal dominant traits with reduced penetrance, there are also autosomal recessive and X-linked forms as well. Among the dominant forms, both genetic and environmental factors are believed to contribute to the reduced penetrance [3]. At the end of 2011, 19 dystonia loci were distinguished genetically, however the genes for only 10 of these were known. In 2012, four genes for primary dystonia (termed isolated dystonia in the new classification system) were identified using next generation sequencing and a fifth DYT locus has been called into question. In addition, a combination of next generation and traditional Sanger sequencing has expanded the phenotypic spectrum associated with some of the dystonia plus and paroxysmal loci (now both fall under the term combined dystonia). As the main focus of this review is the impact of next generation sequencing on dystonia genetics, it is necessary to provide a brief background on previous genetic techniques and an introduction to the technology of the whole exome sequencing.

The majority of dystonia genes identified thus far have been found using a traditional gene discovery approach which included linkage analysis and positional mapping of the disease gene (reviewed in [4]) a process that required large pedigrees with multiple affected members. However, application of this method to dystonia gene discovery was limited by locus heterogeneity of the disease, late age of onset (in focal forms) and reduced penetrance resulting in families with only a few affected members. The novel technology of next generation DNA sequencing allows for rapid, cost-effective sequencing of either the entire human genome or only its protein coding part (exome), and has significantly accelerated gene discovery [5]. The human exome refers to the collection of all exons in the human genome and consists of approximately 164,000 exons distributed across the genome, and comprises a total of 27.9Mb of sequence. Whole exome sequencing detects all exon variants in an individual's genome, in an unbiased manner, including potentially causative variants. As a result, unlike linkage analysis, whole exome sequencing has no prerequisite for the number of affected individuals in a family and could even be used in cases with sporadic disease [5]. Since most Mendelian disorders are caused by rare mutations in the coding region or at splice junctions, exome sequencing readily captures these types of mutations.

Primary dystonia (Isolated dystonia)

Primary torsion dystonia (PTD) is defined as a syndrome in which dystonia is the only clinical sign (except for tremor), and there is no evidence of neuronal degeneration or an acquired cause by history or routine laboratory assessment. The phenotypic spectrum associated with PTD is broad ranging from early-onset generalized to adult-onset focal dystonia. The prevalence of PTD has been estimated at between 330 per million [6] to 152 per million [7] with focal cases constituting the majority. However, these studies are likely to underestimate the true frequency of PTD, because a significant proportion of disease is not diagnosed [8, 9]. Only two PTD genes, *TOR1A* (*DYT1*) for early onset generalized dystonia and *THAP1* (*DYT6*) for adolescent onset with prominent craniocervical and laryngeal involvement were known before last year. The introduction of next generation sequencing together with exome capture techniques tremendously facilitated gene discovery in PTD. Four novel genes, *CIZ1*, *ANO3*, *GNAL* and *TUBB4A* were identified in 2012 using this approach [10–14]. All of these genes, except *TUBB4A*, were found for previously unknown loci. In addition, a combination of genetic methods, including exome sequencing, has called the *DYT7* locus into question [15].

CIZ1 (DYT23)

CIZ1 was detected as a causative dystonia gene in a multigenerational Caucasian pedigree from United States with predominantly cervical dystonia (CD) that was previously described ([16, 17]). The combination of linkage analysis and exome sequencing revealed a p.S264G mutation in *CIZ1* that segregated in this family. Functional studies suggest that this variant alters the splicing of *CIZ1* and subcellular localization of the protein. Screening of an additional 308 CD patients for mutations in *CIZ1* identified two potentially pathogenic variants (p.P47S and p.R672M) [10] but there were no other family members to test segregation. As a large number of missense variants have been reported in *CIZ1* in variant databases of supposedly healthy controls and because only a single family has been shown to segregate a mutation, it has been suggested that validation in a larger population of dystonia patients along with functional tests are needed to prove pathogenicity of this locus [18].

CIZ1 encodes Cip1-interacting zinc finger protein 1, which is expressed in brain [10]. The protein interacts with p21^{Cip1/Waf1} [19] and participates in DNA synthesis and cell-cycle control [20, 21].

ANO3 (DYT24)

Another dystonia gene, *ANO3*, was recently identified by a combination of linkage analysis and exome sequencing in a UK family with autosomal dominant craniocervical dystonia [11]. Linkage analysis revealed five peaks with the LOD scores of 2.01 on chromosomes 4, 5, 6, 7 and 11. Exome sequencing of two affected individuals from the family revealed a heterozygous missense mutation c.1480A>T (p.Arg494Trp) in the *ANO3* gene which resided in one of the linked regions and completely co-segregated with dystonia in this family. Screening of an additional 110 familial and 78 sporadic patients with cervical dystonia with or without dystonic upper-limb tremor, revealed five additional missense mutations in different exons of *ANO3* including one in the 5'UTR. Two of these variants c.1470G>C (p.Trp490Cys) and c.2053A>G (p.Ser685Gly) showed co-segregation with dystonia when tested in additional family members [11].

