Etiology and Pathophysiology of Cervical Dystonia

Qiwen Tang, Linhui Ni, Xinxin Xu, Hanze Chen, and Xingyue Hu*
Department of Neurology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, China
*Correspondence: huxingyue2003@zju.edu.cn

***Abstract*—Although Cervical Dystonia (CD) is regarded as the most common type of focal dystonia, the therapeutic options available for CD are remarkably limited and are unsatisfactory for many patients. Recent functional and clinical studies have made great progress in unraveling the mechanisms underlying CD and other types of dystonia. Much research is still needed; however, the deeper understanding of the etiology of CD is expected to lead to better management of dystonia symptoms and the development of novel therapeutic options. The objective of this review was to summarize the most recent studies examining the pathophysiological features of CD, including genetic mutations, studies about neuronal signaling, and metabolomic studies.**

***Keywords*—cervical dystonia, torticollis, genetic mutations, neuronal signaling, metabolomic studies**

I. Introduction

Dystonia is a syndrome characterized by sustained involuntary muscle contractions, often causing twisting, repetitive movements, and abnormal postures [1]. Cervical Dystonia (CD), also known as spasmodic torticollis, is characterized by involuntary muscle contractions that cause abnormal twisting and turning of the neck and sometimes the shoulders. Recent studies suggest that adult-onset dystonia results from the interaction between genetic predisposition factors and environmental triggers leading to the dysregulation of several processes that, at a certain threshold, manifest as dystonia symptoms [1]-[3]. Although CD is the most prevalent subtype of focal dystonia, knowledge of its underlying pathogenesis remains obscure and even lags behind the rarer subsets of dystonia. Indeed, only a few causative mutations have been identified to date. Due to the lack of knowledge regarding the disease's etiology, most current treatments available for CD are largely ineffective. For instance, botulinum toxin injection is regarded as the most effective treatment for CD, but long-term adherence to this treatment remains low due to unsatisfactory responses and adverse side effects. This highlights the need for a deeper understanding of the underlying mechanisms of CD, which may facilitate treating patients with CD and developing novel treatment options. Thus, in this paper, we review recent studies on the pathophysiological features of CD, including genetic mutations, dysregulation of neuronal signaling, and metabolomics.

Manuscript received May 12, 2022; revised August 11, 2022; accepted September 19, 2022.

II. Genetic Mutations in CD

It is well established that mutations in THAP domain-containing apoptosis-associated protein 1 (*THAP1*), Cip1-interacting zinc finger protein 1 (*CIZ1*), anoctamin-3 (*ANO3*), and G protein subunit $G\alpha_{olf}$ (*GNAL*) are associated with specific forms of CD (DYT6, DYT23, DYT24, and DYT25, respectively). Therefore, the specific mechanisms involving these genes are not elaborated in this review, as they have been extensively reviewed elsewhere [4]-[8].

A. Mutations in COL8A1, DENND1A, and GABBR2

Numerous collaborative studies have identified additional genetic mutations in CD in recent years. A recent multi-center genome-wide association study (GWAS) identified novel genes and variants likely involved in the pathogeny of CD in patients with European ancestry. This study showed that the genes *COL8A1* (rs2219975, chromosome 3) and *DENND1A* were significantly associated with CD risk and that the low-frequency variant (rs147331823) in *GABBR2* was associated with a younger age-at-onset (16.4 ± 2.9 years) of CD [9].

COL8A1, which encodes the α1 (VIII) collagen chain, has been associated with corneal dystrophy and age-related macular degeneration. Although *COL8A1* had not been previously associated with dystonia, biallelic mutations in the *COL6A3* gene, which encodes another member of the collagen family, have been reported to be a potential cause of recessive dystonia; however, the results remain uncertain [10].

DENND1A encodes the DENN domain protein 1A. *DENND1A* is a member of the connecdenn family which act as guanine nucleotide exchange factors in the brain and is involved in vesicle dynamics. Although variants in the guanine nucleotide exchange factors encoded by *GNAL* and *GNAO1* are known to play a role in adult-onset focal and segmental dystonia and childhood-onset chorea and dystonia, *DENND1A* had not been linked to dystonia before [11].

Finally, *GABBR2* encodes Gamma-Aminobutyric Acid Subunit (GABA)-B receptors. Some GABA receptor activators such as baclofen and benzodiazepines have

doi: 10.18178/ijpmbs.12.1.13-20

been successfully used as a treatment for specific types of dystonia. However, *GABBR2* had not previously been linked to any type of dystonia [9]. In conclusion, although these three genes have not previously been linked to dystonia, prior evidence does support their involvement in the etiology of CD. Further functional studies should investigate their specific roles and functions in dystonia.

B. Mutations in NALCN and TOR1A

The Single Nucleotide Polymorphisms (SNPs) rs1338041 and rs61973742 in the *NALCN* gene and the SNPs rs2296793 and rs3842225 in *TOR1A* have been associated with dystonia in populations of multiple ethnicities [12]-[14]. The *NALCN* SNPs rs1338041 and rs61973742 were identified in a GWAS of 212 British patients with CD and 5,173 control subjects [13]. However, another GWAS conducted on 252 patients with CD and 342 unrelated control subjects of Spanish origin failed to replicate these findings. *NALCN* encodes a channel in the voltage-gated sodium/calcium channel family that is universally expressed in the central nervous system [15]. *NALCN* is reported to be a sodium leak channel that regulates the neuronal resting membrane potential and consequently, neuronal excitability [16]; however, the specific characteristics of this channel, such as its voltage sensitivity and ion selectivity, are still unclear [17], [18]. Several functional studies suggest that ion channel dysfunction may be a pathogenic factor in dystonia [19], [20].

