

REVIEW

An Update on the Phenotype, Genotype and Neurobiology of ADCY5-Related Disease

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ABSTRACT: Adenylyl cyclase 5 (ADCY5)-related phenotypes comprise an expanding disease continuum, but much remains to be understood about the underlying pathogenic mechanisms of the disease. ADCY5-related disease comprises a spectrum of hyperkinetic disorders involving chorea, myoclonus, and/or dystonia, often with paroxysmal exacerbations. Hypotonia, developmental delay, and intellectual disability may be present. The causative gene encodes adenylyl cyclase, the enzyme responsible for the conversion of adenosine triphosphate (ATP) to cyclic adenosine-3',5'-monophosphate (cAMP). cAMP is a second messenger that exerts a wide variety of effects via several intracellular signaling pathways. ADCY5 is the most commonly expressed isoform of adenylyl cyclase in medium spiny neurons (MSNs) of the striatum, and it integrates and controls dopaminergic signaling. Through cAMP pathway, ADCY5 is a key regulator of the cortical and thalamic signaling that control initiation of voluntary movements and prevention of involuntary movements. Gain-of-function mutations in *ADCY5* have

been recently linked to a rare genetic disorder called ADCY5-related dyskinesia, where dysregulation of the cAMP pathway leads to reduced inhibitory activity and involuntary hyperkinetic movements. Here, we present an update on the neurobiology of ADCY5, together with a detailed overview of the reported clinical phenotypes and genotypes. Although a range of therapeutic approaches has been trialed, there are currently no disease-modifying treatments. Improved in vitro and in vivo laboratory models will no doubt increase our understanding of the pathogenesis of this rare genetic movement disorder, which will improve diagnosis, and also facilitate the development of precision medicine approaches for this, and other forms of hyperkinesia. © 2021 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society

Key Words: ADCY5; dyskinesia; hyperkinesia; movement disorder; precision medicine

Adenylyl cyclases (ACs) comprise a family of molecules involved in the conversion of adenosine triphosphate (ATP) to cyclic adenosine-3',5'-monophosphate (cAMP),

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a secondary messenger that exerts a wide variety of effects via several intracellular signaling pathways. Isoform 5 of adenylyl cyclase (ADCY5) is highly expressed in the brain and myocardium.¹ Brain-specific expression of ADCY5 is extremely selective, with high levels of expression in the striatum, nucleus accumbens, and olfactory tubercle.^{1,2} This anatomic specificity likely underlies the impact of ADCY5 mutations on the control of movement. Mutations in *ADCY5* have been linked to a range of complex movement disorders often associated with neurodevelopmental phenotypes. There are currently no clear disease-modifying treatments for ADCY5-related disease, although the role of caffeine is currently being explored. Therefore, understanding the molecular mechanisms with appropriate laboratory models is of the utmost importance. Here, we review the neurobiology of ADCY5 as well as the clinical presentation and molecular genetic

features of ADCY5-related movement disorders. We also review the current management of disease as well as possible future therapeutic strategies that could be developed using new in vitro models and genome editing tools.

Biology of Adenylyl Cyclases

The family of human ACs encompasses 10 different isoforms. Of these, nine are membrane-bound, and one is a soluble isoform. The structure of membrane-bound ACs (AC1-AC9) includes an intracellular N-terminus, two repeats of six transmembrane helices domains (TM1 and TM2), two intracellular catalytic domains of 40 kDa each (C1 and C2) and an intracellular C-terminus (Fig. 1). AC activity is regulated by heteromeric G protein-coupled receptors. All isoforms of membrane-bound ACs are stimulated by the GTP-bound α subunit of $G_{s\alpha}$ and inhibited by the α subunit of $G_{i\alpha}$. Once activated, ACs catalyze the conversion of ATP to cAMP and pyrophosphate. The generated cAMP then propagates downstream signaling via specific cAMP-binding proteins (eg, cAMP-dependent kinases, transcription factors, or ion transporters).

Knock-out and overexpression cellular models have provided insights into the tissue distribution and differential expression of the various AC isoforms.³⁻⁶ It is not surprising that given the crucial importance of signaling integration in the brain, all nine membrane-bound ACs are

expressed in the central nervous system. Although some isoforms are widespread (eg, AC6 and AC7), some are only expressed in specific regions (eg, AC3 in the olfactory cilia and AC5 in the striatum) (Supplementary Table S1).

Dopaminergic Signaling in the Striatum

The striatum within the subcortical basal ganglia is a critical component of motor and reward systems. GABAergic medium spiny neurons (MSNs) constitute 95% of the cellular population of the striatum, and they are the central receiving station of the basal ganglia.⁷ They are innervated by excitatory glutamatergic fibers from the cortex and thalamus and by modulatory dopaminergic fibers from the midbrain. MSNs are defined by their expression of the dopamine- and cAMP-regulated phosphoprotein DARPP-32.⁸ There are two distinct populations of MSNs, based on their neurochemical characterization and projection targets; DRD1-expressing MSNs of the striatonigral direct pathway and DRD2-expressing MSNs of the striatopallidal indirect pathway.

