

# Update on the Genetics of Dystonia

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**Abstract** Mainly due to the advent of next-generation sequencing (NGS), the field of genetics of dystonia has rapidly grown in recent years, which led to the discovery of a number of novel dystonia genes and the development of a new classification and nomenclature for inherited dystonias. In addition, new findings from both in vivo and in vitro studies have been published on the role of previously known dystonia genes, extending our understanding of the pathophysiology of dystonia. We here review the current knowledge and recent findings in the known genes for isolated dystonia *TORIA*, *THAPI*, and *GNAL* as well as for the combined dystonias due to mutations in *GCHI*, *ATPIA3*, and *SGCE*. We present confirmatory evidence for a role of dystonia genes that had not yet been unequivocally established including *PRKRA*, *TUBB4A*, *ANO3*, and *TAF1*. We finally discuss selected novel genes for dystonia such as *KMT2B* and *VAC14* along with the challenges for gene identification in the NGS era and the translational importance of dystonia genetics in clinical practice.

**Keywords** Dystonia · Classification · Nomenclature · Novel genes · Next-generation sequencing · Mutation

## Introduction

The term “dystonia” was first coined by Hermann Oppenheim in his landmark article entitled “About a peculiar cramping sickness in children and adolescents (dysbasia lordotica progressiva, dystonia musculorum deformans)” [1]. Intriguingly, despite the absence of an obvious family history, Oppenheim suspected that “hereditary burden likely plays a major role” in the etiology of the disease and thus his paper may also represent the first reference to the role of genetics in dystonia [1, 2•].

Dystonia is a rare movement disorder with an estimated prevalence of 16:100,000 [3]. It is characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements, postures, or both [4•]. Dystonia is a clinically and genetically highly heterogeneous disorder. Dystonic symptoms can present from early infancy to late adulthood and affect one (focal), several (multifocal and segmental), or many (generalized) body parts. Adult-onset focal dystonia is by far the most frequent form of dystonia. In two recent studies of 1500 Chinese and 600 Irish patients with this type of dystonia, respectively, the vast majority of patients had dystonia of the neck (cervical dystonia, 37 [5] vs. 69% [6]) or the eye lid (blepharospasm, 56 [5] vs. 17% [6]). The differences for the prevalence of blepharospasm may be best explained by the exposure to environmental factors such as sun light [7] or air pollution that directly interact with the eyes. Other types of dystonia such as focal hand or leg dystonia (3–7%), spasmodic dysphonia 18 (1–3%), musician’s dystonia (3%), or oromandibular dystonia (1%) were much rarer [5, 6]. Women are affected about twice as often as men.

Of note, although the genetic causes of dystonia—especially for adult-onset focal forms—are largely elusive, a positive family history has been reported in about 20% of the patients [6, 8, 9]. In the past three decades, more than 200 genes have

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been linked to different, mainly childhood-onset generalized forms of dystonia ([www.omim.org](http://www.omim.org)) [10, 11]. This includes forms in which dystonia is the only disease manifestation with the exception of tremor (“isolated dystonia”), forms in which dystonia co-occurs with another movement disorder such as parkinsonism or myoclonus (“combined dystonia”) and disorders in which dystonia is often less prominent and usually one of several disease manifestations (“complex dystonia”) [4, 12]. Notably, most of the genetic forms belong to the latter phenotypic group which, however, also represents the most heterogeneous class in terms of clinical expression.

The genetic nomenclature (DYTs) was originally designed to consecutively number findings from linkage studies in large dystonia families without knowing the underlying gene mutation. Meanwhile, many of the locus symbols were repeatedly confirmed and respective genes with disease-causing mutations identified, e.g., mutations in the *TOR1A* gene at the *DYT1* locus as a cause of autosomal dominant torsion dystonia [13]. The genetic locus symbols (e.g., *DYT1*) were later also used as synonyms for the respective phenotype such as “DYT1 dystonia” or the underlying genetic cause (mutation in the “*DYT1*” gene) which is, however, not recommended by the official HUGO Gene Nomenclature Committee ([www.genenames.org](http://www.genenames.org)). Given the rapidly evolving field of (dystonia) genetics, it has become increasingly impossible to maintain a chronological, numbered system and several problems arose, including erroneous or duplicated loci [14]. Therefore, a new genetic classification and nomenclature scheme based on the gene, mutations which have been confirmed as a genetic cause of dystonia, has been proposed by a dedicated Task Force for the Classification and Nomenclature of Genetic Movement Disorders installed by the International Parkinson’s Disease and Movement Disorder Society. According to the Task Force’s recommendations the phenotype prefix “DYT” for dystonia is followed by the gene name [12]. In the case of *DYT1*, this would, for example, be replaced by DYT-TOR1A.