Although the genetic variants in the *TOR1A* gene have been widely studied, the results remain controversial. The SNP rs3842225 showed a negative association with dystonia in Italian and North American [14], Dutch [21], German [22], and Australian [23] populations, but a positive association was observed in a Russian cohort [12]. Additionally, one study revealed that the *TOR1A* haplotype T2, comprising rs2296793, rs1182, and rs3842225 was strongly associated with susceptibility to sporadic dystonia in an Icelandic population [24], whereas the same haplotype was found to exert the opposite effect in another study including German and Austrian cohorts [25]. Moreover, another study including a cohort of German origin with a sample size twice that of the discovery study failed to replicate the original positive findings [22]. These discrepancies have raised doubts regarding the involvement of *TOR1A* in dystonia.

Also known as DYT-TOR1A dystonia, DYT1 is an early-onset dystonia, which sometimes also presents an adult-onset phenotype. DYT1 is reported to be primarily caused by a 3-bp (GAG) deletion in the *TOR1A* gene [26]. This autosomal dominant mutation typically has a low penetrance (approximately 30–40%). Its high frequency in the Ashkenazi Jewish population, in which it is approximately five times more frequent, may be due to a founder mutation effect [27]. The GAG deletion causes the loss of a glutamic acid residue in the protein torsin A, which belongs to the superfamily of ATPase chaperone-like proteins and is widely expressed in neurons [28]. This amino acid change induces mislocalization of torsin A to the nuclear envelope [29] and accumulation of torsin A in perinuclear membranous inclusions [30]. Such accumulations were detected in the midbrain reticular formation and periaqueductal gray of four patients with DYT1 dystonia [31] and are presumably involved in the pathogenic mechanism of this disorder. Additionally, it has been suggested that the mutation in torsin A may exert a dominant-negative effect on the wild type protein [32]. A recent study showed that striatal dopamine D2 receptors on cholinergic interneurons might also contribute to the pathophysiology of DYT1 dystonia [33]. Finally, torsin A has been linked to phosphatidic acid lipid metabolism through the regulation of lipase activity, which converts phosphatidic acid lipids to diacylglycerol. In patients with DYT1 dystonia, the activity of lipase in neural cells was found to be increased compared with cells from healthy controls. This suggests that suppressing lipase activity might be a suitable therapeutic approach for DYT1 dystonia and other conditions in which *TOR1A* is mutated. Additionally, the results of the same study showed that the level of lipase activity differed between patients with heterozygous and biallelic *TOR1A* mutations, highlighting the need for further assessment of the impact of lipase activity on neuronal function and torsin A function [34]. Another recent study also showed that torsin A plays an essential role in the development of the central nervous system and the acquisition of motor skills at an early age. This adds more evidence to the DYT1-associated striatal cholinergic abnormalities linked to deficits in corticostriatal plasticity, which may contribute to dystonic-like movements [35]. Finally, torsin A overexpression prevents abnormal motor symptoms in DYT1 dystonia, while a relatively low level of torsin B resulted in abnormal nuclear envelope budding in the developing brain [36].

Despite these functional studies indicating a relationship between *NALCN*, *TOR1A*, and CD, the conflicting results from GWAS may indicate false-positive associations. To further evaluate this possibly, two independent studies examined the involvement of genetic variants in these genes in a Chinese cohort of CD patients. The genetic landscape of 201 patients with primary CD and without GAG deletion mutation of torsin A and 289 health controls was evaluated. The results revealed a lack of association between the SNPs rs1338041, rs61973742, rs2296793, and rs3842225 and CD, suggesting that these SNPs are not associated with CD in a Chinese population [37], [38]. Altogether, these studies suggest that the association between these four SNPs varies among groups of different ethnicities, highlighting the need for follow-up studies in different regions.

C. Other Genetic Variants

ANO3 encodes anoctamin 3, a protein that functions as a Ca^{2+}-gated chloride channel and is highly expressed in the striatum. Several variants in *ANO3* have been identified in autosomal-dominant craniometrical dystonia [6]. The c1969G>A mutation was discovered in a family with a history of adult-onset dystonia which began as cervical dystonia [39]. This mutation is located in a transmembrane domain (domain-5) of the anoctamin 3

protein and is thought to alter the hydrophobicity of this domain [40].

Another study reported a case of a patient with CD and cerebellar ataxia in which a previously undescribed pathogenic autosomal dominant mutation in the *CACNA1A* gene was identified. The *CACNA1A* gene encodes the transmembrane pore-forming subunit (α1A) of the P/Q-type voltage-gated Ca^{2+} channel termed $Ca_V2.1$ [41]. The identified mutation (c.4056_4057insG, p. Pro1353Alafs*3) resulted in the insertion of a premature stop codon [42], leading to the production of truncated, incomplete, and non-functional protein product.

Another case report described two sisters (33 and 30 year-old) with phosphomannomutase-2 deficiency-congenital disorder of glycosylation (PMM2-CDG) with cervical dystonia, which is a rare clinical symptom in patients with PMM2-CDG. In both patients, the heterozygous mutations c.422G>A (p.Arg141His) and c.722G>C (p.Cys241Ser) were detected in the *PMM2* gene. According to the American College of Medical Genetics and Genomics guidelines, both mutations were annotated as likely pathogenic. Although the c.422G>A mutation is the most frequent variant in *PMM2*, further studies are needed to characterize its functional consequences [43]. No variants previously associated with dystonia were found to be shared by these two siblings. Even though the *PMM2* gene is not otherwise known to be related to dystonia, this finding indicates that it may be necessary to revise the diagnostic criteria for PMM2-CDG, including the presence or absence of dystonia [44].