Direct pathway MSNs innervate the output nuclei of the basal ganglia, which are the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr). Indirect pathway MSNs project to the external segment of the globus pallidus (GPe) and the subthalamic nucleus (STN). Direct and indirect MSNs in

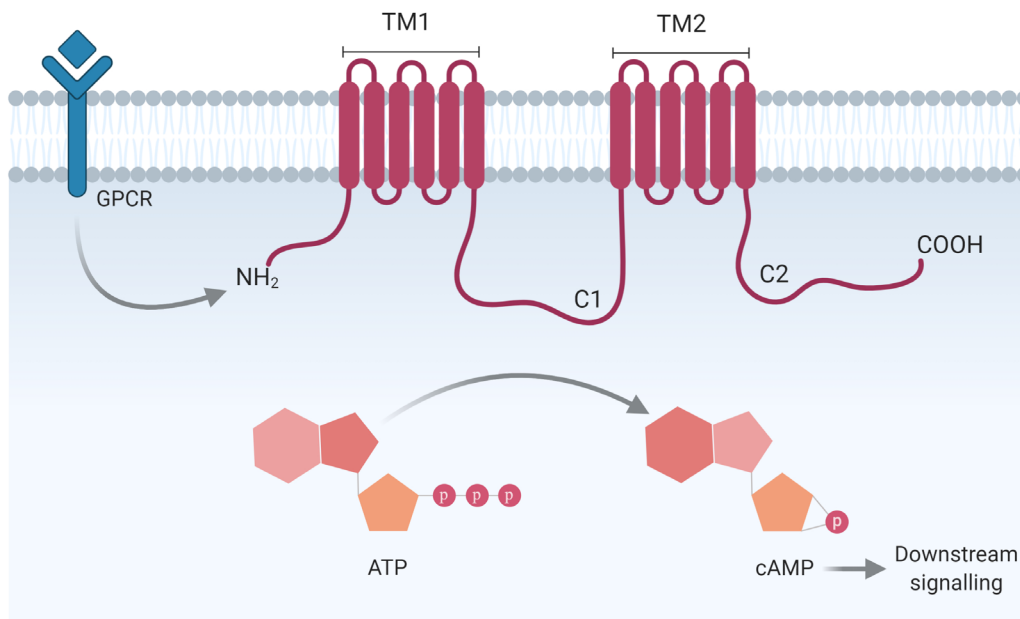


FIG. 1. General structure of adenylyl cyclase proteins. Adenylyl cyclases are transmembrane proteins that consist of two bundles of six transmembrane domains. They are regulated by heterotrimeric G proteins coupled to membrane receptors. G protein complexes consist of α , β , and γ subunits. When the receptor is activated by a hormonal stimulus, it undergoes a conformational change that causes the α subunit to dissociate from the complex and become bound to GTP. The $G\alpha$ -GTP complex binds to and activates adenylyl cyclase. Activated adenylyl cyclase catalyzes the conversion of ATP to cAMP. cAMP is a second messenger which activates downstream signaling regulating several intracellular pathways. GPCR = G-protein coupled receptor; TM = transmembrane domain; C = catalytic domain; GTP = guanosine triphosphate; ATP = adenosine triphosphate; cAMP = cyclic adenosine-3',5'-monophosphate. [Color figure can be viewed at wileyonlinelibrary.com]

the striatum exert opposite effects on the control of movement. Activation of DRD1 stimulates striatopallidal pathway MSNs resulting in disinhibition of thalamocortical neurons and excitation of the motor cortex, which leads to movement. On the other hand, on activation of the indirect pathway, DRD2 inhibits striatonigral pathway MSNs leading to inhibition of thalamocortical neurons and the motor cortex, which leads to suppression of movement and prevention of unwanted movements (Fig. 2A).

ADCY5 is the most expressed AC isoform in MSNs, and it has been estimated that it accounts for more than 80% of cAMP production.^{1,9} ADCY5 is located mostly in DRD1-expressing MSNs.¹⁰ Stimulation of DRD1 activates $G_{s\alpha}$ -mediated ADCY5 activity increasing cAMP levels whereas stimulation of DRD2 activates $G_{i\alpha}$ -mediated ADCY5 activity decreasing cAMP levels.⁹ Increased intracellular cAMP levels are linked to protein kinase A (PKA)-mediated downstream signaling (Fig. 2B). In MSNs, increased levels of cAMP and PKA activity lead to increased phosphorylation of DARPP-32 and transcription factor cAMP-responsive element-binding protein (CREB) with a broad range of downstream effects on neuronal function. Disruption of cAMP signaling therefore contributes to post-synaptic MSN dysfunction, which may underpin movement disorders such as dystonia, chorea, and parkinsonism.¹¹ Together with *ADCY5*, other genes with key roles in MSN-related cAMP signaling (such as *PDE10A*, *GNAO1*, *GNAL1*, and *GPR88*) have also been associated with overlapping motor phenotypes.¹¹

Clinical Features of ADCY5-Related Disease

ADCY5 mutations were first implicated in neurological disorders in 2012,¹² and they are associated with heterogeneous syndromes. Movement disorders are often a prominent feature of the clinical phenotype. Classically, the condition presents in early childhood with an initially fluctuating or paroxysmal hyperkinetic movement disorder characterized by dystonia, chorea, and/or myoclonus. There may be a progression with age from paroxysmal to continuous abnormal movements.¹³ Symptoms vary greatly in severity between patients. Exacerbations can vary in length from minutes to hours or days, and the most widely reported trigger is fatigue. Other triggers include anxiety, excitement, and intercurrent illness. Axial hypotonia is often also present and eye movements may be abnormal.¹⁴ Although most other movement disorders remit during sleep, patients with *ADCY5*-related disorders often experience episodes of abnormal movement throughout the night, resulting in significant disturbance.^{15,16} A recent study confirmed that *ADCY5*-related nocturnal paroxysmal dyskinesia is not directly elicited by sleep

or because of a primary sleep disorder.¹⁵ Rather, patients showed nocturnal paroxysmal dyskinesia that emerged during night-time awakenings with subsequent delayed sleep (as opposed to movements associated with drowsiness or delayed sleep onset). Patients were also found to have long and often violent paroxysmal dyskinesia on morning awakening. Except for sleep efficiency and specific sleep measures related to prolonged nocturnal awakenings, sleep architecture (proportion of each sleep stage, respiratory events, periodic leg movements, and muscle activity) is otherwise normal in patients with *ADCY5* mutations.

