ADCY5-related dyskinesia

Broader spectrum and genotype–phenotype correlations

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Supplemental data at Neurology.org

ABSTRACT

Objective: To investigate the clinical spectrum and distinguishing features of adenylate cyclase 5 (*ADCY5*)–related dyskinesia and genotype–phenotype relationship.

Methods: We analyzed *ADCY5* in patients with choreiform or dystonic movements by exome or targeted sequencing. Suspected mosaicism was confirmed by allele-specific amplification. We evaluated clinical features in our 50 new and previously reported cases.

Results: We identified 3 new families and 12 new sporadic cases with *ADCY5* mutations. These mutations cause a mixed hyperkinetic disorder that includes dystonia, chorea, and myoclonus, often with facial involvement. The movements are sometimes painful and show episodic worsening on a fluctuating background. Many patients have axial hypotonia. In 2 unrelated families, a p.A726T mutation in the first cytoplasmic domain (C1) causes a relatively mild disorder of prominent facial and hand dystonia and chorea. Mutations p.R418W or p.R418Q in C1, de novo in 13 individuals and inherited in 1, produce a moderate to severe disorder with axial hypotonia, limb hypertonia, paroxysmal nocturnal or diurnal dyskinesia, chorea, myoclonus, and intermittent facial dyskinesia. Somatic mosaicism is usually associated with a less severe phenotype. In one family, a p.M1029K mutation in the C2 domain causes severe dystonia, hypotonia, and chorea. The progenitor, whose childhood-onset episodic movement disorder almost disappeared in adulthood, was mosaic for the mutation.

Conclusions: *ADCY5*-related dyskinesia is a childhood-onset disorder with a wide range of hyperkinetic abnormal movements. Genotype-specific correlations and mosaicism play important roles in the phenotypic variability. Recurrent mutations suggest particular functional importance of residues 418 and 726 in disease pathogenesis. ***Neurology*® 2015;85:2026–2035**

GLOSSARY

ADCY5 = adenylate cyclase 5; **ATP** = adenosine-5′-triphosphate; **cAMP** = 3′,5′-cyclic adenosine monophosphate; **ChDys** = chorea-dystonia; **EHC** = essential hereditary chorea; **FDFM** = familial dyskinesia with facial myokymia; **MIP** = molecular inversion probe; **SNP** = single nucleotide polymorphism; **STRP** = short tandem repeat marker.

We previously reported that a p.A726T mutation in adenylate cyclase 5 (*ADCY5*) in one family caused autosomal dominant familial dyskinesia with facial myokymia (FDFM; OMIM 600293) because of the distinctive features of perioral and periorbital twitches, choreiform and dystonic movements of the neck and arms, and increased reflexes.[1] Subsequently, we identified a different *ADCY5* missense mutation, p.R418W, in 2 sporadic cases with axial hypotonia, chorea,

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myoclonus, dystonia, and occasional facial movements, but not obvious myokymia.[2] Adenylate cyclases convert adenosine-5′-triphosphate (ATP) to 3′,5′-cyclic adenosine monophosphate (cAMP), a second messenger in signal transduction.[3] Specificity and modulation of ADCY activity is related to tissue expression patterns and stimulators and inhibitors for each member of the gene family. *ADCY5* is highly expressed in striatum, a region involved in modulating movement, and *ADCY5* knock-out mice display a parkinsonian phenotype.[4] Overexpression of either mutated protein in HEK293 cells increased cAMP accumulation,[2] suggesting a gain-of-function effect.

The variability in adventitious movements, periodicity, and severity in 3 families prompted us to evaluate *ADCY5* in other cases with similar features. To clarify the clinical spectrum and define possible genotype–phenotype relationships, we identified multiple new familial and sporadic cases and show that *ADCY5*-related dyskinesia is more common and constitutes a broader category than previously thought.

METHODS Standard protocol approvals, registrations, and patient consents. Blood samples and skin biopsy were obtained under approved Institutional Review Board protocols.

Patients and samples. Patients with *ADCY5* mutations were identified at multiple sites utilizing different strategies. Some patients had clinical exome sequencing and others had targeted *ADCY5* sequencing based on characteristic phenotypes. Videos and medical records were reviewed and patients were evaluated in Neurology or Genetics Clinics at the University of Washington, Seattle (ID027, Ch4, FDFM, essential hereditary chorea [EHC] family, and chorea-dystonia [ChDys] family); Rady Children's Hospital, University of California San Diego (ID1 and ID032); Johns Hopkins All Children's Hospital, St. Petersburg, Florida (ID016); Lucile Packard Children's Hospital, Stanford University, Palo Alto, California (ID028); Phoenix Children's Hospital, Arizona (ID029); Hôpital de la Pitié Salpêtrière, Paris (ID018, ID019, ID019B, ID035); Hôpital Robert Debré, Paris (ID020); Hôpital Trousseau, Paris (ID021, ID024); and Hôpital Civil de Strasbourg, France (ID023, ID033).

Mutation detection and analysis. For the EHC family, all 21 *ADCY5* exons were Sanger sequenced (table e-1 on the *Neurology*® Web site at Neurology.org). Exome sequences for the ChDys family were obtained as previously described[1] using SeqCap EZ Human Exome Library v3.0 (Roche NimbleGen; Madison, WI) capture and an Illumina (San Diego, CA) HiSeq 1000 system with paired-end 100-base reads. Clinical exome sequencing was performed at Baylor College of Medicine (Houston, TX) (ID016 and ID029), GeneDx (Gaithersburg, MD) (ID028), and ICM (Paris, France) (ID033 and ID035). Mutations in ID032 and case Ch4 were detected by molecular inversion probe (MIP) capture[5] (table e-1) and sequencing on an Illumina MiSeq system. For the remaining sporadic cases, we sequenced exons 2, 10, and 18.