Finally, Ortega-Suero et al. reported a case in which a novel mutation in the *OPA1* gene, which encodes OPA1 mitochondrial dynamin-like GTPase, was associated with dominant optic atrophy and cervical dystonia. The identified variant (c.2060T>A) was located in exon 22 of the gene and caused an amino acid change (p.Val687Glu) in OPA1 [45]. Before this case, only one patient with cervical dystonia and a mutation in *OPA1* a (heterozygous variant c.1345A>C [p.Thr449Pro]) had been reported [46]. The Ortega-Suero et al. study predicted that the substitution of a polar amino acid (threonine) with a nonpolar amino acid (proline) would be deleterious (Table I).

These studies all demonstrate that gene variants play a major role in the etiology of CD. Additional studies in populations of different ethnicities would be of great benefit.

III. Neuronal Signaling Dysregulation in CD

As CD is a neurological disorder, several studies have hypothesized that the dysregulation of neuronal signaling may be a pathological event in CD. Supporting this hypothesis, a link between CD and the Dopamine D2 Receptor (DRD2), Dopamine Transporter (DAT), Serotonin Transporter (SERT), and GABA receptors has been confirmed.

TABLE I. Summary of the Newfound Genetic Mutation in CD

Authors. [Ref.] Year	Region	Gene[SNPs]/C	Coding	Cases/Health	Diagnosis
Sun YV *et al.* [9]. 2021	Europe	COL8A1/C3	one of the alpha chains of type VIII collagen	919/1491	CD
		DENND1A/C9	DENN domain containing protein 1A		CD
		GABBR2/C9	a subunit of GABA-B receptors		Younger age-at-onset CD
Zhou Q *et al.* [37], [38]. 2016	China	NALCN [rs1338041, rs61973742]/C13	a sodium leak channel	201/289	Not in Chinese CD patients
		TOR1A [rs2296793, rs3842225]/C9	the protein torsinA	201/289	Not in Chinese CD patients
Miltgen M *et al.* [39]. 2016	Flanders	ANO3(c.1969G>A)/C11	a predicted $Ca2^{+}$-gated chloride channel	5 patients in a family	The onset begins as CD in their fourth decade
Fuerte-Hortigón A *et al.* [42]. 2020	—	CACNA1A/C19	the transmembrane pore-forming subunit of the Ca2+ channel	a case	CD presenting with cerebellar ataxia
Rossi M *et al.* [44]. 2017	Spain -Italy	PMM2(c.422G>A and c.722G>C)/C16	Phosphom -annomutase 2	2 sisters	PMM2-CDG presenting with CD

Abbreviations: C = Chromosome; CD = cervical dystonia; GABA = gamma-aminobutyric acid; PMM2-CDG = Phosphomannomutase-2 deficiency-congenital disorder of glycosylation; DOA = dominant optic atrophy.

A recent study identified a novel variant in exon 5 of *DRD2* (c.634A> T, p.Ile212Phe) in a four-generation Dutch family with dominantly inherited combined progressive chorea and cervical dystonia. Compared to the wild type receptor, the aberrant receptor increased the efficiency of G protein signaling, while reducing the

activation level of arrestin [47]. Arrestin 2 and arrestin 3 (also referred to as *β*-arrestin 1 and β-arrestin 2, respectively) are non-visual, ubiquitously expressed members of the arrestin family. The recruitment of arrestin 3 has been linked to G protein-coupled receptor signaling and G protein- independent signaling pathways [48]. Arrestin 3 is required for movement regulation; silencing arrestin 3 in animal models reduced the beneficial locomotor effect of levodopa and improved movement disorders, overexpression of arrestin 3 showed the opposite effect [49]. The results of this study instead supported the idea that D2-$I^{212}F$ affected the ability of arrestin 3 to bind to DRD2, thereby indirectly reducing recruitment of arrestin 3. This and the overstimulation of G protein-dependent signaling, lead to a dysfunction of the DRD2 receptor in dopaminergic neurons. The results of another study with 27 patients with CD and 15 age-matched controls supported that depression symptoms commonly occurring in patients with CD were related to abnormal striatal DAT and D2/3 receptor binding [50]. This study hypothesized that dopamine plays a role in the pathogenesis of tremor and jerks in CD, which may be caused by biochemical changes in the nucleus of Cajal, connecting with the substantia nigra pars compacta [51].

A study assessing serotonergic signaling in 14 patients with CD and 12 matched controls identified that SERT binding was a pathogenic event involved in the occurrence of both motor and non-motor symptoms in CD. Moreover, SERT dysfunction was positively correlated with the severity of dystonic motor symptoms, pain scores, and sleep disturbances but negatively correlated with fatigue scores. Additionally, the authors demonstrated that the dysregulation of serotonergic signaling in different parts of the brain, such as the caudate nucleus, the hippocampus, the dorsal and medial raphe nuclei, and the putamen, were linked to different symptoms of the disease. The study suggested a statistically significant positive correlation between the loss of [(11)C]DASB non-displaceable binding potential (BP_{ND}) in the dorsal raphe nucleus and the severity of dystonic symptoms, but not that of psychiatric symptoms [52]. Another study indicated that CD patients with psychiatric comorbidities had reduced SERT BP_{ND} in the midbrain/diencephalon compared to those without psychiatric comorbidities, but dystonia and jerks were unrelated to SERT BP_{ND} [53]. Unfortunately, this study did not clearly identify additional specific areas of the midbrain/diencephalon like the former. Additionally, this study reported a significant positive correlation between extra-striatal SERT and striatal DAT BP_{ND} in CD patients with jerks, but not in those without jerks. SERT is one of the main presynaptic regulators of serotonergic signaling. The relationship between increased SERT BP_{ND} and dystonia, which is supported by additional studies [54], [55], could be explained by the increased serotonin abundance in the synaptic cleft.