The primary disease phenotype has been labelled “familial benign chorea”¹⁷ or “familial dyskinesia with facial myokymia”.¹² The term “myokymia” is technically a misnomer; it describes twitching arising from a pathology of the muscle or neuromuscular junction, whereas the movements seen in *ADCY5*-related conditions are believed to originate from the central nervous system¹⁸. The perioral muscle twitching observed in patients with *ADCY5* gene mutations were initially described as myokymia. However, a subsequent electromyography study showed a complex electrophysiological pattern with no evidence of myokymia.¹⁸ Based on the clinical phenomenology and electrophysiological findings, the facial twitching and truncal jerks in these patients are now considered to be dyskinesia or myoclonus-chorea. Variant presentations reported in a small number of patients include generalized myoclonus-dystonia,¹⁹ spastic paraparesis,²⁰ and, in one report, alternating hemiplegia of childhood.¹³ The course of the condition is generally stable after onset, and life expectancy is believed to be normal.²¹ Although the movement disorder can be significantly disabling, and poorly responsive to many drugs, there are also some reports of spontaneous improvement in adolescence or adulthood.^{14,22} The majority of affected individuals have normal intelligence, but intellectual disability does occur in a minority,¹⁴ and acquisition of early milestones is often delayed by the movement disorder.²¹ There is an impression that the incidence of mood disorder and psychotic illness may be increased, but this has not been reliably quantified.^{23,24} *ADCY5* encodes a specific adenylyl cyclase that is also highly expressed in the myocardium,² and there have been reports of cardiac complications such as congestive heart failure in patients.¹² Brain imaging is normal,²¹ and diagnosis is usually confirmed by genetic testing.

The first brain autopsy findings in a molecularly proven case of *ADCY5*-dyskinesia (age of death, 46 years) have been recently reported.²³ In this study, gross pathology was unremarkable with the exception of mild pallor of the substantia nigra. Compared to control subjects, there was no loss or decrease in size of neurons in the patient. Increased immunoreactivity for *ADCY5* was found in neurons in multiple brain regions. Interestingly, tau deposits were found in the