Next-generation sequencing (NGS) has led to the description of many new dystonia genes [15]. However, several of these findings need to be interpreted with caution. In this article, we will highlight new findings on several of the established genes for isolated and combined forms of dystonia, include selected examples of novel dystonia genes identified in 2015 and 2016, and provide guidelines for distinguishing false-positive from true-positive genetic findings in the era of NGS.

### Current Knowledge and New Findings in “Old” Genes

In this paragraph, we will summarize current knowledge on the three unequivocally established genes for isolated dystonia

including *TOR1A* [13], *THAP1* [16], and *GNAL* [17] (Table 1). These genetic forms are all inherited in an autosomal dominant fashion and found in <5% of dystonia patients. This will be followed by a description of the well-known genes for combined dystonia including *GCHI* [18], *ATPIA3* [19], and *SGCE* [20] (Table 1). For a detailed overview on long-known genetic forms of complex dystonia, we would like to refer the reader to another review article [21].

### DYT-TOR1A

Mutations in the *TOR1A* gene (torsin family 1 member A) encoding TorsinA, a member of the AAA+ superfamily (ATPases associated with a variety of cellular activities), are a cause of early-onset generalized dystonia. The first and so far the only clearly established mutation is a 3-bp deletion in the *TOR1A* gene (c.904\_906delGAG; p.302delGlu) [13]. This mutation is frequently found among Ashkenazi Jewish patients due to a founder effect [22]. Different rare missense and in-frame mutations have not only repeatedly been reported in individual patients [23, 24] but also control and lack unequivocal confirmation of pathogenicity [25], although some functions of TorsinA may be impaired in cellular models [24, 26].

DYT-TOR1A dystonia usually presents as dystonia in an extremity in childhood. The symptoms later progress to other body parts but typically spare the face and neck [22]. There is variable expressivity ranging from severe childhood-onset generalized to late-onset focal dystonia and about two thirds of the mutation carriers remain unaffected throughout their life (reduced penetrance) [27]. Deep brain stimulation has repeatedly shown to be an effective treatment option [28].

Despite linking a mutation in *TOR1A* to dystonia 20 years ago, the role of TorsinA is still largely elusive. TorsinA is mainly located in the endoplasmic reticulum and the perinuclear space and, due to its function as a triple AAA+ protein, is thought to act as a molecular chaperone [29]. It is known that TorsinA needs to bind to LAMP1 (lamina-associated protein) or LULL1 (luminal domain like LAMP1) for activation [30]. This binding is impaired by the GAG deletion [31] as was confirmed by crystallography [32]. It was recently shown that TorsinA is associated with membrane expansion at the inner nuclear membrane and with elevated cellular lipid content suggesting torsins as essential regulators of cellular lipid metabolism. Thus, disturbed lipid biology may be a main contributor to the development of dystonia due to *TOR1A* mutation(s) [33].

There is an ongoing debate where the anatomical cause of dystonia is located with a main focus on brainstem and increasingly on cerebellum [34]. Investigations of *TOR1A* mutation carriers and respective animal models are used to further explore this issue. For example, a resting-state functional MRI study in symptomatic and asymptomatic mutation carriers, as

**Table 1** Isolated and combined forms of dystonia with an established genetic cause

| Acronym           | Phenotype  | Mutational spectrum  | Protein function   |
|-------------------|--|--|--|
| Isolated dystonia |  |  |  |
| DYT-TOR1A         | Early-onset generalized dystonia (also known as Oppenheim dystonia or DYT1 dystonia) | In most cases, the same mutation (c.904_906delGAG; p.302delGlu)          | ATPases associated with a variety of cellular activities, considered to function as a molecular chaperon |
| DYT-THAP1         | Adolescent-onset dystonia with mixed phenotype (or DYT6 dystonia)                    | About 100 different mutations  | Transcription factor   |
| DYT-GNAL          | Adult-onset segmental dystonia   | About 30 different mutations   | Involved in signal transduction  |
| DYT-ANO3          | Late-onset craniocervical dystonia   | Many different mutations, pathogenicity often not clear (no segregation) | Calcium-activated chloride channels  |
| Combined dystonia |  |  |  |
| DYT-GCH1          | Dopa-responsive dystonia (also known as Segawa syndrome or DYT5 dystonia)            | More than 100 different mutations  | Rate-limiting enzyme in the biosynthesis of tetrahydrobiopterin  |
| DYT-ATP1A3        | Rapid-onset dystonia-parkinsonism (or DYT12 dystonia)                                | About 20 different mutations   | Catalytic subunit of an ionic pump   |
| DYT-PRKRA         | Dystonia-parkinsonism (DYT16)  | One confirmed mutation (c.665C > T, p.Pro222Leu)                         | Protein kinase with function in stress response  |
| DYT-SGCE          | Myoclonus dystonia (DYT11)   | About 80 different mutations   | Probably transmembrane protein; function largely unknown   |