The dystonia phenotype associated with *ANO3* mutations comprises mostly a focal or segmental distribution. All of the mutation carriers had dystonia involving the craniocervical region, but some also had laryngeal dystonia, blepharospasm and/or limb tremor. The range of the disease onset varied between early childhood to 40 years [11]. It is interesting that a patient with an *ANO3* mutation was previously diagnosed with essential tremor, raising the question of whether mutations in *ANO3* are also involved in tremor associated with dystonia (TAD).

ANO3 codes for the anoctamin 3 protein, which belongs to a family of genes encoding Ca²⁺ activated Cl⁻ ion channels [22]. *ANO3* is increasingly expressed during brain development from early fetal stage to adolescence in the striatum, where the highest level of expression was observed, but is also found in the neocortex, amygdala and hippocampus [11]. The precise function of anoctamin 3 is not yet known, but it apparently is targeted to the endoplasmic reticulum (ER) rather than to the cell surface like other anoctamins [22]. Patient fibroblasts expressing the p.Trp490Cys mutation exhibited reduced Ca²⁺ signaling, most likely resulting from a smaller calcium pool within the ER [11]. Therefore, it is possible that mutations in *ANO3* lead to abnormal striatal neuron excitability, which clinically manifests as dystonic movements.

GNAL (DYT25)

GNAL was detected as a dystonia causative gene in two moderate size PTD families with mostly segmental dystonia with neck onset [12]. The combination of linkage analysis and exome sequencing revealed a nonsense p.S293X mutation in *GNAL* resulting in a premature stop codon in one family and a missense p.V137M mutation in the other. Screening the coding region of *GNAL* in 39 additional multiplex PTD families revealed novel mutations in six additional families.

The phenotype in *GNAL* carriers is characterized by an average age of onset of 31.3 years, with a range 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 speech involvement. Brachial onset was not observed and eventual arm involvement was seen in only 32%, distinguishing *GNAL* carriers from *THAPI* carriers [12]. *GNAL* encodes the stimulatory alpha subunit of heterotrimeric G-protein, G α olf, which was initially identified as a G-protein mediating odorant signaling in the olfactory epithelium [23]. However, later it was recognized as the main G-protein alpha subunit in the striatum where it couples dopamine type 1 receptors (D1R) of the direct pathway and adenosine A2A receptors (A2AR) of the indirect pathway to the activation of adenylyl cyclase type 5 (AC5) in the medium spiny neurons [24–26]. Heterozygote *GNAL* mice demonstrate a muted response to acute psychostimulant and caffeine exposure [27]. Homozygote null mice are anosmic, hyperactive at baseline and fail to respond to acute psychostimulant exposure, but do not manifest an overt movement disorder [27, 28]. G α olf has been physiologically linked to levodopa-induced dyskinesias (LID) [29, 30] and genetically linked to bipolar disorder, schizophrenia and attention deficit hyperactivity disorder (ADHD) [31–33].

Most recently, using exome sequencing Vemula et al. (2103) identified a novel *GNAL* missense mutation in an African-American family with the majority of members affected with segmental dystonia [34]. The follow-up screening of 760 patients with familial and sporadic forms of cervical dystonia revealed three additional mutations in mainly focal cervical dystonia cases. The majority of *GNAL* carriers had focal cervical dystonia (7 out of 11) and the average age of onset in *GNAL* carriers was later than previously reported (45 years), most probably due to the high prevalence of focal dystonia in this cohort. Interestingly, related to the role of *GNAL* as a mediator of olfactory signaling, microsomia was very prominent in members of the African American family affected with dystonia, however was absent or variable in the Caucasian *GNAL* carriers [34].

Identification of dystonia causative mutations in *GNAL* points to primary abnormalities of D1R and/or A2AR transmission as possibly leading to dystonia and calls for thorough investigation of the role of this pathway in dystonia pathophysiology.

TUBB4A (DYT4)

A single large Australian family with dystonia symptoms ranging from focal to generalized but with prominent “whispering dysphonia” has been designated as *DYT4* [35, 36]. The disease is inherited in an autosomal dominant (AD) manner with reduced penetrance and has a wide range of age at onset (13–37 years) though most cases begin in their twenties. Out of the 191 identified family members [37] more than 30 affected individuals were reported typically presenting with laryngeal dysphonia which progresses to a generalized dystonia with a characteristic “hobby horse” ataxic gait. Recent re-evaluation of the phenotype in this family identified clinical non-motor features, such as thin face and body habitus, which co-segregated with the *DYT4* phenotype [37]. *DYT4* dystonia has long remained one of the few

on the DYT list missing both locus assignment and gene identification. Recently, two research groups independently identified the *TUBB4A* gene on chromosome 19 as causative for *DYT4* dystonia using a combination of linkage analysis and exome sequencing [13, 14].