To investigate shared ancestry in FDFM and EHC, we determined haplotypes surrounding *ADCY5*. We genotyped short tandem repeat markers (STRPs) as described[6] and sequenced portions of the introns flanking exons 5, 6, and 14, containing informative single nucleotide polymorphisms (SNPs) (figure 1A).

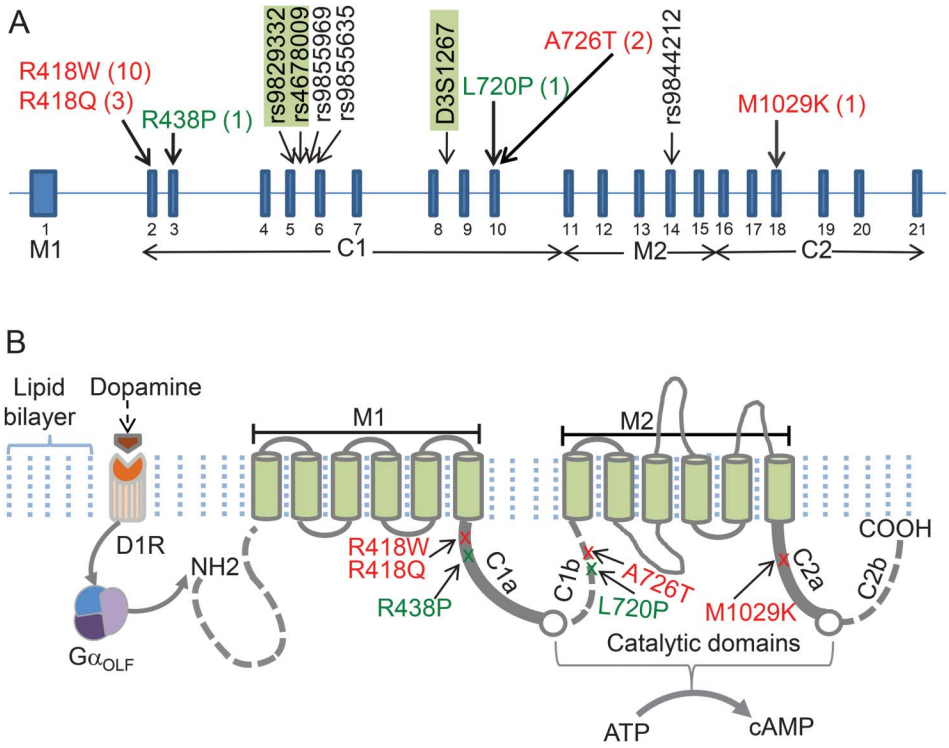
To investigate mosaicism for *ADCY5* mutations, we performed allele-specific PCR amplification with primers containing either the reference sequence or the mutant nucleotide in the 3′-position and a mismatched nucleotide in the preceding position. A common reverse primer was used for both alleles. PCR products were visualized and sequenced.

RESULTS Selected case descriptions. All cases are summarized in table 1.

Simplex cases. *ID016 (p.R418W).* Extreme irritability and exaggerated Moro reflex were noted at 1 week and continued throughout the first year. Hypotonia was apparent by 6 months and motor milestones were significantly delayed. The patient sat at 18 months and walked at 3 years. At 1 year, sudden severe limb movements during sleep developed and subsequently increased in frequency and severity. Similar daytime movements, occasionally painful, sometimes were severe enough to cause falls from her stroller. Minor stresses such as viral infections provoked multiweek episodes. Examination at age 8 years showed normal cognition, marked dysarthria, and moderately severe axial hypotonia especially of the neck. Gait was slow, uncoordinated, and jerky, with a tendency to veer to the side and walk in a circle. She fell frequently. At rest, there were almost continuous sudden myoclonic-like jerks of limbs or trunk. Tendon reflexes were brisk, with normal or occasionally upgoing plantar reflexes.

Several brain MRIs had normal results. Video EEG showed bifrontal irregular bursts of 3–4 Hz delta activity sometimes associated with spike and polyspike activity that did not clearly correlate with muscle jerks and dystonic movements, but one episode of rhythmic head bobbing corresponding with EEG evidence of a seizure was recorded. Comprehensive metabolic evaluation showed only borderline pyruvate and complexes I and III OXPHOS enzyme activities. Despite normal *POLG1* sequence (Medical Neurogenetics, Atlanta, GA) and absence of mitochondrial DNA mutations, a diagnosis of possible myoclonic epilepsy related to mitochondrial OXPHOS disorder was given. Numerous medications were trialed, including CoQ10, carnitine, amantadine, carbamazepine and levodopa-carbidopa. Current medications are levetiracetam, levocarnitine, clonazepam, and melatonin, with moderate control of abnormal movements.