well as healthy controls points to a crucial role of nodes in sensory-motor integration of posterior parietal structures [35]. Results from different *TOR1A* mouse models imply altered maturation of cerebellar synaptic contacts [36].

### DYT-THAP1

Mutations in the *THAP1* gene (*THAP domain containing, apoptosis-associated protein 1*) encoding the transcription factor THAP1 are a cause of adolescent-onset dystonia with mixed phenotype (previously referred to as DYT6) [16]. About 100 different mutations have been reported in *THAP1* including missense, nonsense, and frameshift mutations [37, 38]. DYT-THAP1 dystonia usually presents with dysphonia or writer's cramp in late childhood or adolescence. Over the course of the disease, dystonia spreads to other body parts with prominent craniocervical involvement [39]. As for DYT-TOR1A, the penetrance of DYT-THAP1 is highly reduced (to about 50%) and there is variable expressivity. As in most other dystonia forms, there seems to be no neurodegeneration and no specific disease-related pathology in DYT-THAP1 [40]. Also for this form of dystonia, deep brain stimulation should be considered as a therapeutic option despite an overall less favorable and less consistent response [41].

THAP1 has a DNA-binding THAP domain at the N-terminus as well as a nuclear localization signal (NLS) and several protein-protein interaction motifs towards the C-terminus. Due to posttranslational modification, several THAP1 species exist including a neuron-specific form that may be a key player in controlling neuronal gene transcription [42]. THAP1 regulates its own expression and represses the expression of *TOR1A* in vitro while several THAP1 mutations disrupt this repression [43, 44]. Additional targets of the transcription factor THAP1 remain to be identified. The transcriptional control is mediated by transcriptional cofactors such as HCFC1 (host cell factor C1, unpublished data).

### DYT-GNAL

Heterozygous mutations in the *GNAL* gene (*guanine nucleotide-binding protein subunit alpha L*) encoding the stimulatory  $\alpha$  subunit of the heterotrimeric G protein  $G_{\text{olif}}$  ( $G\alpha_{\text{olif}}$ ) cause cervical or cranial dystonia with a mean onset in the thirties [17]. About 30 different *GNAL* mutations have been reported in dystonia patients. In a recent screening study of 1,000 patients with cervical dystonia, five carriers of pathogenic or likely pathogenic mutations were identified including four missense and a nonsense variant [45]. In the same study, an enrichment of the synonymous substitution

c.1014C.T (p.A338=) was found among patients compared to samples from the exome aggregation consortium (ExAC) [45]. *GNAL* mutations seem to be highly but not fully penetrant [46]. Recently, a first homozygous *GNAL* missense mutation (p.R329W) was found in two sisters with childhood-onset generalized dystonia and mild intellectual disability; the parents were presumably healthy [47•]. Thus, biallelic mutations in *GNAL* also occur but appear to be related to a more severe phenotype, as is also the case for biallelic mutations in *GCHI* [48]. Functional characterization of the homozygous *GNAL* mutation revealed impaired  $G\alpha_{\text{olf}}$  functional coupling to dopamine D1 receptors [47•], while previously described *GNAL* mutations pointed to a strict loss-of-function mechanism [49].

$G\alpha_{\text{olf}}$  is enriched in the striatum where it couples D1 dopamine (D1R) and A2A adenosine (A2AR) receptors to downstream effector molecules. Of note, coupling to A2AR leads to activation of adenylyl cyclase type 5 (AC5) encoded by *ADCY5* [47•], mutations in which are another cause of dystonia and additional movement disorders (see below) [50•].