The missense variant c.4C>G, p.R2G in exon 1 of the *TUBB4A* gene completely co-segregated with dystonia in the family and was found neither in the public databases nor in the healthy control chromosomes screened [13, 14]. Screening of 394 unrelated dystonia patients for mutation in *TUBB4A* revealed an additional missense variant (p.A271T) in a 71-year-old female patient with spasmodic dysphonia, oromandibular dystonia and dyskinesia [14]. Although she had a family history of dystonia, other family members were not available for analysis.

TUBB4A encodes β -tubulin, the main constituent of microtubules, a major cytoskeletal component [38]. The protein is mostly expressed in the brain, however lower levels are also detected in other tissues such as testes, cardiomyocytes and blood [13]. The p.R2G mutation affects an extremely conserved tetrapeptide, MREI, at the N-terminal part of the protein involved in the autoregulation of the β -tubulin mRNA [39]. Site directed mutagenesis studies demonstrated the loss of autoregulatory capability in Arg2 mutants resulting in increases *TUBB4A* transcript levels [40]. In contrast, the expression of the *TUBB4A* was significantly reduced in various cell types from the p.R2G mutation carrier [14]. The reason for this discrepancy is not clear at present and further studies are required to determine the functional consequences of *TUBB4A* mutations.

A new twist was recently added to the *TUBB4A* story when a de novo mutation in this gene was detected in 11 unrelated patients with hypomyelination and atrophy of the basal ganglia and cerebellum (H-ABS) [41], a rare leukodystrophy characterized by developmental delay, extrapyramidal movement disorders (dystonia, choreoathetosis, rigidity, opisthotonus and oculogyric crises), progressive spastic tetraplegia, ataxia and rarely seizures. In contrast to *DYT4* (*TUBB4A*) patients who have no pathological findings on MRI [37], the MRI of the H-ABS patients shows hypomyelination, cerebellar atrophy and absence or disappearance of the putamen. The de novo p.D249N mutation occurs in a functional domain of *TUBB4A* which is responsible for interactions with other tubulins. In addition Asp249 forms a salt bridge with the Arg2 residue mutated in *DYT4*. Further research is required to establish genotype-phenotype relationships in *TUBB4A* mutation carriers and explain how different mutations can result in variability in phenotypic features. Another interesting aspect is the finding of p.D249N mutation mosaicism in the asymptomatic mother of one of H-ABS *TUBB4A* carriers [41], suggesting that rare de novo mutations that are initially phenotypically neutral in a mosaic individual can be disease causing in the subsequent generation if they are inherited. This finding raises the question of whether apparently sporadic cases of focal dystonia could result as the subsequent generation of a de novo mutation carrier. The answer to this question can inform and transform the genetic study design and the analytical pipelines for exome sequence analysis in focal dystonia cases.

DYT7

The *DYT7* locus was mapped to chromosome 18p in a large German family. All affected members had adult-onset cervical dystonia (CD) (mean 43 yrs; range 28 to 70 yrs), although some also had brachial and cranial involvement [42]. This family was recently clinically reevaluated and 6 individuals documented as definitely affected with focal dystonia [15]. Another individual was identified with an alteration of gait and possible CD but without the *DYT7* risk haplotype. In addition, 12 other family members had a diagnosis of questionable CD. Four genes on chromosome 18p were sequenced based on an incidental finding of a small deletion of this region in an unrelated CD case but no mutations were found (one of these was the *GNAL* gene, see above). Exome sequencing of two definitely affected patients

revealed no causative variants in other genes located on chromosome 18p. In addition, copy number variations were ruled out using a SNP array and the *CIZ1* gene (CD phenotype-see above) was sequenced with no mutations identified. Taken together, these data suggest that the original linkage data may have been mistaken and call the *DYT7* locus on chromosome 18p into question [15].

Phenotypic Spectrum

Exome sequencing can also be used to define the phenotypic spectrum of a disease. Two recent examples related to dystonia involve the *ATP1A3* gene, originally described as a cause for rapid onset dystonia parkinsonism (RDP/*DYT12*), and *PRRK2*, a gene implicated in paroxysmal kinesigenic dyskinesia (PKD/*DYT10/DYT19*). RDP is classified as a Dystonia Plus form and PKD is a Paroxysmal Dystonia in the current classification system but both would fall under Combined Dystonia in the new scheme with RDP being combined persistent dystonia and PKD being be combined paroxysmal dystonia [2]

ATP1A3 (DYT12)

ATP1A3 encodes the catalytic subunit of the sodium pump that uses ATP hydrolysis to exchange Na⁺ and K⁺ across the cell membrane to maintaining ionic gradients. In 2004, mutations in the *ATP1A3* gene were first described in cases of rapid-onset dystonia parkinsonism (RDP/*DYT12*) [43]. The disease phenotype is usually characterized by onset of dystonia with parkinsonism (bradykinesia and postural instability) over hours to weeks often triggered by physical or emotional stress with prominent bulbar dystonia that typically follows a rostrocaudal (face>arm>leg) gradient [44]. The typical age of onset is usually in the teens or early twenties but recently two infants with motor delay and ataxia were reported with *ATP1A3* mutations [45]. In addition, non-motor manifestations are also found in RDP patients including an elevated prevalence of mood disorders (50%) and psychosis (19%) compared to familial controls without *ATP1A3* mutations [46].

