MILANO BICOCCA UNIVERSITY

DOCTORATE SCHOOL

School of Medicine and Surgery

PhD PROGRAM IN MOLECULAR AND TRANSLATIONAL MEDICINE - DIMET

XXX CYCLE

The impact of Next Generation Sequencing in rare movement disorders diagnosis: results from a tertiary referral center.

Miryam Carecchio, MD

Matr. no. 798751

Tutor: Valeria Tiranti, PhD

Co-tutor: Nardo Nardocci, MD

Coordinator: Prof. Andrea Biondi

Academic year 2016/2017

To my pediatric patients,

and to their parents,

who taught me what love *is.*

Table of contents

Chapter 1..... 7

General introduction 7

Next Generation Sequencing (NGS)..... 7

NGS in movement disorders 11

Movement disorders 13

Dystonia 16

Genetics of dystonia 19

Chorea 22

Scope of the thesis 24

References 25

Chapter 2.....29

A missense mutation in *KCTD17* causes autosomal dominant myoclonus-dystonia

Chapter 3.....71

The *CACNA1B* R1389H variant is not associated with myoclonus-dystonia in a large European multicentric cohort

Chapter 4.....84

De novo mutations in PDE10A cause childhood-onset chorea with bilateral striatal lesions

Chapter 5.....115

Novel *GNAL* mutation with intra-familial clinical heterogeneity: expanding the phenotype

Chapter 6.....139

Recent advances in genetics of chorea

Chapter 7.....171

DYT2 screening in early-onset isolated dystonia

Chapter 8.....182

ADCY5-related movement disorders: frequency, disease course and phenotypic variability in a cohort of paediatric patients

Chapter 9.....209

Rare causes of early-onset dystonia-parkinsonism with cognitive impairment: a *de novo PSEN-1* mutation

Chapter 10.....222

A *PDE10A de novo* mutation causes childhood-onset chorea with diurnal fluctuations

Chapter 11.....229

Emerging monogenic complex hyperkinetic disorders

Chapter 12.....270

Summary 270

Conclusions and future perspectives 272

References 274

Publications 275

Acknowledgments.....279

Chapter 1

GENERAL INTRODUCTION

NEXT GENERATION SEQUENCING (NGS)

In the past few years, several innovative and powerful techniques for sequencing nucleic acids (DNA and RNA) have become available, rapidly spreading in various field of medical research, as well as in clinical practice. These methods, collectively referred to as Next Generation Sequencing (NGS) or second-generation sequencing, are progressively replacing traditional Sanger sequencing[1], also known as "first-generation sequencing", and have made possible the production of an unprecedent amount of DNA and RNA sequences at relatively low cost, contributing to an enormous expansion of our knowledge on several genetic diseases.

On the basis of the target region to be sequenced, it is possible to distinguish three different techniques[2,3]:

1) Whole Genome Sequencing (WGS), which is the most comprehensive approach, allowing the sequencing of the entire human genome;
2) Whole Exome Sequencing (WES), that allows the analysis of the exome, namely the coding part of the human genome (corresponding to ~2% of the entire genome);
3) Targeted sequencing, a method aimed at sequencing panels of multiple known genes responsible for a specific disease or

group of diseases, either by using commercially available panels or specifically designed ones (customized panels).

Each of the above-mentioned NGS methods is characterized by specific chemistries, sample preparation protocols, and data analysis[4]. Overall, all NGS technologies share a basic conceptual workflow in which an initial high molecular weight DNA sample (eg, human genomic DNA) is shattered into a fragment library, and single strand molecules are amplified and sequenced in parallel.