Figure 1 Organization of *ADCY5* and locations of mutations



(A) Schematic of *ADCY5* exons shows the number of families with each mutation (in parentheses) and the positions of some intragenic short tandem repeat markers (STRPs) and single nucleotide polymorphisms included in the haplotype analysis of the EHC and FDFM families. Markers in green rectangles define distinct haplotypes not shared by the 2 families. Genotypes of flanking STRPs D31271, D3S1278, D3S3606, D3S1292, and D3S1569 were also used (not shown). Mutations seen in multiple affected persons are in red and those identified only in a single individual are in green. In Chen et al.,[2] p.R418 was placed near the end of the sixth helix of the first transmembrane domain; the current consensus is that this residue is in the C1a domain. (B) ADCY5 protein contains 2 membrane-spanning and 2 major bipartite cytoplasmic domains. Binding of dopamine to the excitatory dopamine 1 receptor (D1R) activates the striatal stimulatory heterotrimeric G protein $G\alpha_{OLF}$ that in turn activates ADCY5. On activation, the C1a and C2a regions of ADCY5 form a catalytic pocket in which conversion of adenosine-5′-triphosphate (ATP) binding to 3′,5′-cyclic adenosine monophosphate (cAMP) occurs. EHC = essential hereditary chorea; FDFM = familial dyskinesia with facial myokymia. Modified from Chen et al.[2] with permission (copyright © 2014 American Neurological Association).

ID027 (p.R418W mosaic). A partial description of this case (denoted UW1) is published.[2] Early motor milestones were delayed. Involuntary trunk and limb movements developed at age 5 years. Bouts of movements lasted days or weeks, followed by long quiescent periods. For example, on examination at age 19 years, there were frequent spontaneous dystonic arm, leg, and trunk movements, sustained dystonic posturing of the trunk and legs while walking, and moderately severe dysarthria (video 1). Video EEG documented the movements but showed normal EEG activity. Two months later, gait and speech were normal, there were no adventitious movements, and the examination elicited only occasional slight hesitation in rapid alternating movements and finger-to-nose testing. Tendon reflexes and brain MRI were normal. The patient is one of 5 cases in this study with cognitive deficits. During early childhood, her IQ was estimated at 60, and at 18 she scored 19/30 on the Montreal Cognitive Assessment.

Familial cases. *The EHC family (p.A726T).* This family (figure 2A) was first reported in 1976 as essential benign chorea.[7] Clinical screening of the BCH1 gene *TITF/NKX2-1*[8] was negative. Manifestations are consistent and detailed descriptions of some affected individuals in the EHC family, including IV-5, have been published.[7,9] Episodic jerky movements of the face, upper extremities, and feet began between ages 1 and 5 years. Tendon reflexes were hyperactive with flexor plantar responses, but sometimes normalized in adulthood. Muscle tone was normal. Movements worsened with stress, were socially embarrassing, and were not considered benign by the family. Manifestations were nonprogressive and in at least one individual, III-4, diminished substantially with age. Chlordiazepoxide, phenobarbital, or clonazepam had no benefit. Recent EMG in individual IV-5 showed neither facial myokymia nor peripheral neuropathy, but random generation of motor unit action potentials corresponding to the facial muscle twitches. Video 2 shows EHC IV-5 at 44 and 57 years.

The ChDys family (p.M1029K). Individual II-2 (figure 2B) was first described at age 19 years in 1967 as sporadic paroxysmal choreoathetosis without EEG

Table 1 Clinical features in families with *ADCY5*-related dyskinesia

Family or case	Mutation	No. generations	No. affected and examined (total affected)	Sex, F/M	Age at onset, y	Current age, y	Age at death, y	Cognition/ behavior	Abnormal movements and speech disturbance[a]	Facial twitches	Nocturnal episodes	Painful movements	Paroxysmal episodes[b]	Ambulations	Axial hypotonia	Deep tendon reflexes
FDFM[1,2,6,12]	A726T	5	10 (18)	7/11	2.5–19	20–60	14–81	Normal	Brief jerks hands, limbs, trunk	+	–	–	–	U	–	↑
EHC[7,9,30]	A726T	4	8 (12)[c]	7/5	1–5	4–56	47–75	Normal	Brief jerks hands, limbs, trunk	+	–	–	–	U	–	↑
ChDys[10,11]	$M1029K_{mos}$	3	1	1/0	2	70		Normal	Dystonia, chorea	–	+	–	+	U	–	Normal
	M1029K		3	3/0	0.5	20, 22, 46		Mild ↓/P	Dystonia, chorea	+	+	+	–	C	+	↑
ID019B	$R418W_{mos}$	2	1	0/1	6	71		Normal	Dystonia only	+	+	–	+	U	+	Normal
ID019	R418W		1	1/0	1	37		Normal	Dystonia, myoclonus, chorea, dysarthria	+	+	+	+	U	+	Normal
ID1[2]	R418W	1	1	1/0	0.5	18		Normal	Chorea, dystonia, myoclonus, dysarthria	+	+	+	+	C/W/L	+	↑
ID016	R418W	1	1	1/0	0.5	9		Normal	Brief jerks/myoclonus	–	+	+	+	U	+	↑
ID029	R418W	1	1	0/1	0.5	5		Normal	Myoclonus, chorea	+	+	+	–	W	+	↓
ID032	R418W	1	1	0/1	1.25	8		Mild ↓	Dystonia, myoclonus, chorea, dysarthria	+	+	+	+	L	+	↑
ID023	R418W	1	1	0/1	0.5	25		Normal	Dystonia, myoclonus, chorea, dysarthria	+	+	+	+	W	+	Normal
ID020	$R418W_{mos}$	1	1	0/1	4	7		Normal	Dystonia, chorea	–	+	–	+	U	+	Normal
ID021	$R418W_{mos}$	1	1	0/1	1.3	11		Normal	Dystonia, myoclonus	+	–	–	+	U	+	Normal
ID027[d,2]	$R418W_{mos}$	1	1	1/0	1	20		Mild ↓	Dystonia, brief jerks limbs, trunk	–	+	–	+	U	+	Normal
Ch4	$R418W_{mos}$	1	1	0/1	<20[e]	42		Normal/P	Dystonia, chorea	+	–	–	–	U	–	↑
ID018	R418Q	1	1	1/0	0.2	22		Normal	Myoclonus, chorea, dysarthria	+	–	–	+	U	+	Normal
ID028	R418Q	1	1	1/0	1.0	14		Mild ↓	Chorea, myoclonus, dystonia, mild dysarthria	+	+	–	–	U	+	↑
ID024	$R418Q_{mos}$	1	1	0/1	1.5	13		Mild ↓	Chorea	–	–	–	+	U	–	Normal
ID033	L720P[f]	1	1	0/1	0.2	29		Normal	Myoclonus, dystonia, dysarthria	–	–	–	+	U	+	Normal
ID035	R438P[f]	1	1	1/0	2	34		Normal	Tremulous dystonia	–	–	–	+	U	+	Normal