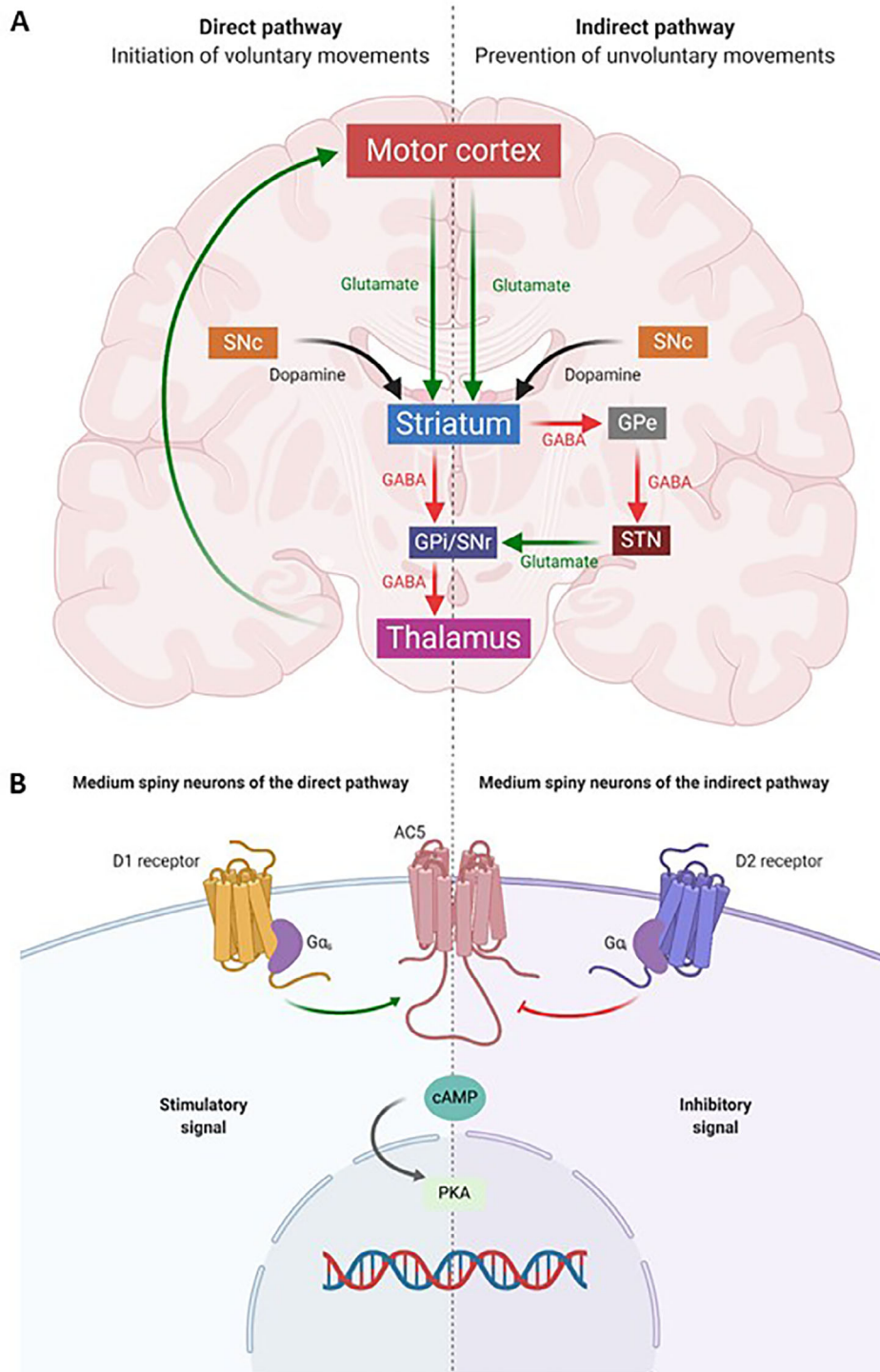


FIG. 2. Basal ganglia motor circuits. **(A)** Direct and indirect pathways of the basal ganglia. Direct and indirect MSNs in the striatum have opposite effects on the control of movement. MSNs of the direct pathway innervate the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr). This results in the disinhibition of thalamocortical neurons and excitation of the motor cortex, which leads to movement. MSNs of the indirect pathway project to the external segment of the globus pallidus (GPe) and the subthalamic nucleus (STN). Activation of indirect pathway leads to the inhibition of thalamocortical neurons and the motor cortex, which leads to suppression of movement and prevention of unwanted movements. **(B)** Role of ADCY5 in the integration of direct and indirect pathway signaling in medium spiny neurons. ADCY5 is mainly expressed on DRD1-MSNs. Activation of DRD1 has a stimulatory effect and activates $G_{\alpha s}$ -mediated ADCY5 activity, increasing intracellular cAMP levels. Increased cAMP levels are linked to PKA-mediated downstream signaling. Activation of DRD2 has an inhibitory effect and activates $G_{\alpha i}$ -mediated ADCY5 activity decreasing cAMP levels. SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; GPe, globus pallidus external segment; GPi, globus pallidus internal segment; STN, subthalamic nucleus. Green arrows, activation; red arrows, inhibition. [Color figure can be viewed at wileyonlinelibrary.com]

deep cortical sulci, midbrain, and hippocampus with minimal amyloid pathology and no Lewy bodies. This was somewhat surprising and further studies on molecularly proven cases of ADCY5-related dyskinesia will be needed to assess whether the disease has a tauopathy component.

Molecular Genetic Features of ADCY5-Related Disease

ADCY5 consists of 1261 amino acids encoded by a 21-exon gene located on chromosome 3p21.1. Mutations in the *ADCY5* gene were initially identified in 2001 in a single five-generation German kindred and formerly described as familial dyskinesia and facial myokymia.²⁵ Inheritance of *ADCY5* mutations is usually autosomal dominant, and no reports of incomplete penetrance have been published to date. There are several reports of somatic mosaicism, where mosaic carriers may present with symptoms, although these are often less severe.²³ Mosaic parents may also be asymptomatic.²⁰ Autosomal recessive inheritance has been reported in two families; in both, heterozygous carriers were asymptomatic and homozygous individuals experienced a phenotype closely resembling the autosomal dominant disease form.^{26,27} Table 1 presents an overview of all the reported mutations and associated phenotypes. The biological basis for the observed clinical heterogeneity needs further investigation because there is no strikingly clear genotype–phenotype correlation. It is possible that the p.R418W mutation is linked to a more severe phenotype, whereas the p.A726T is associated with a milder phenotype. The p.R418W variant, together with other mutations at this residue (p.R418Q, p.R418G) are recurrent mutations in the majority of reported cases, suggesting a mutational hotspot at Arginine 418. In the last few years, exome sequencing has emerged as a very powerful tool to identify causative genes for rare Mendelian diseases.^{12,30} Diagnostic exome sequencing has also provided insights into the molecular diversity of ADCY5-related dyskinesia and identified several de novo mutations or previously undiagnosed cases.^{20,30,32} Interestingly, one of these de novo variants was found again at the 418 site, confirming a markedly increased degree of intrinsic mutability of this genomic site.³²

ADCY5 has two transmembrane domains (TM1 and TM2), comprising six helices of hydrophobic amino acids, and two cytoplasmic domains (C1 and C2). C1 and C2 are brought together to form an ATP-binding site with a catalytic pocket for the hydrolysis of ATP.³³ As illustrated in Figure 3, the majority of the reported mutations are located in C1 and C2 domains, suggesting how they might affect the strength of enzyme-substrate binding or the C1-C2 interaction to form the catalytic

pocket. For example, the most common mutation on residue arginine 418 lies in the cytoplasmic domain C1 and replaces a branched positively charged amino acid with the negatively charged amino acid tryptophan, likely affecting the normal formation of the catalytic pocket. It can be hypothesized that a gain-of-function mutation facilitates the interaction between C1 and C2, leading to enhanced cAMP production. Mutations outside C1 and C2 such as M1029L in the TM2 domain are likely linked to rearrangement of the protein structure, which may eventually lead to C1 and C2 being in closer proximity. Although most of the reported *ADCY5* mutations are missense, some frameshift mutations are reported, such as the deletion p.K694_M696 in the intracellular catalytic portion. It is not clear whether such mutations lead to loss- or gain-of-function; within in the transmembrane domain, it is possible that these mutations could enhance the affinity between C1 and C2, leading to aberrant dimerization and ligand-independent interaction.