Finally, regulation of the inhibitor neurotransmitter GABA is also aberrant in patients with CD. GABA+/Cre levels in the right thalamus of patients with CD were significantly decreased, which was positively correlated with increased CD duration. Additionally, there was a negative correlation between GABA-A availability in the bilateral thalamus and disease duration and motor symptom severity, which may be due to inadequate compensation for the decrease in GABA by GABA-A receptor upregulation [56]. Finally, a study identified a negative correlation between GABA-A availability within the bilateral cerebellum and motor symptom severity in 15 patients with CD and 15 age -and sex-matched controls [57] (Fig. 1).

IV. Metabolomic Studies of CD

The first published metabolomic study for CD analyzed plasma samples from 100 cases of idiopathic CD and an equal number of controls, all recruited from the Emory University Movement Disorders Clinic in the United States [58]. In this study, nine biological processes were found to be strongly associated with CD ($p < 0.05$). The identified pathways were related to dihomo-gamma linoleic acid; the pentose phosphate pathway; arachidonic acid metabolism; leukotriene metabolism; starch and sucrose metabolism; valine, leucine, and isoleucine degradation; galactose metabolism; fructose and mannose metabolism; and propanoate metabolism. Among these, five were linked to carbohydrate metabolism and three were linked to lipid metabolism, which is associated with the production of metabolites mediating inflammatory responses [59]. Together, these results suggest the involvement of immune-related processes in dystonia, as described in some earlier studies [60], [61]. Following this, another study conducted on 50 patients with CD, 66 patients with two other specific dyston]ia subtypes, and 55 healthy control subjects found alterations of dopaminergic- and serotonergic-related compound levels in plasma [55]. Specifically, a significant increase in 3-methoxytyramine (a metabolite of dopamine) and a reduction of tryptophan (the precursor of serotonin) were found in dystonia patients compared with the control subjects. Moreover, patients with CD showed a higher dopamine/levodopa ratio than patients with other subtypes of dystonia. In addition, similar to the results of studies focusing on GABA levels [56], [57], there was a correlation between the concentration of levodopa and the severity of dystonia, as well as non-motor symptoms, depression, and fatigue. Further studies of the enzymes involved in these pathways may lead to the development of better management approaches and provide clinical benefits for both motor and non-motor symptoms in CD and other types of dystonia.

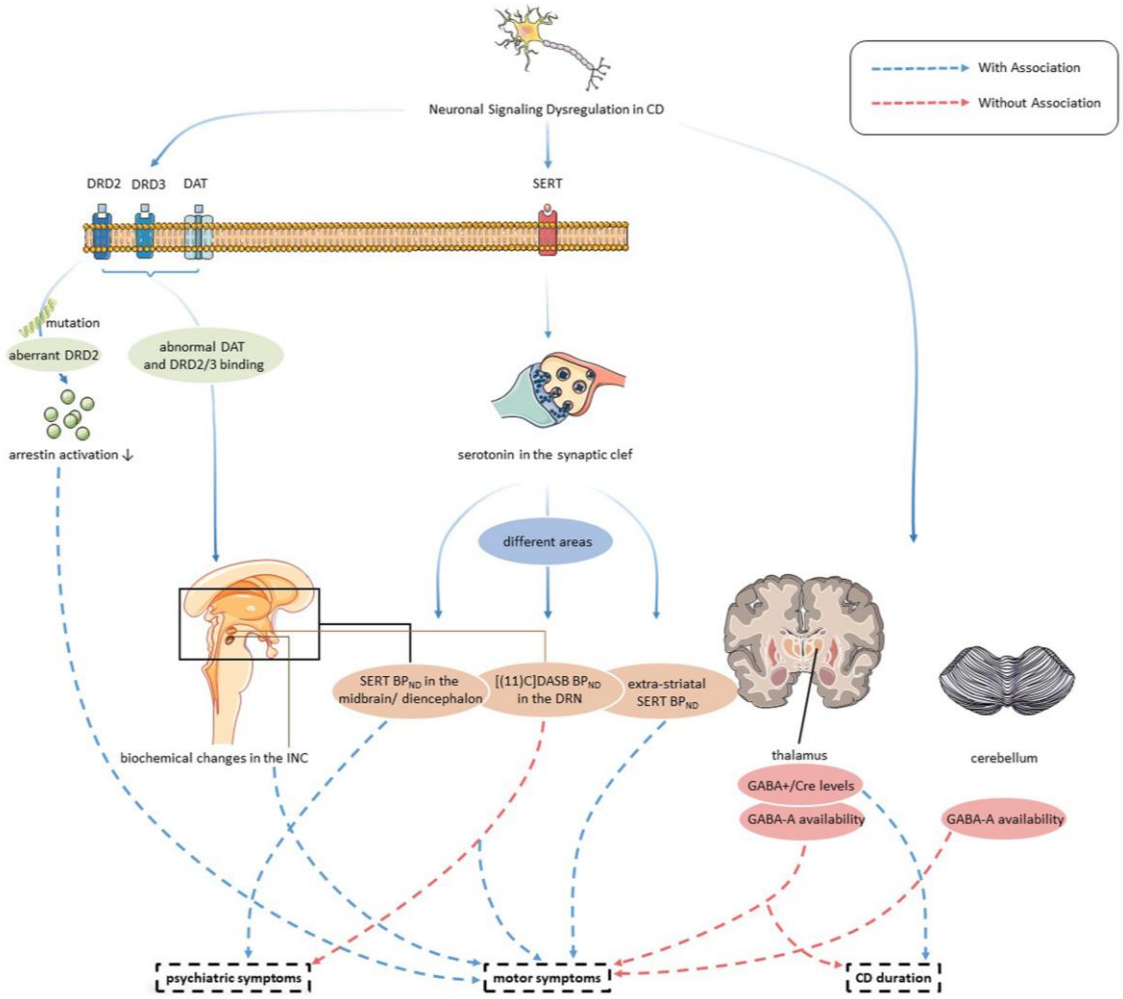

Figure 1. Summary of the newfound neuronal signaling dysregulation in CD.