### DYT-GCHI

Heterozygous mutations in the *GCHI* gene (GTP cyclohydrolase 1), encoding the rate-limiting enzyme in the biosynthesis of dopamine via the biopterin pathway, are a cause of childhood-onset dopa-responsive dystonia (DRD) with diurnal fluctuation [18]. Parkinsonian features often co-occur or may be the only finding [51, 52] and even be associated with a presynaptic dopaminergic deficit as evidenced by SPECT [53]. Further, *GCHI* variants may also represent a risk factor for PD [54]. To date, more than 100 different mutations have been reported including missense, nonsense, frameshift, and splice-site mutations all over the gene, as well as whole-exon or whole-gene deletions [51, 55]. *GCHI* mutation carriers show a penetrance of around 50% which is considerably higher in women compared to men. Recessively inherited (biallelic) mutations in *GCHI* result in a much more severe clinical phenotype including developmental delay and infantile onset [48].

Due to the enzymatic defect in the levodopa biosynthesis, there is a life-long response of DRD to levodopa therapy. Dopamine signaling is important already for intra-uterine (brain) development [56], and prenatal treatment with levodopa in a homozygous *GCHI* mutation carrier was shown to prevent development of the severe phenotype related to biallelic *GCHI* mutations [57]. Of note, biopterin is not only an important intermediate substrate for the biosynthesis of dopamine but also of other neurotransmitters including serotonin. This may explain why non-motor features such as sleep disturbances, mood disorders, or migraine are frequent among patients with DRD [51].

### DYT-ATPIA3

Mutations in the *ATPIA3* gene (ATPase  $\text{Na}^+/\text{K}^+$  transporting subunit alpha 3), encoding an ionic pump, are a cause of rapid-onset dystonia-parkinsonism (RDP) with a characteristic and as the name indicates, sudden onset usually in adolescence or young adulthood [19]. The acute onset is usually associated with a trigger such as fever, physical exertion, or emotional stress [58]. Dystonic symptoms frequently show a rostro-caudal gradient with a strong involvement of the bulbar region and are often accompanied by bradykinesia as a parkinsonian feature [58]. Symptoms usually only slightly respond to levodopa but to a certain degree to benzodiazepine and clonazepam therapy. RDP is inherited in an autosomal dominant manner with reduced penetrance. In addition, mutations in *ATPIA3* have also been linked to a variety of clinical syndromes (pleiotropy) including epileptic or hemiplegic attacks, ataxia, cognitive decline, and other neurological disorders, often with a more severe course and an earlier age at onset [59•].

Proper function of the ionic pump ATP1A3 that uses ATP hydrolysis to exchange  $\text{Na}^+/\text{K}^+$  ions is important to maintain an electrochemical gradient across the plasma membrane that is a prerequisite for electrical excitability and secondary transport in neurons. In a mouse knock-in model, it was recently shown that this ATPase is involved in the control of spatial learning and memory and may be linked to GABA transmission [60].

About 20 different missense mutations were identified in *ATPIA3*. In about 50% of the patients, *ATPIA3* mutations arose de novo or were inherited from parents with mosaics [61]. Most of the mutations are unique but a mutational hot spot seems to be located at protein position D801 [60]. It has been demonstrated that RDP-causing mutations reduce protein levels of ATP1A3 but do not affect the enzymatic activity per se [19].

### DYT-SGCE

Mutations in the *SGCE* gene (sarcoglycan epsilon), coding for the  $\epsilon$  member of the sarcoglycan family, are a frequent cause of myoclonus-dystonia (M-D) characterized by predominant, action-induced, alcohol-responsive myoclonic jerks [20, 62]. Myoclonus as well as dystonia most commonly involves the neck or arms/hands. Onset is usually in childhood or adolescence [63]. Additionally, many carriers of *SGCE* mutations develop psychiatric features such as anxiety-related disorders and alcohol dependence, which is important for counseling and specific treatment [64]. About 80 different mutations have been reported in *SGCE*, many of which are loss-of-function mutations due to frameshift, splice site, nonsense mutations, or deletions of whole exons or even the entire gene. The latter type of mutation often also

involves loss of adjacent genes leading to additional clinical features such as joint problems [62].

As for all autosomal dominantly inherited isolated and combined dystonias, M-D also shows reduced penetrance—but in contrast to the other forms—for *SGCE*, the underlying mechanism has been elucidated: penetrance of *SGCE* mutations is only reduced upon maternal transmission due to maternal genomic imprinting of the *SGCE* gene [65]. This impacts on genetic counseling since it is very unlikely that offspring who inherited the mutation from the mother will develop the disease because only the wildtype allele from the father will be expressed and the mutated, maternal allele will be inactive due to the imprinting mechanism.