It is important to acknowledge that ADCY5-related dyskinesia is not only clinically but also genetically heterogeneous. As discussed, although most of the reported mutations are postulated to lead to a gain-of-function, there are several studies suggesting that, at least for certain mutations, loss-of function may instead be the pathophysiological mechanism, especially in families where autosomal recessive inheritance is observed.^{26,27} For example, the missense mutation described by Bohlega et al on the C1 domain has been predicted to be damaging by different in silico tools.²⁶ Based on the recessive inheritance pattern, it is conceivable that this biallelic change leads to loss of normal protein function. Furthermore, Carapito et al³⁴ reported a single de novo mutation (c.2088 + 1G > A in a 5' donor splice-site of *ADCY5*) segregating with disease. This mutation is predicted to induce mRNA degradation, suggesting that *ADCY5* haploinsufficiency may also be a possible mechanism of disease. Therefore, it appears that ADCY5-related dyskinesia can result from either a gain- or loss-of-function mechanism, although the underlying pathogenic processes accounting for these differences are not yet fully understood. Further investigation is needed to better delineate the link between *ADCY5* mutations, effect on protein function and different disease phenotypes.

Proposed Molecular Mechanisms

Given the relatively recent identification of pathogenic mutations in *ADCY5*, there are only very few reports of in vitro functional studies assessing the impact of mutant protein function. Chen and colleagues performed some pivotal in vitro functional studies using HEK293 cells overexpressing *ADCY5*. They showed

TABLE 1. Overview of reported *ADCY5* mutations with associated clinical phenotype

Variant			Features			
Transcript	cDNA	Protein	Inheritance	Mutation type	Clinical phenotype	References
NM_183357.2	c.409_428del	p.G137Cfs*184	Autosomal recessive Compound heterozygous	Frameshift	Generalized dystonia with superimposed myoclonus	27
NM_183357.2	c.3037C > T	p.R1013C	Autosomal recessive Compound heterozygous	Missense	Generalized dystonia with superimposed myoclonus	27
NM_183357.2	c.1252C > T	p.R418W	Autosomal dominant/de novo	Missense Gain of function	Infantile- or early childhood-onset axial hypotonia with limb hypertonia, intermittent tremors, paroxysmal dyskinesia, myoclonus both at rest and with activity, involuntary choreic and dystonic movements, occasional facial movements but not obvious myokymia Normal brain MRI Delayed motor and speech milestones Mild cognitive delay Abnormal saccades Nonparoxysmal generalized chorea (Benign hereditary chorea, BHC) Sleep disturbances Dystonia	14,16,17,18,20,21, 28,29,30,31,47,48,49 Chen DH et al. 2015 Meijer et al. 2016 Friedman et al. 2016
NM_183357.2	c.1253G > A	p.R418Q	Autosomal dominant	Missense	Generalized chorea, mild myoclonic jerks Delayed motor milestones	21 Chen et al. 2015 Chang et al. 2016 Friedman et al. 2016
NM_183357.2	c.1252C > G	p.R418G	Autosomal dominant	Missense	Delayed motor and speech milestones Axial hypotonia, mild generalized chorea and dystonic posturing of the limbs (tiptoe walking) Anxiety and obsessive compulsive disorders	18,20,21
NM_183357.2	c.1313G > C	p.R438P	Autosomal dominant	Missense	Paroxysmal dyskinesia, axial hypotonia, dystonia, tremor Normal motor and speech development	Friedman et al. 2016
NM_183357.2	c.1378A > T	p.I460F	De novo	Missense	Lower face dyskinesias, tongue thrusting, dysarthric speech, phasic retro- and laterocollis, and axial dystonia. Abnormal gait	32
NM_183357.2	c.1425C > G	p.I475M	N/A	Missense	N/A	Only reported in ClinVar (by Ambray Genetics)
NM_183357.2	c.1646 + 1G > A	Altered splicing	N/A	Frameshift	N/A	Only reported in ClinVar (by Ambray Genetics)
NM_183357.2	c.1762G > A	p.D588N	Autosomal recessive	Missense	Axial hypotonia with dystonia Facial and oral twitching, myoclonus, dysarthria Delayed motor and speech milestones Normal cognitive function	26

(Continues)

TABLE 1. Continued

Variant			Features			
Transcript	cDNA	Protein	Inheritance	Mutation type	Clinical phenotype	References
NM_183357.2	c.2088 + 1G > T	Altered splicing	Autosomal dominant	Frameshift ADCY5 haploinsufficiency	Mild choreiform movements associated with dystonia No facial myokymia Normal psychomotor development	Chen et al. 2012 Carapito et al. 2014
NM_183357.2	c.2176G > A	p.A726T	Autosomal dominant	Missense	Familial dyskinesia with facial myokymia (FDFM), dystonic movements of neck and arms, perioral and periorbital twitches	18 Fernandez et al. 2001 Chen et al. 2012
NM_183357.2	c.2722G > A	p.E908K	Autosomal dominant (mosaic asymptomatic parent)	Missense	Axial hypotonia with dystonia Delayed motor and speech milestones Spastic paraparesis with hyperreflexia, hypertonia in the legs, and extensor plantar reflexes	20
NM_183357.2	c.2080_2088del	p.K694_M696	Autosomal dominant	Frameshift deletion	Severe choreoathetosis involving face, limbs and trunk Profound axial and appendicular hypotonia with no dystonia or myoclonus Significantly delayed cognitive function Orolingual dyskenesia	29
NM_183357.2	c.3086 T > A	p.M1029K	Autosomal dominant	Missense	Severe dystonia, hypotonia, chorea Mild cognitive impairment Familial dyskinesia with facial myokymia (FDFM)	19 Chen DH et al. 2015
NM_183357.2	c.2180G > A	p.R727K	Autosomal dominant	Missense	N/A	Zech et al. 2017
NM_183357.2	c.1196C > T	p.P399L	Autosomal dominant	Missense	N/A	Zech et al. 2017
NM_183357.2	c.1400A > G	p.N467S	Autosomal dominant	Missense	N/A	Zech et al. 2017
NM_183357.2	c.3177_3182delTGA	p.D1060del	Autosomal dominant	In-frame deletion	N/A	Zech et al. 2017
NM_183357.2	c.3625A > G	p.M1209V	Autosomal dominant	Missense	N/A	Zech et al. 2017
NM_183357.2	c.3045C > A	p.D1015E	N/A	Missense	Paroxysmal paralysis Paroxysmal chorea Mild hypotonia Repeated attacks of hemiplegia involving either side of the body Mild developmental delay	Westenberger et al. 2016
NM_183357.2	c.3074A > T	p.E1025V	N/A	Missense	Paroxysmal paralysis Paroxysmal dystonia Repeated attacks of hemiplegia involving either side of the body Mild developmental delay	Westenberger et al. 2016