Note: The aberrant receptor caused by a novel variant in exon 5 of DRD2 (c.634A> T, p.Ile212Phe) reduced the activation level of arrestin, which was associated with motor symptoms in the patients with CD. Abnormal striatal DAT and D2/3 receptor binding were hypothesized to be related to tremor and jerks in CD, may caused by biochemical changes in the nucleus of Cajal. The dysregulation of serotonergic signaling in different parts of the nervous system was linked to different symptoms of CD, such as the midbrain/diencephalon and psychiatric symptoms, the DRN and the severity of dystonic symptoms as well as extra-striatal changes and jerks, which could be explained by the increased serotonin abundance in the synaptic cleft. GABA+/Cre levels in the thalamus were positively correlated with CD duration, but not GABA-A availability. It was also identified that GABA-A availability in both the thalamus and cerebellum had negative correlations with motor symptom severity. Abbreviations: CD = cervical dystonia; DRD2= dopamine D2 receptor; DRD3= dopamine D3 receptor ; DAT= dopamine transporter ; SERT= serotonin transporter ; INC= interstitial nucleus of Cajal ; BP_{ND}= non-displaceable binding potential ; DRN= dorsal raphe nucleus ; GABA = gamma-aminobutyric acid.

V. Conclusions and Recommendations

Since CD is considered the most prevalent subtype of focal dystonia, extensive efforts have been dedicated to achieving a deeper understanding of the pathophysiological mechanisms underlying CD in recent years. These efforts have especially focused on the genetic factors and the dysregulation of neuronal signaling and metabolism. However, despite recent advances, a considerable number of genetic variants and molecular mechanisms remain obscure or in need of further validation by functional studies. Furthermore, the differences in the genetic landscape between populations of different ethnicities should be considered to achieve a more comprehensive understanding of the pathology of the disease. This will not only deepen the current knowledge about the disease but may also open up new avenues of research, such as establishing animal and cellular models, and could lead to novel and advanced treatments of CD and other forms of dystonia, ultimately improving the quality of life of patients suffering from this condition.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

HXY and TQW conceived the review; TQW collected and analyzed all the bibliography; TQW, NLH, XXX and CHZ wrote the original manuscript; HXY supervised and edited the manuscript. All authors had approved the final version.

ACKNOWLEDGMENT

This study was funded by the Natural Science Foundation of Zhejiang Province of China (Nos. LY19H090026). Fig. 1 was produced with modified elements of Servier Medical Art (https://smart.servier.com), licensed under a Creative Commons Attribution 3.0 Unported License.