First, DNA fragments are generated by mechanical or enzymatic methods. In exome sequencing and resequencing of customized gene panels, an additional crucial step involves the enrichment of the target DNA fragments (capturing). Libraries are then obtained and platform-specific adaptors are added to both ends of each fragment. This step allows the fragments to be more easily PCR amplified using just one pair of primers or to be hybridized to a surface using complementary adaptors. Then the sequences of the fragments are read cyclically and in parallel using different chemistries that results, in most cases, in fluorescent or electrical signals. Last, this signal is detected by an imaging or a different sensing system coupled with a computer, which allows one to simultaneously ascertain the sequences of millions of reads in parallel. The large amount of data generated is then processed using bioinformatic tools.

For DNA resequencing, bioinformatic analysis includes the alignment of the raw reads against the reference sequence alignment and the comparison of aligned reads against the reference to obtain a list of genomic variations (variant calling).

Subsequently, descriptive information is added to the identified variants (variant annotation)[5].

Candidate variants individuated by NGS are then prioritized applying bioinformatic filters that progressively reduce the number of potentially disease-causing variants (**Figure 1**).

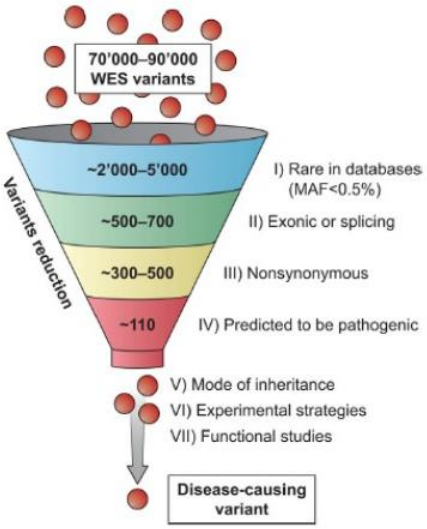


Figure 1. Schematic representation of a general filtering workflow for NGS variants (from Olgiati S. *et al*., 2016[5]).

The first filtering step consists in selecting only variants with a minor allele frequency (MAF) <1%, as variants causing human diseases transmitted in a mendelian fashion are usually rare. Subsequently, additional filtering strategies are applied to narrow down the candidate variants until a very limited number is left. These tools include filters that recognize nonsynonymous and splicing variants, the analysis of evolutionary conservation of the affected aminoacid throughout species

(based on the principle that a highly-conserved aminoacid is likely to have a relevant role for the protein function), *in silico* analyses (that predict functional consequences of a mutation at a protein level) and interrogation of publicly available population and disease-specific database (ExAc, gnomAD, LOVD, etc)[5]. This process allows to confidently identify and report single nucleotide variants (SNVs) and short insertions and deletions (Indels). Large genomic rearrangements and copy number variants (CNVs) can also be identified using dedicated software to analyze NGS data, but other techniques such as CGH-array and Multiplex Ligation-dependent probe Amplification (MLPA) are preferable and have a larger diffusion in diagnostic laboratories[6]. After all this process, variants surviving the filtering might still need a more precise characterization to conclusively define their pathogenicity with respect to the patient's clinical picture. Segregation of the variant with disease-status within the patient's family, replication of the same finding (i.e. same mutation or same mutated gene found in additional subjects with a consistent phenotype) and functional studies in cellular or animal models are often needed to fully support the pathogenicity of a specific variant. Most importantly, hypothesizing an *a priori* pattern of inheritance based on the patient's family tree is of great importance to filter variants in genes with a dominant or recessive inheritance. Yet, at the end of this long and complex process, available evidence may not be sufficient to discriminate whether a variant is pathogenic or not. In this case, variants will be classified as "variants of unknown significance" (VUS).

It is important to note that each filtering step and tool used has some limitations and may give rise to false positive or false negative results.

For this reason, well trained personnel is required when performing NGS and a tight and constant collaboration between the laboratory and clinicians is essential to reach definite conclusions and formulate a reliable diagnosis.