that two recurrent *ADCY5* mutations (p.A726T and p.R418W) cause a significant gain-of-function, with an enhancement of cAMP production in response to β-adrenergic stimulation compared to wild-type AC5, supporting their causative role in the pathogenesis of

the disease.²⁸ Recently, Doyle et al³⁵ expanded on this, characterizing five recurrent *ADCY5* gain-of-function mutations. Using a newly developed HEK293 line depleted of other predominant adenylyl cyclase isoforms, they demonstrated that *ADCY5* mutants show

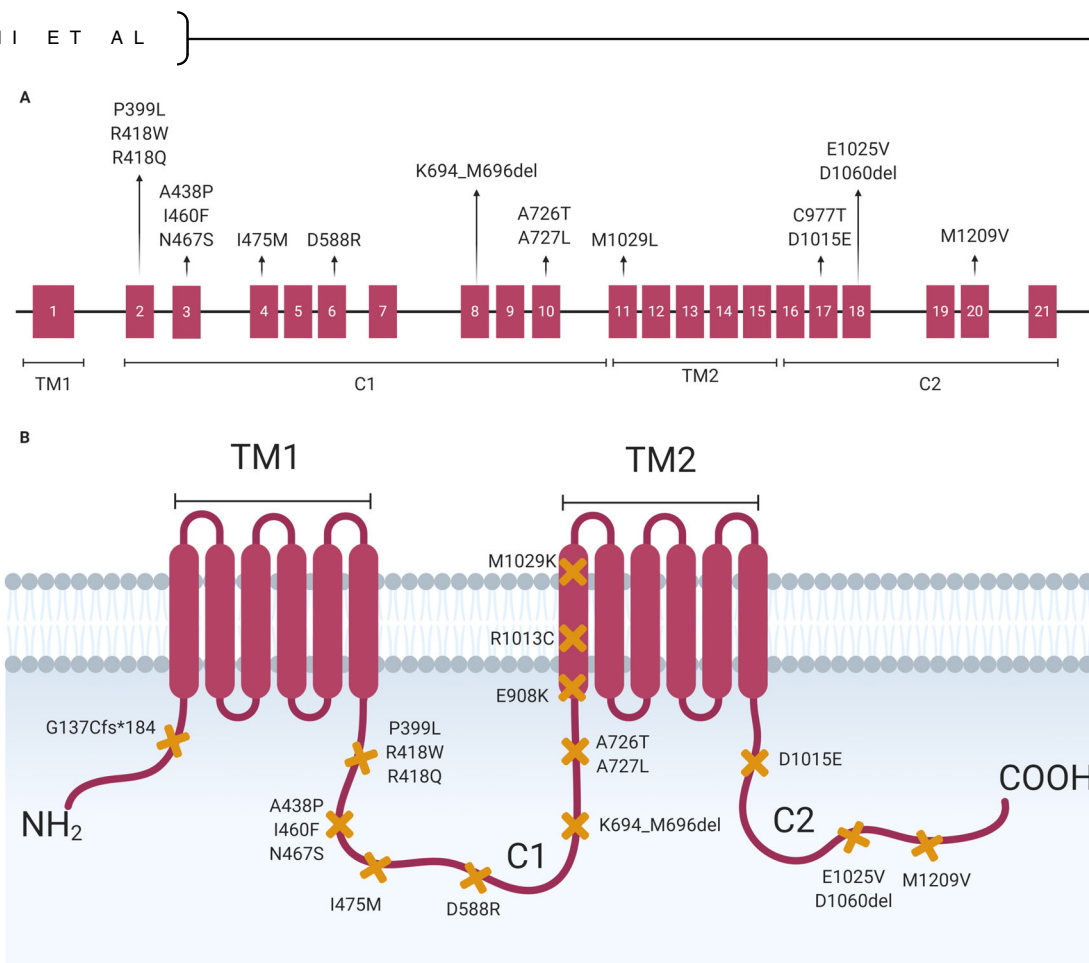


FIG. 3. Localization of *ADCY5* mutations. **(A)** Schematic representation of *ADCY5* mutations in the gene. Localization of the reported patients' mutations in the exons of *ADCY5* gene. **(B)** Localization of *ADCY5* mutations on the domains of the protein. TM, transmembrane domain; C1 and C2, cytoplasmic domains. [Color figure can be viewed at wileyonlinelibrary.com]

an enhanced response to $G_{\alpha s}$ -stimulation. They further demonstrated that increased cAMP at the membrane results in increased downstream target gene transcription, providing potential insights into pathogenic molecular mechanisms. The increased cAMP promotes the dissociation and activation of protein kinase A catalytic subunits, which translocate into the nucleus and phosphorylate several proteins, including the CREB. This stimulates an altered transcription which leads to a hyperactivation of the direct pathway (Fig. 4).