REFERENCES

[1] S. Fahn, C. Marsden, and D. Calne, "Classification and investigation of dystonia," *Movement Disorders*, vol. 2, pp. 332-58, 1987.
[2] P. Gonzalez-Alegre, "Advances in molecular and cell biology of dystonia: Focus on torsinA," *Neurobiology of Disease*, vol. 127, pp. 233-241, Jul. 2019.
[3] A. Molloy, *et al.*, "An evaluation of the role of environmental factors in the disease penetrance of cervical dystonia," *Journal of Neurology, Neurosurgery, and Psychiatry*, vol. 86, no. 3, pp. 331-335, Mar. 2015.
[4] S. Giri, *et al.*, "Genetic screening of THAP1 in primary dystonia patients of India," *Neuroscience Letters*, vol. 637, pp. 31-37, 2017.
[5] J. Xiao, *et al.*, "Mutations in CIZ1 cause adult onset primary cervical dystonia," *Annals of Neurology*, vol. 71, no. 4, pp. 458-469, Apr. 2012.
[6] G. Charlesworth, *et al.*, "Mutations in ANO3 cause dominant craniocervical dystonia: Ion channel implicated in pathogenesis," *Am. J. Hum. Genet.*, vol. 91, no. 6, pp. 1041-1050, 2012.
[7] L. T. Jiang, *et al.*, "The expanding clinical and genetic spectrum of ANO3 dystonia," *Neuroscience Letters*, vol. 746, p. 135590, 2021.
[8] K. R. Kumar, *et al.*, "Mutations in GNAL: A novel cause of craniocervical dystonia," *JAMA Neurology*, vol. 71, no. 4, pp. 490-494, 2014.
[9] Y. V. Sun, *et al.*, "A multi-center genome-wide association study of cervical dystonia," *Movement Disorders: Official Journal of the Movement Disorder Society*, vol. 36, pp. 2975-2801, 2021.
[10] K. Lohmann, *et al.*, "The role of mutations in COL6A3 in isolated dystonia," *Journal of Neurology*, vol. 263, no. 4, pp. 730-734, 2016.
[11] H. A. Jinnah and Y. V. Sun, "Dystonia genes and their biological pathways," *Neurobiology of Disease*, vol. 129, pp. 159-68, 2019.
[12] S. L. Timerbaeva, N. Y. Abramycheva, O. Y. Rebrova, and S. N. Illarioshkin, "TOR1A polymorphisms in a Russian cohort with primary focal/segmental dystonia," *The International Journal of Neuroscience*, vol. 125, no. 9, pp. 671-677, 2015.
[13] K. Y. Mok, *et al.*, "Genomewide association study in cervical dystonia demonstrates possible association with sodium leak channel," *Movement Disorders: Official Journal of the Movement Disorder Society*, vol. 29, no. 2, pp. 245-251, 2014.
[14] J. Clarimon, *et al.*, "Assessing the role of DRD5 and DYT1 in two different case-control series with primary blepharospasm," *Movement Disorders: Official Journal of the Movement Disorder Society*, vol. 22, no. 2, pp. 162-166, 2007.
[15] B. J. Liebeskind, D. M. Hillis, and H. H. Zakon, "Phylogeny unites animal sodium leak channels with fungal calcium channels in an ancient, voltage-insensitive clade," *Molecular Biology and Evolution*, vol. 29, no. 12, pp. 3613-3616, 2012.
[16] B. Lu, *et al.*, "The neuronal channel NALCN contributes resting sodium permeability and is required for normal respiratory rhythm," *Cell*, vol. 129, no. 2, pp. 371-83, 2007.
[17] H. C. Chua, M. Wulf, C. Weidling, L. P. Rasmussen, S. A. Pless, "The NALCN channel complex is voltage sensitive and directly modulated by extracellular calcium," *Science Advances*, vol. 6, no. 17, article eaaz3154, 2020.
[18] M. Bouasse, H. Impheng, Z. Servant, P. Lory, and A. Monteil, "Functional expression of CLIFAHDD and IHPRF pathogenic variants of the NALCN channel in neuronal cells reveals both gain- and loss-of-function properties," *Scientific Reports*, vol. 9, no. 1, pp. 11791, 2019.
[19] A. Zachou, D. Palaiologou, E. Kanavakis, and E. Anagnostou, "Retrocollis as the cardinal feature in a de novo ITRP1 variant," *Brain & Development*, vol. 44, no. 5, pp. 347-352, 2022.
[20] R. Cocoș, *et al.*, "CACNA1B gene variants in adult-onset isolated focal dystonia," *Neurological Sciences: Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology*, vol. 42, no. 3, pp. 1113-1137, 2021.
[21] J. L. Groen, *et al.*, "Is TOR1A a risk factor in adult-onset primary torsion dystonia?" *Movement Disorders: Official Journal of the Movement Disorder Society*, vol. 28, no. 6, pp. 827-831, 2013.
[22] S. Hague, *et al.*, "Lack of association with TorsinA haplotype in German patients with sporadic dystonia," *Neurology*, vol. 66, no. 6, pp. 951-952, 2006.
[23] J. R. Newman, *et al.*, "Common polymorphisms in dystonia-linked genes and susceptibility to the sporadic primary dystonias," *Parkinsonism & Related Disorders*, vol. 18, no. 4, pp. 351-357, 2012.
[24] J. Clarimon, *et al.*, "Torsin A haplotype predisposes to idiopathic dystonia," *Annals of Neurology*, vol. 57, no. 5, pp. 765-767, 2005.
[25] C. Kamm, *et al.*, "Strong genetic evidence for association of TOR1A/TOR1B with idiopathic dystonia," *Neurology*, vol. 67, no. 10, pp. 1857-1859, 2006.
[26] L. J. Ozelius, *et al.*, "The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein," *Nature Genetics*, vol. 17, no. 1, pp. 40-48, 1997.
[27] C. S. Schwarz and S. B. Bressman, "Genetics and treatment of dystonia," *Neurologic Clinics*, vol. 27, no. 3, pp. 697-718, 2009.
[28] S. J. Augood, *et al.*, "Distribution and ultrastructural localization of torsinA immunoreactivity in the human brain," *Brain Research*, vol. 986, no. 1-2, pp. 12-21, 2003.
[29] R. E. Goodchild and Dauer, "Mislocalization to the nuclear envelope: An effect of the dystonia-causing torsinA mutation," in *Proc. the National Academy of Sciences of the United States of America*, 2004, pp. 847-52.
[30] J. Hewett, *et al.*, "Mutant torsinA, responsible for early-onset torsion dystonia, forms membrane inclusions in cultured neural cells," *Human Molecular Genetics*, vol. 9, no. 9, pp. 1403-1413, 2000.
[31] K. S. McNaught, *et al.*, "Brainstem pathology in DYT1 primary torsion dystonia," *Annals of Neurology*, vol. 56, no. 4, pp. 540-547, 2004.
[32] A. R. Chase, E. Laudermilch, and C. Schlieker, "Torsin ATPases: Harnessing dynamic instability for function," *Frontiers in Molecular Biosciences*, vol. 4, p. 29, 2017.
[33] Y. Liu, H. Xing, F. Yokoi, D. E. Vaillancourt, and Y. Li, "Investigating the role of striatal dopamine receptor 2 in motor coordination and balance: Insights into the pathogenesis of DYT1 dystonia," *Behavioural Brain Research*, vol. 403, p. 113137, 2021.
[34] A. Cascalho, *et al.*, "Excess Lipin enzyme activity contributes to TOR1A recessive disease and DYT-TOR1A dystonia," *Brain: A Journal of Neurology*, vol. 143, no. 6, pp. 1746-1765, 2020.
[35] J. Li, D. S. Levin, A. J. Kim, S. S. Pappas, and W. T. Dauer, "TorsinA restoration in a mouse model identifies a critical therapeutic window for DYT1 dystonia," *The Journal of Clinical Investigation*, vol. 131, no. 6, article e139606, 2021
[36] J. Li, C. C. Liang, S. S. Pappas, and W. T. Dauer, "TorsinB overexpression prevents abnormal twisting in DYT1 dystonia mouse models," *eLife*, vol. 9, 2020.
[37] Q. Zhou, *et al.*, "Association analysis of NALCN polymorphisms rs1338041 and rs61973742 in a Chinese population with isolated cervical dystonia," *Parkinson's Disease*, vol. 2016, p. 9281790, 2016.
[38] Q. Zhou, *et al.*, "Association analysis of TOR1A polymorphisms rs2296793 and rs3842225 in a Chinese population with cervical dystonia," *Neuroscience Letters*, vol. 612, pp. 185-188, 2016.
[39] M. Miltgen, *et al.*, "Novel heterozygous mutation in ANO3 responsible for craniocervical dystonia," *Movement Disorders: Official Journal of the Movement Disorder Society*, vol. 31, pp. 1251-1252, 2016.
[40] D. Paulet, M. Claustres, and C. Béroud, "Hydrophobic pulses predict transmembrane helix irregularities and channel transmembrane units," *BMC Bioinformatics*, vol. 12, p. 135, 2011.
[41] D. Pietrobon, "Calcium channels and channelopathies of the central nervous system," *Molecular Neurobiology*, vol. 25, no. 1, pp. 31-50, 2002
[42] A. Fuerte-Hortigón, *et al.*, "Novel CACNA1A variant may cause cervical dystonia and cerebellar ataxia syndrome," *Journal of the Neurological Sciences*, vol. 415, p. 116909, 2020.