Abbreviations: C = wheelchair; ChDys = chorea-dystonia family; EHC = essential hereditary chorea family; FDFM = familial dyskinesia with facial myokymia; mos = mosaic for the mutation; L = limited, can walk short distances unassisted; P = psychosis with auditory hallucinations in 1 family member; U = unassisted; W = walker.

[a] Waxing and waning of types, frequency, and severity of abnormal movements characterize many of these individuals.

[b] Episodes of dramatic worsening that can last minutes to hours and have a definable abrupt onset and end.

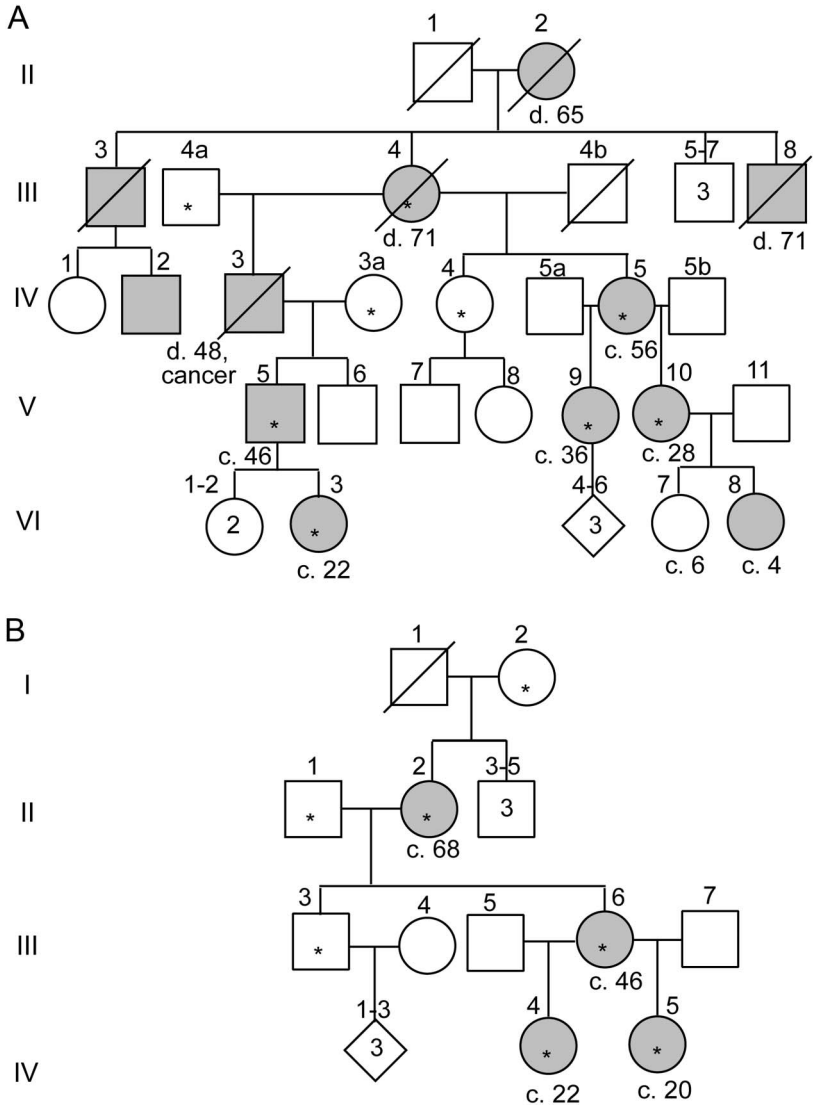
[c] Detailed clinical data of 3 affected individuals in the EHC family[9] and 6 affected individuals in the FDFM family[1,12] have been published previously.

[d] UW1 in Chen et al.[2]

[e] The patient was psychotic at the time of ascertainment and is lost to follow-up.

[f] Until corroboration in other cases or by functional studies, these are classified as variants of uncertain significance.

Figure 2 Pedigrees of 2 families with mutations in *ADCY5*



Asterisks denote individuals who provided DNA samples and all of these who were affected were examined. The family-specific mutation segregated completely with affectation status; no nonmanifesting carriers were identified. Age at death (d) or current age (c) is shown. (A) Family EHC with autosomal dominant essential chorea and p.A726T *ADCY5* mutation, updated from the diagram published in 1976.[7] Medical records of individuals II-2 and III-3 had been reviewed and individual IV-3 had been examined previously.[7] Individual VI-8 was examined but not sampled. Clinical descriptions of III-4, IV-5, and V-5 were detailed previously (denoted II-3, III-5, and IV-2).[9] (B) Family ChDys, first described in 1967, with severe chorea and dystonia and p.M1029K *ADCY5* mutation. In individual II-2, the mutation was detectable only by allele-specific amplification. EHC = essential hereditary chorea; ChDys = chorea-dystonia.

abnormality.[10] Choreiform movements with dystonic trunk posturing began at 6 months, occurred as frequently as 20 times per day, and could last for hours and be so severe that the patient was unable to stand. Anxiety or sudden activity but not passive motion precipitated episodes. However, she also had asymptomatic periods of several days. Chlordiazepoxide decreased attack frequency, but diphenylhydantoin, phenobarbital, reserpine, ethosuximide, imipramine, and haloperidol were unhelpful and diphenhydramine worsened the movements. In the 3rd decade, gradual improvement in frequency and severity of episodes occurred.[11] Neurologic examination at age 30 years had normal results except for mild jerks of trunk, hands, and face. By age 56, the patient had only subtle truncal jerks, and by age 68, her neurologic examination was essentially normal.