In contrast to the gain-of-function effects of missense mutations, an *ADCY5* knock-out mouse generated by homologous recombination exhibited a hypokinetic phenotype with parkinsonism features.³⁶ Interestingly, the same *ADCY5* knock-out mouse was also used to study ageing and longevity, showing that *ADCY5* disruption increases lifespan by 30% through oxidative stress protection.^{37,38} Inhibition of *ADCY5* activates SIRT1/FoxO3a and Raf/MERK/ERK pathway that upregulates the antioxidant mitochondrial enzyme MnSOD, resulting in resistance to oxidative stress during ageing.³⁷ It is known that increased levels of cAMP are associated with oxidative stress.³⁹ Therefore, a

mechanism by which an overactivation of *ADCY5* could lead to neuronal dysfunction may be through increased oxidative stress in MSNs, potentially leading to reactive oxygen species (ROS)-induced cell death. The absence of neuronal loss in both available imaging studies and on post-mortem analysis would however not corroborate this theory. It is possible that single-photon emission computed tomography or positron emission tomographic neuroimaging might offer better resolution than magnetic resonance imaging (MRI) to investigate neuronal degeneration in *ADCY5* patients.⁴⁰ Another potential mechanism for neuronal dysfunction could be ATP depletion as a result of increased cAMP production, leaving the cells depleted of energy. Further in vitro and in vivo models are needed to test these proposed hypotheses and to better delineate the molecular mechanisms of disease at both the neuronal and systems level.

The enzyme adenylyl cyclase 5 receives signals from striatal GPCRs, including dopamine receptors DRD1, DRD2, and the A2A adenosine receptor.⁹ A potential reason why stress may trigger worsening of the symptoms lies in the hypothesized molecular mechanism.

Current and Future Therapeutic Perspectives

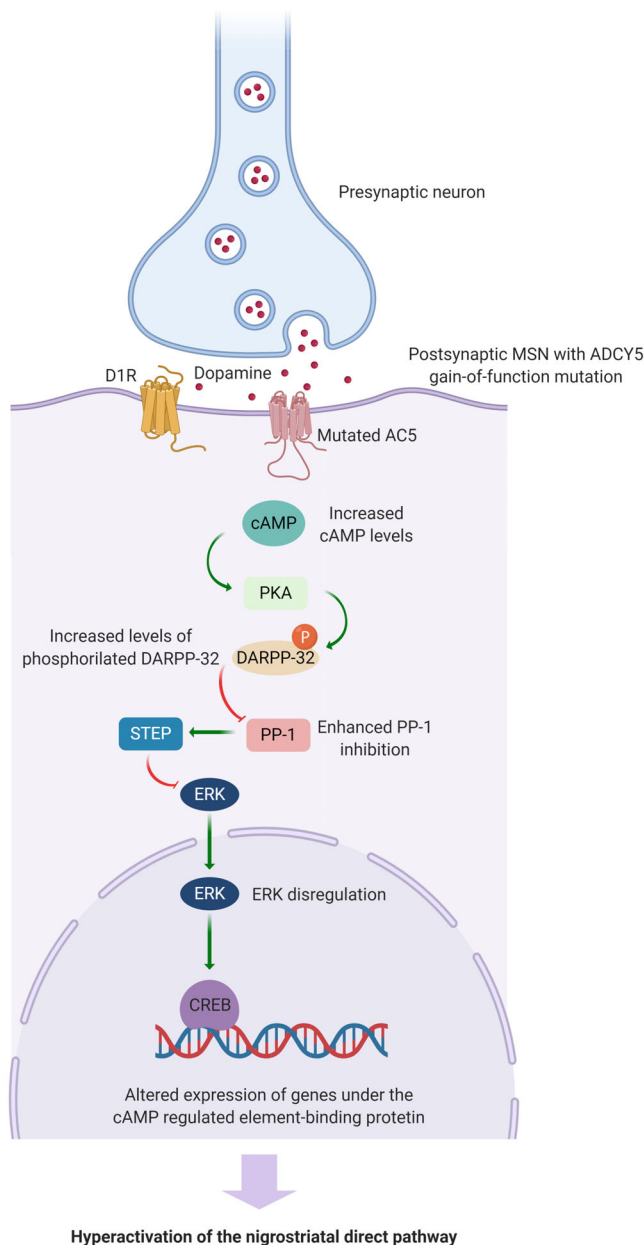


FIG. 4. Disrupted cAMP pathway in medium spiny neurons with mutated ADCY5. ADCY5 gain-of-function mutations lead to increased intracellular cAMP levels. This, in turn, leads to increased activation of PKA and increased levels of phosphorylated DARPP-32. Eventually, there is a dysregulation of the ERK pathway and an altered expression of genes under the transcription factor cAMP-responsive element-binding protein (CREB) with a broad range of downstream effects on neuronal function and a hyperactivation of the direct pathway. PKA, protein kinase A; DARPP-32, -dopamine- and cAMP-regulated neuronal phosphoprotein; PP-1, protein phosphatase 1; STEP, striatal enriched phosphatase; ERK, extracellular signal-regulated kinase; CREB, cAMP-response element binding protein. [Color figure can be viewed at wileyonlinelibrary.com]

Stress increases striatal dopamine synthesis and release, enhancing D1R sensitivity and activating ADCY5 through $G_{\alpha s}$. Mutated ADCY5 with gain-of-function could increase ATP binding to the catalytic pocket, increasing downstream levels of cAMP and subsequent cellular activity.²⁴