Recently, using exome sequencing in trios, several groups identified de novo mutations in *ATP1A3* as a cause of Alternating Hemiplegia of Childhood (AHC) [47–49]. The diagnostic criteria for AHC include: (1) paroxysmal episodes of hemiplegia; (2) episodes of bilateral hemiplegia, or quadriplegia; (3) other paroxysmal manifestations, such as abnormal eye movements, nystagmus, strabismus, dystonia, tonic spells, or autonomic disturbance which can occur during hemiplegia or as isolated events; (4) evidence of permanent neurological dysfunction which can manifest as mental retardation, seizures, ataxia disorders, choreoathetosis, developmental delay, and/or persistent motor deficits such as spastic diplegia/quadruplegia or hypotonia; (5) sleep during a paroxysmal attack relieves symptoms, although attacks may resume soon after awakening; (6) the first signs of dysfunction occur prior to the age of 18 months; and (7) not attributable to other disorders [50].

A total of 11 novel mutations (9 missense mutations, a 3-bp in-frame deletion and a 3-bp in-frame insertion) have been reported in 20 RDP families, including 8 de novo cases, 8 that occur in families, and 4 with unknown origin of the mutation [45, 51, 52]. In AHC patients, a total of 27 different *ATP1A3* mutations have been reported, including ten that are recurrent and two (D801N and E815K) that explain more than 50% of AHC patients [47–49]. For the most part, none of the mutations overlap between RDP and AHC although the same amino acid is mutated to distinct residues in several cases (i.e. D801Y in RDP and D801N in AHC). However, there is one multiplex family where the same mutation (D923N) leads to both RDP (one typical and one atypical case) and AHC (one typical and 3 AHC-like cases) phenotypes [52]. There are several other examples of patients with intermediate phenotypes [46, 48] suggesting that these disorders are different manifestations along a clinical spectrum [53]. Studies to determine exactly how these mutations disrupt the Na K pump may shed

light on these phenotypic differences but other factors including the timing of the expression of the mutation and environmental factors may also play a role. Moreover, it would not be surprising if a wider range of phenotypes associated with *ATP1A3* mutations become apparent as next-generation sequencing becomes more widely used in clinical practice.

PRRT2 (DYT10/DYT19)

Paroxysmal kinesigenic dyskinesia (PKD) is characterized by brief (less than 1 min) frequent (up to 100 times per day) attacks of choreoathetoid and/or dystonic movements induced by sudden voluntary movements or startle [54]. Patients remain conscious and the neurological examination is normal. The disease is inherited in autosomal dominant manner and the prevalence is estimated at 1:150,000 individuals [54]. Two thirds of the PKD patients have family history and sporadic cases are reported more frequently in males. Two loci for PKD (*DYT10* and *DYT19*) were previously mapped on chromosome 16 very close to each other [55, 56]. Recently, exome sequencing in PKD families identified mutations in the proline-rich transmembrane protein 2 (*PRRT2*) as a cause of PKD in these families [57–59] as well as in a cohort of PKD with Infantile Convulsions [60]. At present, *PRRT2* is the major gene associated with PKD and is responsible for both *DYT10* and *DYT19* [61]. In the Chinese population, three different studies identified *PRRT2* mutations in a total of 16 out of 17 families and 10 out of 29 sporadic cases [57–59]. In a European population of 34 PKD index cases selected based on the criteria defined by Bruno et al [54], *PRRT2* mutations were reported in 65%, including 13 out of 14 familial cases (93%) and nine out of 20 sporadic cases (45%) [62]. In an ethnically diverse cohort of PKD/IC families, 24/25 clinically well documented families had a *PRRT2* mutation while 28/78 less well clinically characterized families also possessed a mutation [60]. In all of these studies, the majority of PKD cases harbor the same insertion mutation c.649_650insC resulting in truncation of the encode protein [57–62]. Age of onset in *PRRT2* mutation carriers is younger (range: 6–14 years) than in non-*PRRT2* PKD cases (range: 9.5–19 years). A small number of patients with the typical PKD phenotype do not harbor mutations in *PRRT2* suggesting existence of at least one more PKD causative gene. *PRRT2* is highly expressed in the basal ganglia and disrupted neurotransmitter release has been suggested as a possible pathogenic mechanism [57–60].