In fact, in clinical practice, when using customized gene panels, some apparently pathogenic variants in known disease-causing genes can emerge, yet the patient's clinical phenotype may be completely unrelated; for example, a possibly pathogenic variant in a dystonia-related gene with dominant inheritance may be individuated in a patient with classical Parkinson's disease. In this case, formulating a precise clinical diagnosis is of vital importance to exclude a role of the variant in the pathogenesis of the patient's disease and to attribute any significance to it. Also, a detailed family history is always of substantial importance when translating NGS results in clinical practice.

NGS IN MOVEMENT DISORDERS

The interpretation of NGS results in the clinical setting represents the modern and most challenging frontier of translational medicine, especially when the analysis of newly discovered genes becomes available for diagnostic purposes. In fact, clinicians not only need highly-qualified biologists to perform genetic analyses, but they must also regularly interact with the lab and play an active role in the interpretation of results. This implies continuous updates in clinical genetics, molecular biology techniques and, needless to say, the ability of clinicians to perform a deep phenotypic characterization of patients.

This is of even higher importance in the field of movement disorders, where a correct differential diagnosis and subsequent diagnostic workflow strictly relies on a detailed classification of patients' phenomenology (e.g. chorea vs dystonia).

NGS has had an enormous impact in the field of movement disorders for several different reasons. First, its application has allowed the discovery of a rapidly growing number of genes responsible for monogenic diseases, a mandatory step to understand underlying pathophysiological mechanisms and to develop future targeted therapies. Examples of recently-identified genes through NGS include *GNAL*, *ANO3*, *ADCY5*, and *PDE10A*[7-10]. Second, the phenotypic spectra of previously discovered genes have largely expanded (such as in dyskinetic-epileptic encephalopathies)[11], exceeding the initial descriptions, and sometimes apparently different diseases have been linked to a single gene (e.g. Rapid-Onset Dystonia Parkinsonism and Alternating Hemiplegia of Childhood linked to *ATP1A3* mutations)[12-14]. Third, NGS has offered the opportunity to perform comprehensive genetic analyses in patients affected by diseases caused by several different genes (hereditary spastic paraparesis, ataxias, motor neuron disease, fronto-temporal dementia), allowing clinicians to save time during the diagnostic work-up with a reasonably low cost as compared to the traditional gene-by-gene approach by Sanger sequencing. Nevertheless, if a patient displays a phenotype highly suggestive of a specific underlying genotype (e.g. DYT1-related dystonia; DOPA-responsive dystonia due to *GHC1* mutations) Sanger sequencing still results the preferable method to use in order to save time and reduce costs.

Given the genetic heterogeneity of the majority of movement disorders, especially dystonia, the advent of NGS has been of particular importance in this specific subfield of neurology.

Despite the profound changes that NGS has produced in clinical practice, the spreading of gene panels for movement disorders in routine diagnostics seems not to have significantly increased the proportion of patients receiving a molecular diagnosis so far. Despite a good cost profile and a shorter duration of the diagnostic workup, gene panel analysis led to a definite genetic diagnosis in only 14.8% of 61 patients affected by dystonia in a recent study by van Egmond *et al.*[15]. These results do not significantly differ from the diagnostic yield (11.4%) obtained in our lab in the past three years on a group of 221 patients with genetically undiagnosed movement disorders of various types analyzed by means of customized gene panels[16]. The large proportion of patients with movement disorders that are left without a genetic diagnosis even after NGS can be explained in several different ways, including technical limitations of NGS that can miss pathogenic variants (due to unsatisfactory depth of coverage or presence of large genomic deletions) and the existence of several, still unknown genes.

MOVEMENT DISORDERS

The term "movement disorders" refers to a heterogeneous group of neurological conditions characterized by the production of abnormal voluntary movements or involuntary movements. These conditions can have a genetic basis, or be secondary to various types of damage of the central nervous system with structural and functional alterations of the basal ganglia circuit and other cerebral regions. A large number of

movement disorders, such as Parkinson's disease, are thought to have a multifactorial etiology, with a genetic component that does not follow classical mendelian rules of inheritance.