To date, there are no disease-modifying therapies for ADCY5-related disease that show proven long-term efficacy. A good response to treatment with benzodiazepines (clonazepam or clobazam) has been reported in some patients with ADCY5-related dyskinesia,^{21,31} and there has also been a case report of positive response to methylphenidate.⁴¹ Deep brain stimulation has led to significant, although partial, improvement in a number of cases.²⁹ Most recently, some patients have reported a dramatic improvement following drinking coffee, suggesting that caffeine may be a useful treatment for some.⁴² The rationale underlying this phenomenon is that caffeine is an antagonist of the adenosine A2A receptors (A2AR) (localized preferentially in striatal neurons expressing dopamine D2 receptors) that activate ADCY5.⁴³ Therefore, caffeine likely inhibits ADCY5 by inhibiting A2A receptors, leading to clinical improvement in patients with gain-of-function mutation and ADCY5 overactivity. A pilot study on caffeine efficiency in ADCY5-related dyskinesia (NCT04351360, 17/04/2020 on <http://ClinicalTrials.gov>) has been recently started to determine the percentage of responders to caffeine. The primary outcome measure is an improvement in overall involuntary movements of 40% or more; the results of this trial are eagerly awaited.

Of note, an aggravating factor that is consistently observed across affected individuals is the presence of anxiety and exposure to typical life stressors. Further research will be needed to determine whether the number and frequency of movements might be reduced with better stress management techniques or limitation of stress-inducing activities.

Another recent insight into a targeted therapeutic approach has been provided by the functional in vitro studies of Doyle and colleagues³⁵ with their work on P-site inhibitors. P-site inhibitors are adenosine nucleotide analogues that bind to the catalytic pocket of adenylyl cyclase. It has been shown that the inhibitor SQ 22.536 is able to hinder ADCY5 activity in ADCY5-overexpressing HEK cells. However, SQ 22.536 has no ADCY5 specificity, and it is anticipated that the lack of specificity would lead to detrimental side effects because of the inhibition of other ADCY isoforms. Further research is needed to identify better ADCY5-specific P-site inhibitors.

Another therapeutic avenue could involve RNA manipulation techniques. For example, small interfering RNA or antisense oligonucleotides are powerful tools to reduce the expression of a single gene; ideally, dominant, gain-of-function disease such as ADCY5-related dyskinesia could be treated using such approaches that specifically silence the mutant allele while leaving the expression of the wild-type allele unperturbed.

Although several therapeutic approaches to manage the manifestation of disease have been attempted, these treatments are still not entirely specific in targeting the core underlying pathogenesis of this disorder. Hence, better models enabling a deeper understanding of the molecular mechanisms involved in the pathogenesis of the disease and its impact on MSNs are of the utmost importance. In this respect, *in vitro* models with neurons derived from human induced pluripotent stem cells (hiPSCs) can not only shed light on the molecular mechanisms but also drive the development of new therapeutic strategies in a patient-specific manner, as already done for other pediatric neurological disorders.⁴⁴ Recently, DARPP32-expressing MSNs have been successfully differentiated from human hiPSCs.⁸ Genome editing tools such as the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system can be used to correct the mutations in patient-derived cells or to generate isogenic control lines, allowing detection of disease-specific phenotypes.⁴⁴ Therefore, patient-derived MSNs represent an unprecedented humanized tool to decipher the exact pathogenesis of ADCY5-related dyskinesia and identify potential drug targets for pre-clinical and clinical studies. Crosstalk between neurons and other cell types with synaptic connectivity is extremely important for neuronal networks, and co-culture *in vitro* systems are now widely used to study cell interactions and improve the maturation of hiPSC-derived cells. hiPSC-derived MSNs with ADCY5 mutations can be potentially co-cultured with hiPSC-derived cholinergic interneurons⁴⁵ to better mimic the native microenvironment and the impact of cAMP dysregulation not only on MSNs but also on other neuronal subtypes. Besides 2D monolayer cultures, 3D brain tissue-like systems, either scaffold-free (eg, organoids) or scaffold-based (eg, using biomaterials) are emerging as novel model systems to investigate human brain development and disease and could be used to elucidate the molecular and cellular dysfunction in ADCY5-related disorders.

Phenotypic data obtained from these advanced *in vitro* models could then be integrated with data obtained from *in vivo* models and human patients. As previously discussed, an ADCY5 knock-out mouse model has been used to study motor dysfunction in parkinsonism disorders.³⁶ An ADCY5 knock-in mouse with constitutively active ADCY5 is still lacking. It could be generated with CRISPR-Cas9, and may potentially recapitulate the motor features of patients with gain-of-function mutations. In addition to rodents, other easily manipulable species could be engineered to generate transgenic animals for disease modelling. For example, *Drosophila melanogaster* is a simple, yet powerful, *in vivo* system used to model Parkinson's disease.⁴⁶ This simple organism could be used to recapitulate the pathogenic mutations of ADCY5 and provide insights into the pathobiology and genotype/phenotype relationships in ADCY5-related disorders.

Conclusions

ADCY5-related dyskinesia is an evolving new genetic disorder with a prominent motor phenotype, and one of the many post-synaptic disorders now associated with altered cAMP signaling. Functional studies have shown increased adenylyl cyclase activity as a pathophysiological factor in ADCY5-related dyskinesia with gain-of-function mutations. As additional families are characterized, the full spectrum of ADCY5 mutations and their relationship to the phenotype of ADCY5-related dyskinesia will be better elucidated. Better cellular and animal disease models, such as the ones discussed in this review will provide the basis for superior precision medicine approaches, therefore paving the way for new treatments for ADCY5-related dyskinesia and other similar genetic movement disorders. ■

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K.B.: 1C, 2B
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