The exact function of  $\epsilon$ -sarcoglycan has not yet been elucidated at the cellular level. It is known to have a transmembrane domain and to be located at the plasma membrane. Other sarcoglycans form the dystrophin-glycoprotein complex that links the cytoskeleton to the extracellular matrix and mutations in these genes cause limb-girdle muscular dystrophies [66]. The sarcoglycan complex has extensively been studied in muscle cells. However, the disease-related role of  $\epsilon$ -sarcoglycan has to be related to expression in the brain. In contrast to other sarcoglycans,  $\epsilon$ -sarcoglycan is also expressed in astrocytes, endothelial cells, and pericytes. It may play an important role at the blood-brain barrier (BBB) [67]. Of note, dysfunction of the BBB has also been implicated in primary familial brain calcification (PFBC) due to mutations in *PDGFB* [68] and its receptor *PDGFRB* [69], where dystonia is part of the phenotypic spectrum [70].

### Confirmatory Evidence for a Role of “Older” Genes in Dystonia

Several genes for dystonia seem to be extremely rare and could not be unequivocally confirmed for many years. This includes for instance the *PRKRA* [71] or *TUBB4A* genes [72, 73]. Rare variants in other genes such as *ANO3* [74] have not only been identified in a number of patients but also frequently in controls [75]. A third scenario is clear linkage finding but lack of knowledge of the exact underlying genetic cause, as for X-linked dystonia-parkinsonism (XDP [76]) despite extensive analyses. In this section, we will briefly illustrate these four examples.

#### DYT-PRKRA

The first biallelic mutations in the *PRKRA* gene (protein activator of interferon-induced protein kinase EIF2AK2) were reported in 2008 [71] in patients with a dystonia-parkinsonism syndrome. No convincing biallelic mutations were published for the next 7 years. However, recently, a

number of reports described single families with mainly homozygous mutations in *PRKRA* [77] (Table 1). Most of the reported patients carry the same missense mutation (c.665C>T, p.Pro222Leu, rs121434410) that seems to result from a shared founder [77]. The phenotype includes early-onset generalized dystonia, often with laryngeal dystonia; tongue protrusion, prominent oro-mandibular involvement, dysphagia, and retrocollis. Parkinsonian features are mild (or even absent) and do not respond to levodopa therapy [77]. *PRKRA* was reported as a stress-response gene and encodes a protein kinase with largely unknown function [71].

#### DYT-TUBB4A

Mutations in the *TUBB4A* gene (tubulin beta 4A class IVa) have been described in 2013 in a large Australian DYT4 family [72, 73]. The phenotype is rather unique including severe generalized dystonia with spasmodic dysphonia and craniocervical dystonia as well as extrusional tongue dystonia and a “hobby horse gait” in some patients [78]. Thus, it is not surprising that *TUBB4A* mutations are an extremely rare cause of dystonia [79] and only one novel 3-bp in-frame deletion was recently found in a patient with cervical dystonia (unpublished data). Similarly, as for *ATPIA3* mutations, there is a broad clinical spectrum linked to *TUBB4A* mutations ranging from isolated dystonia to hypomyelination and brain atrophy with the latter being by far the most common disease manifestation. Whether this represents pleiotropy or a continuous phenotypic spectrum is still under debate [80]. Beta-tubulin is an essential component of microtubules that form the cytoskeleton and serve diverse cellular functions.

#### DYT-ANO3

Mutations in the *ANO3* gene (*anoctamin 3*) have first been reported in 2012 in patients with predominantly craniocervical dystonia with a broad range of ages of onset [74]. Mutations were detected in about 1% of dystonia patients including small families with segregation of *ANO3* variants (Table 1). Notably, a large number of missense variants can be found in variant databases and in healthy individuals [75]. However, a pathogenic role of *ANO3* mutations has recently been supported by the description of additional families and a de novo mutation in a patient with dystonia and myoclonic jerks [11]. Indeed, the presence of de novo mutations in a given disease gene usually supports pathogenicity of the variant [81]. *ANO3* encodes a transmembrane protein that belongs to a family of calcium-activated chloride channels and thus may play a role in signal transduction, an important pathway involved in dystonia [15].