[43] C. A. González-Domínguez, *et al.*, "Non-functional alternative splicing caused by a Latino pathogenic variant in a case of PMM2-CDG," *Molecular Genetics and Metabolism Reports*, vol. 28, p. 100781, 2021.
[44] M. Rossi, A. Medina Escobar, L. Ameghino, and M. Merello, "Expanding the phenotype of phosphomannomutase-2 gene congenital disorder of glycosylation: Cervical dystonia," *Journal of the Neurological Sciences*, vol. 378, pp. 52-54, 2017.
[45] G. Ortega-Suero, M. Fernández-Matarrubia, E. López-Valdés, and J. Arpa, "A novel missense OPA1 mutation in a patient with dominant optic atrophy and cervical dystonia," *Movement Disorders Clinical Practice*, vol. 6, pp. 171-173, 2019.
[46] P. Liskova, *et al.*, "Novel OPA1 missense mutation in a family with optic atrophy and severe widespread neurological disorder," *Acta Ophthalmol.*, vol. 91, no. 3, pp. e225-e231, 2013.
[47] M. C. M. van der Weijden, *et al.*, "A gain-of-function variant in dopamine D2 receptor and progressive chorea and dystonia phenotype," *Movement Disorders: Official Journal of the Movement Disorder Society*, vol. 36, no. 3, pp. 729-739, 2021.
[48] P. Y. Jean-Charles, S. Kaur, and S. K. Shenoy, "G protein-coupled Receptor signaling through β-arrestin-dependent mechanisms," *Journal of Cardiovascular Pharmacology*, vol. 70, no. 3, pp. 142-158, 2017.
[49] N. M. Urs, *et al.*, "Targeting β-arrestin2 in the treatment of L-DOPA-induced dyskinesia in Parkinson's disease," in *Proc. the National Academy of Sciences of the United States of America*, vol. 112, no. 19, 2015, pp. E2517-E2526.
[50] E. Zoons, *et al.*, "The relationship between the dopaminergic system and depressive symptoms in cervical dystonia," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 44, no. 8, pp. 1375-1382, 2017.
[51] A. G. Shaikh, D. S. Zee, and H. A. Jinnah, "Oscillatory head movements in cervical dystonia: Dystonia, tremor, or both?" *Movement Disorders: Official Journal of the Movement Disorder Society*, vol. 30, no. 6, pp. 834-842, 2015.
[52] M. Smit, *et al.*, "Relationships between serotonin transporter Binding in the raphe nuclei, basal ganglia, and hippocampus with clinical symptoms in cervical dystonia: A [(11)C]DASB positron emission tomography study," *Frontiers in Neurology*, vol. 9, p. 88, 2018.
[53] E. Zoons, *et al.*, "Lower serotonin transporter binding in patients with cervical dystonia is associated with psychiatric symptoms," *EJNMMI Research*, vol. 7, no. 1, p. 87, 2017.
[54] J. Y. Lee, *et al.*, "Putaminal serotonergic innervation: Monitoring dyskinesia risk in Parkinson disease," *Neurology*, vol. 85, no. 10, pp. 853-860, 2015.
[55] E. R. Timmers, *et al.*, "Dopaminergic and serotonergic alterations in plasma in three groups of dystonia patients," *Parkinsonism & Related Disorders*, vol. 91, pp. 48-54, 2021.
[56] C. L. Groth, *et al.*, "Cervical dystonia is associated with aberrant inhibitory signaling within the thalamus," *Frontiers in Neurology*, vol. 11, p. 575879, 2020
[57] B. D. Berman, *et al.*, "GABA(A) receptor availability changes underlie symptoms in isolated cervical dystonia," *Frontiers in Neurology*, vol. 9, p. 188, 2018.
[58] C. Liu, *et al.*, "A metabolomic study of cervical dystonia," *Parkinsonism & Related Disorders*, vol. 82, pp. 98-103, 2021.
[59] X. Wang, H. Lin, and Y. Gu, "Multiple roles of dihomo-γ-linolenic acid against proliferation diseases," *Lipids in Health and Disease*, vol. 11, p. 25, 2012.
[60] A. Hinduja, S. Chokroverty, P. Hanna, and R. P. Grewal, "Dystonia with superimposed myasthenia gravis: An experiment in nature," *Movement Disorders: Official Journal of the Movement Disorder Society*, vol. 23, no. 11, pp. 1626-1627, 2008.
[61] F. Cavallieri, C. Godeiro, J. C. Lino, Jr., and E. Moro, "Cervical dystonia in a case of longstanding secondary progressive multiple sclerosis," *Revue Neurologique*, vol. 175, no. 4, pp. 269-171, 2019.