Mutations in the *PRRT2* gene have also been found in patients with a wide array of phenotypes (reviewed in [63]), including:

- Benign Familial Infantile Seizures: an autosomal dominant disorder with non-febrile convulsions that begins between 2 and 12 month of age, responds well to anticonvulsant treatment, and remits by 2 years of age.
- Infantile Convulsions with Choreoathetosis (ICCA) with or without paroxysmal kinesigenic dyskinesia: (an autosomal dominant disorder characterized by infantile seizures, paroxysmal dyskinesia or both.
- Episodic Ataxia (EA): a rare disorder characterized by attacks of ataxia and usually caused by mutations in *KCNA1* or *CACNA1A*; half of the individuals who did not have mutation in these two genes, had mutation in *PRRT2*.
- Hemiplegic Migraine (HM): a rare subtype of migraine with aura and attacks associated with transient weakness or hemiparesis.
- Benign paroxysmal torticollis of infancy

There is no obvious genotype-phenotype correlation observed yet, since phenotypes vary not only between families but also between carriers of the same mutation within family [64]. However, the shared feature of all the above phenotypes seems to be the paroxysmal nature

of the symptoms. It is worth noting that in a family carrying two copies of a *PRRT2* mutation, homozygous mutation carriers had a more severe phenotype than their heterozygous carrier relatives, suggesting that dosage can play a role in the severity of the phenotype [65].

Conclusion

Exome sequencing is a useful research tool for dystonia gene discovery which has allowed geneticists to more than double the number of genes responsible for primary dystonia in the past year. Both the phenotypic variability associated with mutations in the same gene (*ATPIA3*, *PRRT2*, *TUBB4A*) and the overlapping clinical phenotypes among the primary dystonia genes (*DYT1*, *DYT6*, *CIZ1*, *ANO3*, *GNAL*) makes molecular diagnosis of inherited dystonia challenging and expensive. The price of Sanger sequencing for the currently identified dystonia genes will soon be more expensive than clinical exome sequencing. However, clinical exome sequencing in a single dystonia patient also has drawbacks as sorting through the novel variants to determine which one is pathogenic is currently very difficult and will lead to many 'variants of unknown significance'. Instead, the community should work towards a database of novel variants identified in dystonia patients coupled to detailed clinical phenotypes that will allow investigators to identify rare variants that are either present in multiple patients or groups of rare variants that concentrate in particular genes. Moreover, detailed phenotypic information will be useful to more fully describe the associated clinical features in those patients who possess novel variants in known dystonia genes.

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References

1. Fahn S, Bressman SB, Marsden CD. Classification of dystonia. *Adv Neurol.* 1998; 78:1–10. [PubMed: 9750897]
2. Albanese A, Bhatia K, Bressman SB, et al. Phenomenology and classification of dystonia: A consensus update. *Mov Disord.* 2013; 28:863–73. [PubMed: 23649720]
3. Defazio G, Berardelli A, Hallett M. Do primary adult-onset focal dystonias share aetiological factors? *Brain.* 2007; 130:1183–93. [PubMed: 17242025]
4. Fuchs T, Ozelius LJ. Genetics of dystonia. *Semin Neurol.* 2011; 31:441–8. [PubMed: 22266882]
5. Ku CS, Cooper DN, Polychronakos C, et al. Exome sequencing: dual role as a discovery and diagnostic tool. *Ann Neurol.* 2012; 71:5–14. [PubMed: 22275248]
6. Nutt JG, Muenter MD, Aronson A, et al. Epidemiology of focal and generalized dystonia in Rochester, Minnesota. *Mov Disord.* 1988; 3:188–94. [PubMed: 3264051]
7. Epidemiological Study of Dystonia in Europe (ESDE) Collaborative Group. A prevalence study of primary dystonia in eight European countries. *J Neurol.* 2000; 247:787–92. [PubMed: 11127535]
8. Risch N, de Leon D, Ozelius L, et al. Genetic analysis of idiopathic torsion dystonia in Ashkenazi Jews and their recent descent from a small founder population. *Nat Genet.* 1995; 9:152–9. [PubMed: 7719342]
9. Muller J, Kiechl S, Wenning GK, et al. The prevalence of primary dystonia in the general community. *Neurology.* 2002; 59:941–3. [PubMed: 12297587]
10. Xiao J, Uitti RJ, Zhao Y, et al. Mutations in *CIZ1* cause adult onset primary cervical dystonia. *Ann Neurol.* 2012; 71:458–69. [PubMed: 22447717]
11. Charlesworth G, Plagnol V, Holmstrom KM, et al. Mutations in *ANO3* Cause Dominant Craniocervical Dystonia: Ion Channel Implicated in Pathogenesis. *Am J Hum Genet.* 2012;