The proband's daughter, III-6, had hypotonia without pathologic reflexes. Motor milestones were delayed. She crawled at 2 years and walked at 2.5 years. By age 4 years, she manifested dysarthria and marked chorea of all extremities, face, and tongue.[11] Movements were continuous while awake and absent during sleep. In contrast to her mother, disease progressed, and by age 8 years, she had severe movements, poor head control, and contractures of knees and ankles, and required a wheelchair. Tendon reflexes were brisk with ankle clonus. In her 20s, triggers such as stress, hunger, and heat precipitated painful episodes of arm flexion and internal rotation and hip flexion, with limited responsiveness for up to 30 minutes despite remaining alert. Unpredictable episodes of severe jerks and spasms continued, as did frequent dystonic tongue thrusting and severe dysarthria. In her 40s, she was hospitalized with a psychotic depression, and she has had several recurrences of psychosis with delusions and auditory hallucinations. She graduated from high school in special education, but at age 50 years had a Mini-Mental State Examination score of 16/26. Brain MRI was normal.

The granddaughters (IV-4 and IV-5) are now 22 and 20 years old. They were hypotonic from infancy, with delayed motor milestones. IV-4 never walked. IV-5 at age 8 had bilateral ankle-foot orthotics and used a wheelchair at school. At 8 and 9 years, respectively, they had marked dystonia and jerky movements of the trunk and all limbs. Muscle tone was decreased but episodically increased with movements. Upper extremity tendon reflexes were normal but there were unsustained ankle clonus and variable Babinski responses. In IV-4, brain MRI at age 1 year was normal, as were muscle and skin biopsies at age 3. Their intellectual abilities were considered normal and they graduated from high school in special education primarily because of their movement disorder. Video 3 shows patient IV-4 at 8 and 20 years.

FDFM (p.A726T). Clinical descriptions of this family, with essentially the same manifestations as EHC, were published, with detailed descriptions of 7 affected people.[6,12] In 2014, repeat hand and face EMG of IV-4[6] showed frequent involuntary bursts of motor units provoked by needle insertion and volition, similar to those in EHC IV-5. These bursts were not typical of myokymic discharges because they were often stimulus provoked, highly variable in duration, and not rhythmic. Most EEGs

in this family have been normal but spike and polyspike activities were recorded in 2 individuals, one treated with mysoline.[12] Various anxiolytics and antiepileptics have produced no improvement (e.g., chlordiazepoxide, primidone, amitriptyline, trifluoperazine, diphenylhydantoin). Acetazolamide and propranolol have been of modest anecdotal benefit in a few persons.

Detection of *ADCY5* mutations and investigation of mosaicism. In EHC, Sanger sequencing revealed the c.2176G>A, p.A726T mutation previously found in FDFM. To investigate the possibility of a shared ancestor in FDFM, of German ancestry, and EHC, of English ancestry, we genotyped one intragenic and 5 flanking STRPs and 5 intronic SNPs. Distinct haplotypes span a 28-kb region containing *ADCY5*, demonstrating that the mutations arose independently (figure 1A).

In the ChDys family, we sequenced 6 exomes (figure 2B) and prioritized rare nonsynonymous variants carried by all 4 affected individuals. *ADCY5* was absent from this list of genes. However, direct review revealed a missense mutation (c.3086T>A, p.M1029K) in 3 affected individuals, but not II-2. Coverage of this position was poor (range 5–9 reads) and marked by the GATK strand-bias filter. The mutation affects a highly conserved residue (PhastCons[13] 1.0 and GERP[14] 4.53), has a nonconservative Grantham score[15] of 95, is not present in the Exome Variant Server or the 1000 Genomes database, and is classified as possibly damaging by PolyPhen2[16,17] (0.576) and deleterious by SIFT[18] (0.02). Given the clinical history of II-2, we considered the possibility of somatic mosaicism. Sanger sequencing of exon 18 in DNA from uncultured peripheral blood and skin again detected only the wild-type allele. Allele-specific amplification followed by sequencing revealed the mutant allele in blood, skin, and cultured fibroblasts, confirming that she is mosaic (figure 3A).