## DYT-TAF1

The exact mutation causing X-linked dystonia-parkinsonism (XDP; DYT3; lubag) is not yet known but several variants in a disease haplotype segregate with the disease. XDP is the only known X-linked form of an isolated or combined dystonia, endemic to an island of the Philippines, shows neurodegeneration, and seems to be fully penetrant [82]. The disease-linked haplotype could recently be narrowed to a region <300 kb [83] containing the *TAF1* gene (TATA box-binding protein-associated factor 1). All seven known disease-specific change (DSC) affect intronic or intergenic regions of or surrounding *TAF1* but despite genome sequencing, no additional rare, protein-changing variant in the linked region was found among XDP patients [83]. This supports the hypothesis that changes in the expression of certain isoforms of TAF1 may be causative for XDP [84]. This is further underlined by the detection of missense mutations in *TAF1* in patients with a severe neurodevelopmental disorder including dystonic features [85••]. *TAF1* encodes one subunit of the transcription factor complex IID. Of note, TATA box-binding protein (TBP), which is encoded by the *TBP* gene that is mutated in spinocerebellar ataxia type 17 (SCA17), is also part of this transcription factor complex, and dystonia is part of the phenotypic spectrum of SCA17 [86].

## Novel Genes for Monogenic Dystonia

Due to the advent of next-generation sequencing, new dystonia genes are currently being reported almost monthly. Many of these genes are responsible for complex forms of dystonia, often accompanied by developmental delay and seizures. Because of space constraints, the authors can only illustrate here a few selected examples not necessarily representing the full spectrum of these novel genes implicated in dystonia.

### Mutations in *ADCY5* in Patients with Choreatic and Dystonic Features (CHOR/DYT-ADCY5)

Although initially described in 2012 in a single family with dyskinesia and facial myokymia [87], mutations in the *ADCY5* gene (adenylate cyclase 5) garnered increasing attention of movement disorder specialists and researchers when it was found in a family with chorea and dystonia in 2015 [88]. Since then, more than 20 mutation carriers have been reported often with de novo mutations and a wide range of clinical manifestations including childhood-onset paroxysmal or persistent choreatic and/or dystonic features, and developmental delay in some cases [50•, 89]. *ADCY5* encodes an enzyme that is responsible for the synthesis of cAMP, plays a role in signaling, and is functionally linked to  $G\alpha_{\text{olf}}$ . Mutations in

*ADCY5* cluster within regions encoding the C1 or C2 domain of the protein.

### Mutations in *GNBI* in Patients with Developmental Delay, Hypotonia, and Dystonia

Several de novo mutations in the *GNBI* gene (G protein subunit beta 1), encoding a subunit of a guanine nucleotide-binding proteins, have been reported in patients with neurodevelopmental disability and a wide range of additional symptoms and signs including hypotonia, seizures, and dystonia [90••]. This gene has been independently confirmed and another de novo mutation was reported in a patient with prominent dystonic postures [91]. Notably, almost all mutations in *GNBI* are located in exons 6 and 7 and thus cluster at the binding surface for interactions with  $G\alpha$  and various downstream effectors. Guanine nucleotide-binding protein form heterotrimeric complexes consisting of the subunits  $\alpha$ ,  $\beta$ , and  $\gamma$  and play a role in signaling such as GNAL and ADCY5.

### Mutations in *GNAO1* in Patients with Developmental Delay and Dystonia

More than 20 patients with mostly de novo mutations in the *GNAO1* gene (G protein subunit alpha o1) have been reported in the literature [92]. As for other genes of complex dystonia, mutations in *GNAO1* are also associated with a wide range of phenotypes including epileptic encephalopathy [93], developmental delay without seizures, and chorea as well as dystonia in a subset of patients. There seems to be a genotype-phenotype correlation with mutations at positions Arg209 and Glu246 leading to the milder phenotype without seizures [92]. Like *GNAL* and *GNBI*, *GNAO1* also encodes a subunit of a G protein and thus functions in signaling.