91:1041–50. This paper describes a novel dystonia causative gene, ANO3, identified by exome sequencing in familial dystonia. The dystonia phenotype associated with ANO3 mutations comprises mostly a focal or segmental distribution with wide range of onset (between early childhood and 40 years). All of the mutation carriers had dystonia involving the craniocervical region, but some also had laryngeal dystonia, blepharospasm and/or limb tremor. One mutation positive patient was previously diagnosed with essential tremor, raising the possibility that mutations in ANO3 may also cause tremor associated with dystonia (TAD). [PubMed: 23200863]

- 12•• Fuchs T, Saunders-Pullman R, Masuho I, et al. Mutations in GNAL cause primary torsion dystonia. *Nat Genet.* 2012; 45:88–92. This paper describes the identification of GNAL by exome sequencing. GNAL mutations were found only in familial cases and the associated phenotype is characterized by average age of onset 313, mostly focal to segmental distribution with rare generalization, onset in the neck in 82% of carriers and prominent neck involvement (93% of carriers) at the final exam. [PubMed: 23222958]
- 13•• Hersheson J, Mencacci NE, Davis M, et al. Mutations in the autoregulatory domain of beta-tubulin 4a cause hereditary dystonia. *Ann Neurol.* 2012 Dec 13. Epub ahead of print. Exome sequencing identifies TUBB4A as a dystonia causative gene for the previously described DYT4 locus. This locus and phenotype were described in a single Australian family with ‘whispering dysphonia’ that progresses to a generalized dystonia with a characteristic “hobby horse” ataxic gait.
- 14•• Lohmann K, Wilcox RA, Winkler S, et al. Whispering dysphonia (DYT4 dystonia) is caused by a mutation in the TUBB4 gene. *Ann Neurol.* 2012 Dec 13. Epub ahead of print. Exome sequencing identifies TUBB4A as a dystonia causative gene for the previously described DYT4 locus. This locus and phenotype were described in a single Australian family with ‘whispering dysphonia’ that progresses to a generalized dystonia with a characteristic “hobby horse” ataxic gait. The mutations in TUBB4A are not expected to be responsible for a significant proportion of dystonia, since screening of the gene in 394 unrelated dystonia patients revealed only one additional putative mutation.
15. Winter P, Kamm C, Biskup S, et al. DYT7 gene locus for cervical dystonia on chromosome 18p is questionable. *Mov Disord.* 2012; 27:1819–21. [PubMed: 23115116]
16. Uitti RJ, Maraganore DM. Adult onset familial cervical dystonia: report of a family including monozygotic twins. *Mov Disord.* 1993; 8:489–94. [PubMed: 8232359]
17. Arman PR, del Grosso N, Valente EM, et al. Primary torsion dystonia: the search for genes is not over. *J Neurol Neurosurg Psychiatry.* 1999; 67:395–7. [PubMed: 10449567]
18. Klein C, König IRK, Lohmann K. Exome sequencing for gene discovery: time to set standard criteria. *Ann Neurol.* 2012; 72:627–8. [PubMed: 23109159]
19. Mitsui K, Matsumoto A, Ohtsuka S, et al. Cloning and characterization of a novel p21(Cip1/Waf1)-interacting zinc finger protein, Ciz1. *Biochem Biophys Res Commun.* 1999; 264:457–64. [PubMed: 10529385]
20. Coverley D, Marr J, Ainscough J. Ciz1 promotes mammalian DNA replication. *J Cell Sci.* 2005; 118:101–12. [PubMed: 15585571]
21. Rahman F, Ainscough JF, Copeland N, Coverley D. Cancer-associated missplicing of exon 4 influences the subnuclear distribution of the DNA replication factor CIZ1. *Hum Mutat.* 2007; 28:993–1004. [PubMed: 17508423]
22. Duran C, Qu Z, Osunkoya AO, et al. ANOs 3–7 in the anoctamin/Tmem16 Cl⁻ channel family are intracellular proteins. *Am J Physiol Cell Physiol.* 2012; 302:C482–93. [PubMed: 22075693]
23. Jones DT, Reed RR. Golf: an olfactory neuron specific-G protein involved in odorant signal transduction. *Science.* 1989; 244:790–5. [PubMed: 2499043]
24. Drinnan SL, Hope BT, Snutch TP, Vincent SR. G(olf) in the basal ganglia. *Mol Cell Neurosci.* 1991; 2:66–70. [PubMed: 19912784]
25. Kull B, Svenningsson P, Fredholm BB. Adenosine A(2A) receptors are colocalized with and activate g(olf) in rat striatum. *Mol Pharmacol.* 2000; 58:771–7. [PubMed: 10999947]
26. Corvol JC, Studler JM, Schönn JS, et al. Gα(olf) is necessary for coupling D1 and A2a receptors to adenylyl cyclase in the striatum. *J Neurochem.* 2001; 76:1585–8. [PubMed: 11238742]