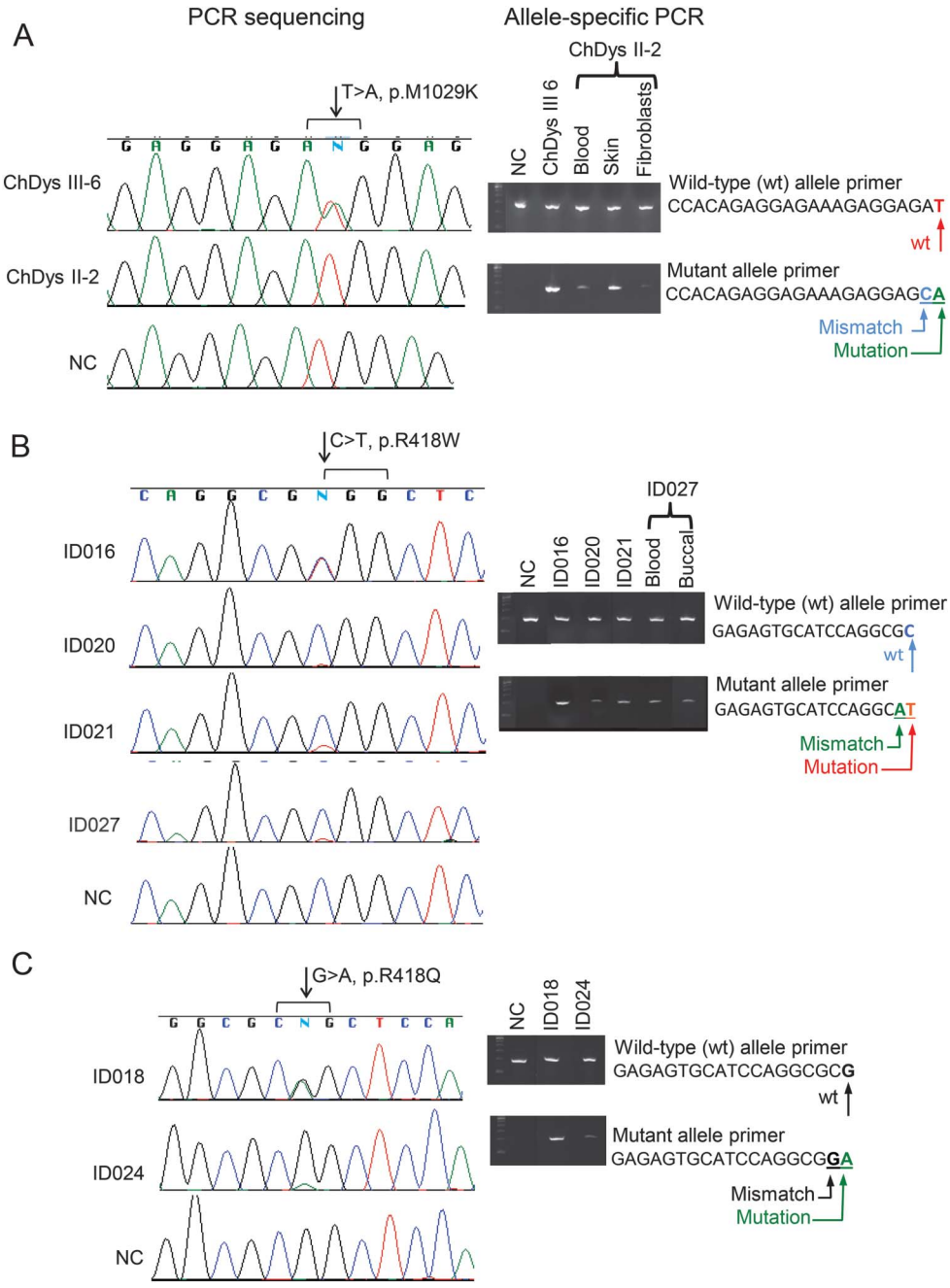
We previously reported a de novo *ADCY5* missense mutation, c.1252C>T, p.R418W, in ID1 and ID027.[2] Whereas the exomes of ID1, ID016, and ID029 contained the variant allele in approximately half the reads, strand bias (7 variants of 32 reads) in ID027 raised the possibility that the patient might be mosaic for the mutation. In the clinical laboratory, Sanger sequencing using 2 sets of primers ruled out uneven allele amplification due to primer placement. Sequence electropherograms of exon 2 documented the lower peak height of the variant T compared to that of wild-type C, whereas these were equal in ID016, who carries the same mutation (figure 3B). The mutation was not carried by the parents of ID016 or ID029. Targeted sequencing of the 3 exons that contained the known mutations showed that ID019, ID020, ID021, and ID023 also carry p.R418W. The very low signals for the variant nucleotide in ID020, ID021, and the more mildly affected father of ID019 were confirmed by allele-specific amplification to reflect somatic mosaicism (figure 3B). MIP capture and sequencing identified p.R418W in ID032 and reported 1 variant read of 18 in Ch4. A very low level mosaicism was confirmed in Ch4 by allele-specific amplification only with overexposure of the blot. His MRI showed decreased signal intensity in globus pallidus.

Three individuals carried a different de novo mutation in the same residue, p.R418Q. Exome sequencing of ID028 obtained 8 variant and 18 reference reads; however, by Sanger sequencing, the heights of the mutant and reference nucleotide peaks were equal at the clinical laboratory and in our laboratory. Targeted sequencing identified the mutation in ID018 and ID024. Allele-specific amplification demonstrated mosaicism in ID024 (figure 3C). By clinical exome sequencing in patients with dystonia, we identified 2 novel de novo *ADCY5* variants, p.L720P (ID033) and p.R438P (ID035), predicted to be pathogenic by Sift and PolyPhen and not found in the ExAC database. Figure 1B shows locations of mutations and variants in protein domains.