From a clinical point of view, movement disorders are traditionally categorized in two groups, according to the main type observed on neurological examination: 1) hypokinetic movement disorders, characterized by an insufficient production of movement; and 2) hyperkinetic movement disorders, characterized by an excess of movement.

Hypokinetic movement disorders refer to parkinsonism, also called akinetic-rigid syndrome, that includes bradykinesia as an obligatory feature, muscular rigidity and often rest tremor and gait disturbances. The most common cause of parkinsonism in adulthood is Parkinson's disease, whereas in children parkinsonism is very rare and its differential diagnosis as well as some phenomenological features largely differ from adults. For example, rest tremor is rarely observed in children, whereas bradykinesia and loss of postural reflexes are the most common clinical features, often causing a delay in the achievement of motor milestones, that can be challenging to recognize.

Hyperkinetic movement disorders include five different categories:

1) Tremor, which is a rhythmic oscillation of a body part;
2) Tics, that are partially suppressible movements or vocalizations of different degrees of complexity;
3) Chorea, characterized by continuous and brief involuntary movements, typically flowing from one body part to another in an unpredictable way in terms of timing, speed and direction;
4) Myoclonus, characterized by brief, shock-like jerks;

5) Dystonia, characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive and patterned movements and/or postures[17].

Patients often present with a mixed movement disorder (e.g. dystonia and parkinsonism), in which case individuating the main movement disorder can be very helpful to establish a list of differential diagnoses and a diagnostic workup.

The research work I carried out during my PhD course was mainly focused on pediatric hyperkinetic movement disorders, especially chorea and dystonia.

NGS is particularly helpful in this age group; in fact, genetically-determined movement disorders are relatively frequent in the under-18 population, complex and partially overlapping phenotypes are frequently observed and a significant proportion of patients has no definite genetic diagnosis despite a long and complex diagnostic workup. Movement disorders with onset in infancy, childhood and adolescence can present alone or in combination with other neurological and systemic features that sometimes constitute syndromic associations suggestive of specific diagnoses (e.g. intellectual disability, post-natal microcephaly, epilepsy and movement disorders in congenital Rett syndrome due to *FOXG1* mutations)[18]. In clinical practice, however, it is not infrequent to see children affected by "pure" movement disorders with no identifiable cause.

DYSTONIA

Dystonia is a hyperkinetic movement disorder characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive movements, postures or both. Dystonic movements are typically patterned, twisting and may be tremulous. Dystonic tremor is typically jerky, variable in amplitude and worsened or brought about by specific positions or tasks. Dystonia is often initiated or worsened by voluntary action and associated with overflow muscle activation.

Several studies have assessed the prevalence of dystonia in the general population. A metanalysis by Steeves *et al*. calculated an overall prevalence of primary (isolated) dystonia of 16.43 per 100.000, but figures are likely to be underestimated[19].

Different classification systems for dystonia exist. Dystonia can be classified according to its body distribution (focal, multifocal, segmental, hemidystonia, generalized), age at onset (childhood vs adult onset), or etiology. Former etiological classification included four etiological categories:

1) Primary dystonia, if no identifiable cause of dystonia or evidence of neurodegeneration is present; primary dystonia can be further subdivided in pure, plus (in association with other signs) and paroxysmal and the cause is either genetic or unknown;
2) Secondary (or symptomatic) dystonia, if it is due to recognizable exogenous factors (e.g., perinatal injury, drugs, cerebral lesions, etc.);
3) Heredodegenerative, if dystonia is present as part of a widespread neurodegenerative syndrome; in such case, dystonia

is often accompanied by additional neurological signs and symptoms (pyramidal tract signs, parkinsonism, dementia, epilepsy, visual disturbances, etc).

In 2013 a new classification of dystonia was adopted[20], replacing the previous categories of primary, secondary and heredodegenerative dystonia. In the new classification system, dystonia is classified along two main axes: clinical characteristics (axis 1) and etiology (axis 2) (**Table 1**).