27. Corvol JC, Valjent E, Pascoli V, et al. Quantitative changes in Galphao1f protein levels, but not D1 receptor, alter specifically acute responses to psychostimulants. *Neuropsychopharmacology*. 2007; 32:1109–21. [PubMed: 17063155]
28. Belluscio L, Gold GH, Nemes A, Axel R. Mice deficient in G(olf) are anosmic. *Neuron*. 1998; 20:69–81. [PubMed: 9459443]
29. Alcacer C, Santini E, Valjent E, et al. Galpha(olf) mutation allows parsing the role of cAMP-dependent and extracellular signal-regulated kinase-dependent signaling in L-3,4-dihydroxyphenylalanine-induced dyskinesia. *J Neurosci*. 2012; 32:5900–10. [PubMed: 22539851]
30. Corvol JC, Muriel MP, Valjent E, et al. Persistent increase in olfactory type G-protein alpha subunit levels may underlie D1 receptor functional hypersensitivity in Parkinson disease. *J Neurosci*. 2004; 24:7007–14. [PubMed: 15295036]
31. Berrettini WH, Vuoristo J, Ferraro TN, et al. Human G(olf) gene polymorphisms and vulnerability to bipolar disorder. *Psychiatr Genet*. 1998; 8(4):235–8. [PubMed: 9861642]
32. Segurado R, Detera-Wadleigh SD, Levinson DF, et al. Genome scan meta-analysis of schizophrenia and bipolar disorder, part III: Bipolar disorder. *Am J Hum Genet*. 2003; 73:49–62. [PubMed: 12802785]
33. Laurin N, Ickowicz A, Pathare T, et al. Investigation of the G protein subunit Galphao1f gene (GNAL) in attention deficit/hyperactivity disorder. *J Psychiatr Res*. 2008; 42:117–24. [PubMed: 17166517]
34. Vemula SR, Puschmann A, Xiao J, et al. Role of Galpha(olf) in familial and sporadic adult-onset primary dystonia. *Hum Mol Genet*. 2013; 22:2510–9. [PubMed: 23449625]
35. Parker N. Hereditary whispering dysphonia. *J Neurol Neurosurg Psychiatry*. 1985; 48:218–24. [PubMed: 3156966]
36. Ahmad F, Davis MB, Waddy HM, et al. Evidence for locus heterogeneity in autosomal dominant torsion dystonia. *Genomics*. 1993; 15:9–12. [PubMed: 8432555]
37. Wilcox RA, Winkler S, Lohmann K, Klein C. Whispering dysphonia in an Australian family (DYT4): a clinical and genetic reappraisal. *Mov Disord*. 2011; 26:2404–8. [PubMed: 21956287]
38. Mandelkow E, Mandelkow EM. Microtubules and microtubule-associated proteins. *Curr Opin Cell Biol*. 1995; 7:72–81. [PubMed: 7755992]
39. Yen TJ, Gay DA, Pachter JS, Cleveland DW. Autoregulated changes in stability of polyribosome-bound beta-tubulin mRNAs are specified by the first 13 translated nucleotides. *Mol Cell Biol*. 1988; 8:1224–35. [PubMed: 2835666]
40. Yen TJ, Machlin PS, Cleveland DW. Autoregulated instability of beta-tubulin mRNAs by recognition of the nascent amino terminus of beta-tubulin. *Nature*. 1988; 334:580–5. [PubMed: 3405308]
41. Simons C, Wolf NI, McNeil N, et al. A De Novo Mutation in the beta-Tubulin Gene TUBB4A Results in the Leukoencephalopathy Hypomyelination with Atrophy of the Basal Ganglia and Cerebellum. *Am J Hum Genet*. 2013; 92:767–73. [PubMed: 23582646]
42. Leube B, Rudnicki D, Ratzlaff T, et al. Idiopathic torsion dystonia: assignment of a gene to chromosome 18p in a German family with adult onset, autosomal dominant inheritance and purely focal distribution. *Hum Mol Genet*. 1996; 5:1673–7. [PubMed: 8894706]
43. de Carvalho Aguiar P, Sweadner KJ, Penniston JT, et al. Mutations in the Na⁺/K⁺-ATPase alpha3 gene ATP1A3 are associated with rapid-onset dystonia parkinsonism. *Neuron*. 2004; 43:169–75. [PubMed: 15260953]
44. 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; 130:828–35. [PubMed: 17282997]
45. Brashear A, Mink JW, Hill DF, et al. ATP1A3 mutations in infants: a new rapid-onset dystonia-Parkinsonism phenotype characterized by motor delay and ataxia. *Dev Med Child Neurol*. 2012; 54:1065–7. [PubMed: 22924536]
46. Brashear A, Cook JF, Hill DF, et al. Psychiatric disorders in rapid-onset dystonia-parkinsonism. *Neurology*. 2012; 79:1168–73. [PubMed: 22933743]
- 47••. Heinzen EL, Swoboda KJ, Hitomi Y, et al. De novo mutations in ATP1A3 cause alternating hemiplegia of childhood. *Nat Genet*. 2012; 44:1030–4. This is the first paper (along with [48]) to

identify mutations in the ATP1A3 gene in patients with Alternating Hemiplegia of Childhood. This expands the phenotypic spectrum associated with mutations in this gene beyond the original description of rapid onset dystonia parkinsonisms (RDP) and suggests these disorders are part of the same clinical spectrum. [PubMed: 22842232]