DISCUSSION We show that *ADCY5* mutations in the cytoplasmic domains cause a childhood-onset mixed hyperkinetic movement disorder with no or slow progression. Prior to molecular characterization, the varied character of the movement semiology, which may include chorea, dystonia, or myoclonus, sometimes affecting the face, led to diagnoses such as benign hereditary chorea, paroxysmal dyskinesia, cerebral palsy, or mitochondrial disease. Clues to the diagnosis are as follows: (1) axial hypotonia, sometimes accompanied by weakness; (2) facial chorea or dystonia; (3) nocturnal paroxysmal dyskinesia, often pronounced; (4) movement-related pain; (5) dramatic fluctuations in frequency and severity of movements; (6) no or mild cognitive impairment; (7) normal MRI; and (8) little or no progression. Some patients have paroxysms that can last for minutes to weeks, with or without provocation by movement. Other triggering factors have been prolonged periods of inactivity, fatigue, intercurrent illness, or other stressor. The waxing-waning pattern makes it difficult to attribute exacerbations or remissions to environmental factors or medications. However, clonazepam markedly reduced the nighttime sleep-disrupting myoclonic episodes in several patients and levetiracetam reduced movements in a few.

Although there is variability, a pattern of phenotypes associated with mutations in different domains

Figure 3 Allele-specific amplification documents mosaicism of *ADCY5* mutations in representative cases



Wild-type (wt) alleles (top panels) were amplified using wt-primer and mutant alleles (lower panels) amplified using primers containing the mutant nucleotide in the 3′ position and one mismatched nucleotide in the preceding position. (A) Sequence chromatograms for 2 members of the ChDys family. The mutant nucleotide A is detected in patient III-6 but not in her affected mother II-2. Allele-specific PCR in II-2 amplified the mutant allele from blood, skin, and fibroblast cells. The analysis is not quantitative, but the very faint mutant band in fibroblasts suggests a selective advantage for the wt allele during culture. (B) On the sequence chromatograms, in contrast to C and T peaks of equal intensity in ID016, much smaller signals are present for the mutant T allele in ID020, ID021, and ID027. Presence of the mutant allele was confirmed for all by allele-specific amplification. (C) Mosaicism for the mutant A nucleotide is shown for ID024, in contrast to the peaks of equal height for the wt and mutant alleles in ID018. In all panels, for the mosaics, the faint gel signals are consistent with the small mutant allele peaks in the chromatograms. Absence of a mutant allele in a normal control (NC) demonstrates the specificity of the allele-specific amplification. ChDys = chorea-dystonia.

emerged. C1b domain mutation A726T is associated with the mildest disease. The myokymia thought to characterize FDFM could not be confirmed on review of the original EMG tracings, nor was it observed on repeat study or in an individual in the EHC family with similar movements. These facial movements are likely chorea or dystonia. In the ChDys family with a p.M1029K mutation in C2a very near the

catalytic domain, the 3 nonmosaic mutation carriers have had severe generalized unremitting dystonia and chorea, without myoclonic movements. Mutations in C1a and C2a cause moderate to severe disorders. It is of note that 13 of the 14 C1a mutations are de novo and 6 of these are mosaic. Mosaicism often results in milder disease that may remit for extended periods. Among the 14 cases with residue 418 mutations, 78% had onset in infancy, 71% have nocturnal movements, 79% have paroxysmal worsening, 86% have axial hypotonia, 43% have painful dystonia, 71% have facial movements, and 36% have increased reflexes.

Axial hypotonia is a common finding in patients with all the mutations, except those in C1b. The hypotonia is presumably central in origin, a process that has been reported to affect the axial muscles as well as the limbs, although strength is usually preserved.[19] Study of more families will be needed to determine if the seizures documented in 3 individuals, the mild cognitive impairment in 5, the psychosis in 2, or the cardiomyopathy in 1[1] are part of the disease spectrum.

The range of disease severity related to mutation location may reflect the function of *ADCY5* domains. On activation, C1a and C2a form the catalytic ATP–binding pocket. The C2b domain may affect this activity less directly. The mutational hot spot suggests arginine 418 has an important functional role.

Altered striatal dopamine signaling is one hypothesis for various forms of dystonia, including defects in the biosynthesis or transport of dopamine,[20] DYT1 *Torsin A*-related dystonia,[21,22] DYT25 *GNAL*-related dystonia,[23,24] and adult-onset focal dystonia.[25] ADCY5 is the principal adenylate cyclase integrating signals from multiple receptors including D1 and D2 striatal dopamine receptors.[26] Based on increased cAMP accumulation in our functional studies of 2 *ADCY5* mutations[2] and the decrease in spontaneous movement in *ADCY5*-null mice,[4] we suspected that increased adenylate cyclase activity might be a pathophysiologic factor in *ADCY5*-related dyskinesia. Recently, however, a splice site mutation in *ADCY5* was identified in a father and son with chorea and dystonia.[27] It is difficult to reconcile the similar manifestations caused by this loss-of-function mutation and the possible gain-of-function missense mutations in our patients. The missense mutations may have a different effect in vivo than what we observed in vitro. Functional studies in neuronal cells may shed light on this question.

To encompass the broader phenotype, we suggest changing the name from FDFM to *ADCY5*-related dyskinesia.[28] *ADCY5*-related dyskinesia should be considered in patients without a diagnosis who manifest any of the cardinal features, whether episodic or constant, and with or without a positive family history. With the caveat that pathogenicity of the 2 novel variants requires corroboration, with 18 families described herein and 3 other published families,[27,29] it appears this disorder is less rare than initially thought. As additional families are found, the full spectrum of mutations and their relationship to phenotype will be elucidated. Increased understanding of the pathogenesis of *ADCY5*-related dyskinesia will facilitate development of pharmacologic treatments for all forms of dystonia that involve the same or related pathways.