Clinical characteristics include age at onset, body distribution, temporal pattern and associated features. In the pediatric population, the distribution of dystonia at onset usually affects a leg or an arm and tends to generalize. The temporal pattern refers to the disease progression and the variability of dystonic symptoms over the day. The identification of a specific pattern of variability of dystonic symptoms is an important clue for diagnosis: diurnal fluctuations strongly suggest dopa-responsive dystonia, a paroxysmal occurrence suggests paroxysmal dyskinesias. According to the current classification the term *isolated* dystonia refers to conditions where dystonia is the only motor feature apart from tremor; the association with another movement disorders such as parkinsonism or myoclonus, or other neurologic or systemic disorders distinguishes the *combined* dystonias.

According to etiology, dystonia is classified on the basis of the underlining brain pathology into degenerative, non-degenerative or without structural lesions; it is defined inherited (with various patterns), acquired (due to various brain lesions or insult) or idiopathic (sporadic or familial), due to unknown cause.

Axis I *Clinical characteristics*	**Axis II** *Etiology*
Clinical characteristics of dystonia **Age at onset** - Infancy (birth to 2 years) - Childhood (3–12 years) - Adolescence (13–20 years) - Early adulthood (21–40 years) - Late adulthood (>40 years) **Body distribution** - Focal - Segmental - Multifocal - Generalized (with or without leg involvement) - Hemidystonia **Temporal pattern** - Disease course - Static - Progressive **Variability** - Persistent - Action-specific - Diurnal - Paroxysmal **Associated features** *Isolated dystonia or combined with another movement disorder* - Isolated dystonia - Combined dystonia *Occurrence of other neurological or systemic manifestations* - List of co-occurring neurological manifestations	**Nervous system pathology** Evidence of degeneration Evidence of structural (often static) lesions No evidence of degeneration or structural lesion **Inherited or acquired** *Inherited* - Autosomal dominant - Autosomal recessive - X-linked recessive - Mitochondrial *Acquired* - Perinatal brain injury - Infection - Drug - Toxic - Vascular - Neoplastic - Brain injury - Psychogenic *Idiopathic* - Sporadic - Familial

Table 1. Current classification of dystonia (from Albanese A. *et al.*[20])

GENETICS OF DYSTONIA

The first gene to be causally link to isolated dystonia was *TOR1A* (DYT1) in 1997[21]. An in-frame 3-bp deletion (GAG) in this gene, encoding the protein TorsinA, was initially found as a frequent cause of childhood-onset generalized dystonia in the Ashkenazi Jewish population, where its frequency reaches 1:9000 cases[22]. This mutation, causing a single glutamic acid residue loss, has been demonstrated to recur independently also in different ethnic groups, sometimes arising *de novo*, and is transmitted as an autosomal dominant trait with reduced penetrance (30%)[23].

In the past 20 years, the list of genes associated with isolated and combined dystonia as well as other hyperkinetic movement disorders, mainly with onset in childhood, has enormously expanded, with a marked acceleration in the past 5 years thanks to the advent of NGS. The list of DYT loci is continuously being updated, and *KMT2B*, the most recently discovered dystonia-related gene, was assigned the DYT28 locus at the beginning of 2017[24,25].

A comprehensive review of all the genes responsible for isolated and combined dystonia and their clinical characteristics goes beyond the scope of the present thesis.

An updated overview of all the genes causally linked to dystonia with their core phenotypic features is provided in **Table 2**.