### Mutations in *VAC14* in Patients with Sudden Onset of Developmental Regression and Dystonia

Biallelic mutations in the *VAC14* gene (Vac14, PIKFYVE complex component) comprising of four different mutations have recently been reported in two unrelated children from two different families. The disease had a sudden onset of a progressive neurological disorder and regression of developmental milestones at the age of 1–3 years. Both children developed a movement disorder with dystonia, became nonambulatory and nonverbal, and exhibited striatal abnormalities on MRI [94]. *VAC14* encodes a subunit of a trimolecular complex (together with PIKFYVE and FIG4) that regulates the level of  $PI(3,5)P_2$  which is a signaling lipid localized to the cytoplasmic surface of endolysosomal vesicles. Of note, mutations of FIG4 lead to neurological disorders such as Charcot-Marie-Tooth type 4J neuropathy [94]. Although we were also able to identify a compound-

heterozygous missense mutations in a patient with dystonia, anarthria, tetraspasticity, and supranuclear palsy (unpublished data), the role of *VAC14* mutations in dystonia awaits further confirmation.

### Mutations in *TBCD* in Patients with Developmental Delay, Seizures, Microcephaly, and Dystonia

Two homozygous missense mutations in the *TBCD* gene (tubulin-folding cofactor D) have recently been described in four patients from two small consanguineous families [95]. The phenotype of an infantile neurodegenerative disorder was characterized by loss of developmental milestones at the age of 1–2 years, seizures, acquired microcephaly, and dystonia [95]. *TBCD* seems to be important for cortical cell proliferation and radial migration in the developing mouse brain [95]. Interestingly, *TBCD* encodes a tubulin-specific chaperon that is required for the de novo assembly of the  $\alpha$ -/ $\beta$ -tubulin heterodimer. Of note, the dystonia gene *TUBB4A* encodes a  $\beta$ -tubulin. Independent replication of *TBCD* mutations in (complex) dystonia patients is warranted.

### Loss-of-Function Mutations in *KMT2B* in Patients with Dystonia

Very recently, two groups independently identified mainly truncating but also missense mutations in *KMT2B* as a cause of early-onset generalized dystonia often accompanied by other symptoms including intellectual disability, microcephaly, psychiatric features, dysmorphia, or skin lesions [96••, 97••]. The majority of the mutations occurred de novo, but rarely autosomal dominant inheritance with variable expressivity was also observed. *KMT2B* mutations may account for up to 10% of early-onset generalized dystonia [96••] but further validation is warranted. *KMT2B* is a variable gene and variants that only mildly impact on the functionality of the encoded protein, such as missense changes, may not be linked to dystonia. Of note, patients with copy number variations on chromosome 19q13 including the *KMT2B* gene also developed dystonic features [96••, 97••]. *KMT2B* encodes the lysine-specific histone methyltransferase 2B and thus links disordered histone modification and chromatin states to the disease mechanism of dystonia.

### Challenges for Gene Identification in the Era of Next-Generation Sequencing

In the past two decades, a number of dystonia genes have been reported with an exponential increase during the past 5 years due to the introduction of NGS techniques. With this high-throughput approach, the challenge is not any longer to identify a pathogenic mutation at all but to select the pathogenic


variant from thousands of candidates [98, 99]. There are a number of potential pitfalls associated with NGS, which may easily lead to false-positive and false-negative results. Accordingly, not all of the reported dystonia genes hold up and, in some cases, there is even convincing evidence of false-positive reports [100]. There are hints that can help to distinguish true-positive from false-positive new disease genes with high likelihood but not with certainty. These hints include (I) independent confirmation (which may lag behind the initial description of the gene for several years as in the case of *PRKRA*), (II) functional studies (which may be difficult if the function of the protein is not yet clear such as for *TOR1A*), and (III) variability of the gene. For the latter, absolute numbers can help since it is per se more likely to find more (rare) variants in a large gene (Fig. 1).

### Genetic Risk Factors for Dystonia

Although genetic risk factors probably play a major role in dystonia, their elucidation is still limited. A number of common genetic variants (polymorphisms) have been investigated as genetic risk factors in candidate gene association studies. This included variants within genes from the dopamine pathway such as dopamine transporter and receptor genes, brain-derived neurotrophic factor (BDNF), and genes involved in monogenic forms of dystonia; however, the results of these studies were overall inconclusive [101]. An alternative way to identify genetic risk variants in a hypothesis-free approach is large-scale genome-wide association studies (GWAS). Surprisingly, only two small GWAS have been reported for dystonia so far. In one study, variants in the sodium leak channel (*NALCN*) were found as a risk factor in cervical dystonia [102]; in another study, a variant in arylsulfatase G (*ARSG*) gene was reported to be more frequent among patients with musician's dystonia [103]. However, replication of these results and additional studies are clearly warranted.