- 48•. 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; 11:764–73. This is the first paper (along with [47]) to identify mutations in the ATP1A3 gene in patients with Alternating Hemiplegia of Childhood. This expands the phenotypic spectrum associated with mutations in this gene beyond the original description of rapid onset dystonia parkinsonisms (RDP) and suggests these disorders are part of the same clinical spectrum. [PubMed: 22850527]
49. 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. [PubMed: 23409136]
50. Bourgeois M, Aicardi J, Goutieres F. Alternating hemiplegia of childhood. *J Pediatr.* 1993; 122:673–9. [PubMed: 8496742]
51. Ozelius LJ, Lubarr N, Bressman SB. Milestones in dystonia. *Mov Disord.* 2011; 26:1106–26. [PubMed: 21626555]
52. Roubergue A, Roze E, Vuillaumier-Barrot S, et al. The multiple faces of the ATP1A3-related dystonic movement disorder. *Mov Disord.* 2013 Mar 8. Epub ahead of print.
53. Ozelius LJ. Clinical spectrum of disease associated with ATP1A3 mutations. *Lancet Neurol.* 2012; 11:741–3. [PubMed: 22857851]
54. Bruno MK, Hallett M, Gwinn-Hardy K, et al. Clinical evaluation of idiopathic paroxysmal kinesigenic dyskinesia: new diagnostic criteria. *Neurology.* 2004; 63:2280–7. [PubMed: 15623687]
55. Tomita H, Nagamitsu S, Wakui K, et al. Paroxysmal kinesigenic choreoathetosis locus maps to chromosome 16p11.2-q12. 1. *Am J Hum Genet.* 1999; 65:1688–97. [PubMed: 10577923]
56. Valente EM, Spacey SD, Wali GM, et al. A second paroxysmal kinesigenic choreoathetosis locus (EKD2) mapping on 16q13-q22. 1 indicates a family of genes which give rise to paroxysmal disorders on human chromosome 16. *Brain.* 2000; 123:2040–5. [PubMed: 11004121]
57. Chen WJ, Lin Y, Xiong ZQ, et al. Exome sequencing identifies truncating mutations in PRRT2 that cause paroxysmal kinesigenic dyskinesia. *Nat Genet.* 2011; 43:1252–5. [PubMed: 22101681]
58. Li J, Zhu X, Wang X, et al. Targeted genomic sequencing identifies PRRT2 mutations as a cause of paroxysmal kinesigenic choreoathetosis. *J Med Genet.* 2012; 49:76–8. [PubMed: 22131361]
59. Wang JL, Cao L, Li XH, et al. Identification of PRRT2 as the causative gene of paroxysmal kinesigenic dyskinesias. *Brain.* 2011; 134:3493–3501. [PubMed: 22120146]
60. Lee HY, Huang Y, Bruneau N, et al. Mutations in the novel protein PRRT2 cause paroxysmal kinesigenic dyskinesia with infantile convulsions. *Cell Rep.* 2012; 1:2–12.
61. Gardiner AR, Bhatia KP, Stamelou M, et al. PRRT2 gene mutations: from paroxysmal dyskinesia to episodic ataxia and hemiplegic migraine. *Neurology.* 2012; 79:2115–21. [PubMed: 23077024]
62. Meneret A, Grabli D, Depienne C, et al. PRRT2 mutations: a major cause of paroxysmal kinesigenic dyskinesia in the European population. *Neurology.* 2012; 79:170–4. [PubMed: 22744660]
63. Meneret A, Gaubebout C, Riant F, et al. PRRT2 mutations and paroxysmal disorders. *Eur J Neurol.* 2013; 20:872–8. [PubMed: 23398397]
64. Silveira-Moriyama L, Gardiner AR, Meyer E, et al. Clinical features of childhood-onset paroxysmal kinesigenic dyskinesia with PRRT2 gene mutations. *Dev Med Child Neurol.* 2013; 55:327–34. [PubMed: 23363396]
65. Labate A, Tarantino P, Viri M, et al. Homozygous c. 649dupC mutation in PRRT2 worsens the BFIS/PKD phenotype with mental retardation, episodic ataxia, and absences. *Epilepsia.* 2012; 53:e196–9. [PubMed: 23126439]