Qiwen Tang is from Shaoxing, China. She was born on July 27, 1998, who is now a MM candidate in neurology, class of 2024, in Department of Neurology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, in Hangzhou, China. Her major field of study is neurology.
She is currently the master of medicine candidate in neurology, class of 2024, in School of Medicine, Zhejiang University, Hangzhou, China. She has published articles: Beneficial Effects of Total Phenylethanoid Glycoside Fraction Isolated from Cistanche deserticola on Bone Microstructure in Ovariectomized Rats (New York, United States, Hindawi Pub. Corp., 2019). Her current and previous research interests are movement disorders and botulinum toxin.

Linhui Ni is from Taizhou, China. She was born on April 3, 1992, who is now a MD candidate in Neurology, class of 2022, in Department of Neurology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, in Hangzhou, China. Her major field of study is neurology.
She is currently the doctor of medicine candidate in neurology, class of 2022, in School of Medicine, Zhejiang University, Hangzhou, China. She has published articles: Pathological Gait Signatures of Post-stroke Dementia With Toe-Off and Heel-to-Ground Angles Discriminate From Alzheimer's Disease (Switzerland, Lausanne: Frontiers Research Foundation, 2021), Unilateral whisker pad injection of botulinum toxin type a enhances spatial learning in mice (Oxford, UK, Rapid Communications of Oxford Ltd., 2018), Inhibition of endoplasmic reticulum stress reverses synaptic plasticity deficits in striatum of DYT1 dystonia mice (United States, Albany, NY : Impact Journals, LLC, 2021). Her current and previous research interests are movement disorders and the central effect of botulinum toxin type A.

Xinxin Xu is from Quzhou, China. She was born on Feb. 21, 1996, who is now a MM candidate in Neurology, class of 2022, in Department of neurology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, in Hangzhou, China. Her major field of study is neurology.
She is currently the master of medicine candidate in neurology, class of 2022, in School of Medicine, Zhejiang University, Hangzhou, China. She has published articles: Pathological Gait Signatures of Post-stroke Dementia with Toe-Off and Heel-to-Ground Angles Discriminate from Alzheimer's Disease (Switzerland, Lausanne: Frontiers Research Foundation, 2021). Her current and previous research interests are botulinum and cholinergic-related cognition.

Hanze Chen is from Xuzhou, China. He was born on Feb. 20, 1995, who is now a MD candidate in neurology, class of 2023, in Department of Neurology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, in Hangzhou, China. His major field of study is neurology.
He is currently the doctor of medicine candidate in neurology, class of 2023, in School of Medicine, Zhejiang University, Hangzhou, China. He has published articles: Association between skipping breakfast and risk of cardiovascular disease and all cause mortality: A meta-analysis (Edinburgh, England, Edinburgh; New York: Churchill Livingstone, 2020), Anger, hostility and risk of stroke: a meta-analysis of cohort studies (Berlin, Germany, Berlin ; New York, Springer-Verlag, 2019), 25-Hydroxyvitamin D Levels and the Risk of Dementia and Alzheimer's Disease: A Dose-Response Meta-Analysis (Switzerland, Lausanne : Frontiers Research Foundation, 2018). His current and previous research interests are stroke, inflammation and dementia.

Xingyue Hu is from Shaoxing, China. He was born on Jan. 7, 1962, who holds PhD and MD degree in neurology from School of Medicine, Zhejiang University, Hangzhou, China. His major field of study is Neurology.
He is currently the CHIEF of Department of Neurology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, China. He has published articles: Evidence for polymerase gamma, POLG1 variation in reduced mitochondrial DNA copy number in Parkinson's disease (England, Kidlington, Oxford, U.K. ; Tarrytown, NY : Elsevier Science, 2015), Late-onset primary dystonia

in Zhejiang province of China: a service-based epidemiological study (Milano, Italy, Springer-Verlag Italia, 2016), Influence of injection of Chinese botulinum toxin type A on the histomorphology and myosin heavy chain composition of rat gastrocnemius muscles (Hangzhou, China, Zhejiang University Press, 2013). His current and previous research interests are movement disorders and botulinum toxin.

Prof. Hu is currently serving as the vice chairman of Zhejiang Chinese Society of Neurology, the vice chairman of Neurology Sub-Association of Chinese Medical Doctor Association, the chairman of Precision Medicine Society of Neurology, Zhejiang Society for Mathematical Medicine, the member of Group of Parkinson Disease and Movement Disorder of Neurology Branch of Chinese Medical Association, the member of Professional Committee on Parkinson's Disease and Movement Disorders of Chinses Medical Doctor Association, the vice chairman of General Practice and Primary Care of Chinese Stroke Association, the executive member of Stroke Prevention Project Committee, National Health and Family Planning Commission of the People' s Republic of China. He leaded several projects funded by National Natural Science Foundation of China and Zhejiang Provincial Natural Science Foundation, and published five books and dozens of papers indexed by SCI. He also won three provincial prizes for progress in science and technology.