WEB-BASED RESOURCES

Exome Variant Server (EVS): evs.gs.washington.edu/

Exome Aggregation Consortium (ExAC): exac.broadinstitute.org/

PolyPhen-2 (Polymorphism Phenotyping v2): http://genetics.bwh.harvard.edu/pph2/

Sorting Intolerant From Tolerant: http://sift.jcvi.org/www/SIFT_intersect_coding_submit.html

AUTHOR CONTRIBUTIONS

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REFERENCES

1. Chen YZ, Matsushita MM, Robertson P, et al. Autosomal dominant familial dyskinesia and facial myokymia: single exome sequencing identifies a mutation in adenylyl cyclase 5. Arch Neurol 2012;69:630–635.
2. Chen YZ, Friedman JR, Chen DH, et al. Gain-of-function ADCY5 mutations in familial dyskinesia with facial myokymia. Ann Neurol 2014;75:542–549.
3. Hanoune J, Pouille Y, Tzavara E, et al. Adenylyl cyclases: structure, regulation and function in an enzyme superfamily. Mol Cell Endocrinol 1997;128:179–194.
4. Iwamoto T, Okumura S, Iwatsubo K, et al. Motor dysfunction in type 5 adenylyl cyclase-null mice. J Biol Chem 2003;278:16936–16940.
5. O'Roak BJ, Stessman HA, Boyle EA, et al. Recurrent de novo mutations implicate novel genes underlying simplex autism risk. Nat Commun 2014;5:5595.
6. Raskind WH, Matsushita M, Peter B, et al. Familial dyskinesia and facial myokymia (FDFM): follow-up of a large family and linkage to chromosome 3p21-3q21. Am J Med Genet B Neuropsychiatr Genet 2009;150B:570–574.
7. Bird TD, Carlson CB, Hall JG. Familial essential ("benign") chorea. J Med Genet 1976;13:357–362.
8. Breedveld GJ, van Dongen JW, Danesino C, et al. Mutations in TITF-1 are associated with benign hereditary chorea. Hum Mol Genet 2002;11:971–979.
9. Fernandez M, Raskind W, Matsushita M, Wolff J, Lipe H, Bird T. Hereditary benign chorea: clinical and genetic features of a distinct disease. Neurology 2001;57:106–110.
10. Perez-Borja C, Tassinari AC, Swanson AG. Paroxysmal choreoathetosis and seizures induced by movement (reflex epilepsy). Epilepsia 1967;8:260–270.
11. Bird TD, Carlson CB, Horning M. Ten year follow-up of paroxysmal choreoathetosis: a sporadic case becomes familial. Epilepsia 1978;19:129–132.
12. Fernandez M, Raskind W, Wolff J, et al. Familial dyskinesia and facial myokymia (FDFM): a novel movement disorder. Ann Neurol 2001;49:486–492.
13. Siepel A, Bejerano G, Pedersen JS, et al. Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. Genome Res 2005;15:1034–1050.
14. Cooper GM, Stone EA, Asimenos G, Green ED, Batzoglou S, Sidow A. Distribution and intensity of constraint in mammalian genomic sequence. Genome Res 2005;15:901–913.
15. Grantham R. Amino acid difference formula to help explain protein evolution. Science 1974;185:862–864.
16. Sunyaev S, Ramensky V, Koch I, Lathe W III, Kondrashov AS, Bork P. Prediction of deleterious human alleles. Hum Mol Genet 2001;10:591–597.
17. Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. Nucleic Acids Res 2002;30:3894–3900.
18. Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res 2003;31:3812–3814.
19. Peredo DE, Hannibal MC. The floppy infant: evaluation of hypotonia. Pediatr Rev 2009;30:e66–e76.
20. Marecos C, Ng J, Kurian MA. What is new for monoamine neurotransmitter disorders? J Inherit Metab Dis 2014;37:619–626.
21. Asanuma K, Ma Y, Okulski J, et al. Decreased striatal D2 receptor binding in non-manifesting carriers of the DYT1 dystonia mutation. Neurology 2005;64:347–349.
22. Zhang L, McCarthy DM, Sharma N, Bhide PG. Dopamine receptor and Galpha(olf) expression in DYT1 dystonia mouse models during postnatal development. PLoS One 2015;10:e0123104.
23. Fuchs T, Saunders-Pullman R, Masuho I, et al. Mutations in GNAL cause primary torsion dystonia. Nat Genet 2013;45:88–92.
24. Vemula SR, Puschmann A, Xiao J, et al. Role of Galpha (olf) in familial and sporadic adult-onset primary dystonia. Hum Mol Genet 2013;22:2510–2519.
25. Berman BD, Hallett M, Herscovitch P, Simonyan K. Striatal dopaminergic dysfunction at rest and during task performance in writer's cramp. Brain 2013;136:3645–3658.
26. Lee KW, Hong JH, Choi IY, et al. Impaired D2 dopamine receptor function in mice lacking type 5 adenylyl cyclase. J Neurosci 2002;22:7931–7940.

27. Carapito R, Paul N, Untrau M, et al. A de novo ADCY5 mutation causes early-onset autosomal dominant chorea and dystonia. Mov Disord 2015;30:423–427.
28. Shaw C, Hisama F, Friedman J, Bird TD. *ADCY5*-related dyskinesia. In: Pagon RA, Adam MP, Ardinger HH, et al, eds. GeneReviews. Seattle, WA: 1993–2015. Available at: http://www.ncbi.nlm.nih.gov/books/NBK263441/.
29. Mencacci NE, Erro R, Wiethoff S, et al. *ADCY5* mutations are another cause of benign hereditary chorea. Neurology 2015;85:80–88.
30. Bird TD, Hall JG. Additional information on familial essential (benign) chorea. Clin Genet 1978;14:271–272.

ADCY5-related dyskinesia: Broader spectrum and genotype–phenotype correlations

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