### Perspectives

The identification of new pathogenic variants expanded our understanding of disease mechanisms and the pathophysiological basis of dystonia [15] and also impacts on the diagnostic yield of genetic testing. The more genes are known, the more patients can receive a genetic diagnosis and information about the origin of their disease. This is of high clinical importance, as uncertainty is sometimes even more difficult to cope with than an established diagnosis even of a disease with an unfavorable outcome. With the increasing number of genetic causes of dystonia, it is also becoming more and more efficient to use exome sequencing for diagnostic work-up rather than single gene analyses and multiple MRI scans and



|      | Gene           | Mode of inheritance | Type of dystonia | # rare var. | Replication | Functional | Status |
|------|----------------|---------------------|------------------|-------------|-------------|------------|--------|
| 1994 | <i>GCH1</i>    | AD                  | Combined         | 42          | ✓           | ✓          | ✓      |
| 1997 | <i>TOR1A</i>   | AD                  | Isolated         | 103         | ✓           | ✓          | ✓      |
| 2001 | <i>SGCE</i>    | AD                  | Combined         | 150         | ✓           | ✓          | ✓      |
| 2004 | <i>ATP1A3</i>  | AD                  | Combined         | 129         | ✓           | ✓          | ✓      |
| 2009 | <i>THAP1</i>   | AD                  | Isolated         | 49          | ✓           | ✓          | ✓      |
| 2012 | <i>CIZ1</i>    | AD                  | Isolated         | 272         | X           | X          | ?      |
|      | <i>ANO3</i>    | AD                  | Isolated         | 262         | ✓           | (✓)        | ✓      |
| 2013 | <i>GNAL</i>    | AD                  | Isolated         | 48          | ✓           | ✓          | ✓      |
|      | <i>CACNA1B</i> | AD                  | Combined         | 574         | X           | X          | ?      |
| 2015 | <i>COL6A3</i>  | AR                  | Isolated         | 1,408       | X           | X          | ?      |
|      | <i>HPCA</i>    | AR                  | Isolated         | 42          | X           | X          | ?      |
|      | <i>KCTD17</i>  | AD                  | Combined         | 64          | ?           | X          | ?      |
|      | <i>RELN</i>    | AD                  | Combined         | 1,083       | ?           | X          | ?      |

**Fig. 1** Current status of replication/confirmation of reported isolated and combined dystonia genes. The figure illustrates the growing number of known dystonia genes in chronological order. While the upper five genes were all identified before the era of NGS, have been confirmed unequivocally in replication studies, and functional changes of mutant proteins have been demonstrated, this picture changed after the introduction of NGS to the field as indicated by the red arrow. Several of the novel dystonia genes have not yet been confirmed in independent studies. For *CIZ1*, *CACNA1B*, *COL6A3*, and *HPCA*, subsequent screening studies did not identify any additional causative mutations (as indicated by the cross). A possible indication with respect to credibility of

other often non-specific ancillary tests [98]. Especially in childhood-onset diseases, exome sequencing of parent-child trios have proven a powerful tool [104]. Not only do such trio analyses allow for the detection of de novo mutations but they also enable easy detection of biallelic, including compound-heterozygous, mutations.

In recent years, much emphasis has been placed on the identification of new genes, however, the functional characterization of these genes to understand the disease mechanisms underlying dystonia lags behind, calling for concerted efforts in this regard.

## Conclusions

More than 20 years of research in dystonia genetics have led to the identification of a number of monogenic causes of dystonia and thereby provided important insights into its pathophysiology. There are a rapidly increasing number of novel dystonia genes for which careful validation and functional studies are warranted. The expanded knowledge of genetic causes of dystonia improves genetic testing and enables genetic counseling and gene-specific treatment in some cases.

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novel genes may be reflected by the number of rare variants within a given gene. The numbers in the fourth column are taken from the database of the Exome Aggregation Consortium (ExAC at <http://exac.broadinstitute.org/>) and are based on more than 60,000 individuals. Of note, while the confirmed genes (as indicated by a *check mark*) bear an average of 112 rare variants, this number increases for the yet unconfirmed genes (indicated by a *question mark*) to 574 variants (adapted from [15]), suggesting that additional follow-up investigations are warranted. *Check marks* indicate “confirmed” while *crosses* indicate lack of replication or confirmatory functional studies

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## Compliance with Ethical Standards

**Conflict of Interest** Katja Lohmann declares no conflict of interest.

Christine Klein has received personal fees from Centogene and from the Else Kroener Fresenius Foundation.

**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.

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