Type of dystonia	Disease (MIM)	Gene	Locus	Main clinical features	MOI
Isolated	DYT1 (128100)	*TOR1A*	9q34	Childhood or adolescent-onset in the lower limbs, generalization with caudo-cranial gradient, sparing of oro-mandibular and laryngeal region	AD
	DYT2 (224500)	*HPCA*	1p35.1	Early-onset generalized dystonia with slowly progressive course and marked involvement of upper body in adulthood	AR
	DYT4 (128101)	*TUBB4A*	19p13.12-13	Adult-late onset laryngeal dysphonia progressing to generalized dystonia; characteristic "hobbyhorse" ataxic gait	AD
	DYT6 (602629)	*THAP1*	8p11.21	Adolescent-onset, initial cranial-cervical involvement, subsequent generalization with minor involvement of lower limbs	AD
	DYT13 (607671)	-	1p36.32-p36.13	Prominent cranial-cervical and arm involvement, occasional generalization (single Italian family)	AD
	DYT23 (614860)	*CIZ1*	9q34	Adult onset isolated cervical dystonia	AD
	DYT24 (615034)	*ANO3*	11p14.2	Adult-onset, focal or segmental distribution, dystonic tremor and myoclonic jerks can be prominent	AD
	DYT25 (615073)	*GNAL*	18p11	Mostly adult-onset, focal (cervical) or segmental dystonia	AD
	DYT27 (616411)	*COL6A3*	2q37.3	Early-onset (20 years) focal/segmental dystonia in the craniocervical region and upper limbs	AR
	DYT28 (617284)	*KMT2B*	19q13.12	Early-onset, generalized dystonia with initial lower limb involvement and marked spreading to oro-mandibular and laryngeal regions, possible additional features (low IQ, short stature, mild dysmorphisms)	AD

Type of dystonia		Disease (MIM)	Gene	Locus	Main clinical features	MOI
Combined	Dystonia-parkinsonism	DYT5a (218230)	*GCH1*	14q22.2	Dopa-responsive dystonia. Dystonia and parkinsonism with circadian fluctuations; excellent response to LD	AD, AR
		DYT5b (605407)	*TH*	11p15.5	Dopa-responsive dystonia. Delayed milestones, reduced IQ, dysautonomic features, oculogyric crises	AR
		Not assigned	*SPR*	2p13.2	Dopa-responsive dystonia, psychomotor retardation	AR
		DYT3 (314250)	*TAF1*	Xq13.1	Dystonia-parkinsonism; frequent in Philippines	XL
		DYT16 (612067)	*PRKRA*	2q31.2	Generalized dystonia with minor parkinsonian features. Prominent oro-facial and cervical involvement	AR
		DYT12 (128235)	*ATP1A3*	19q13.2	Rapid-onset dystonia-parkinsonism triggered by identifiable stressors; prominent bulbar involvement and marked asymmetry.	AD
	Myoclonus dystonia	DYT11 (159900)	*SGCE*	7q21.3	Alcohol-responsive myoclonus with mild dystonia; upper body distribution; frequent psychiatric comorbidity	AD
		DYT26 (616398)	*KCTD17*	22q12.3	Myoclonus-dystonia with progression of dystonia in adulthood; no improvement with alcohol.	AD
Paroxysmal dyskinesia		DYT8 (118800)	*PNKD*	2q35	Paroxysmal non-kinesigenic dyskinesia	AD
		DYT10 (128200)	*PRRT2*	16p11.2	Paroxysmal kinesigenic dyskinesia	AD
		DYT18 (612126)	*SLC2A1*	1p34.2	Paroxysmal exertion-induced dyskinesia; epilepsy and mental retardation frequent in childhood	AD

Table 2. Genes and loci associated with dystonia. MOI: mode of inheritance; AD: autosomal dominant; AR: autosomal recessive; XL: X-linked; LD: levodopa: IQ: intelligence quotient.

CHOREA

Chorea is a hyperkinetic movement disorder characterized by brief, continuous, patternless involuntary movements that flow from a body part to another in an unpredictable way. Similarly to dystonia, chorea can present with different anatomical distributions, being focal, generalized or with a hemisomatic presentation[17].

A variety of acquired causes of chorea exist, from structural lesions of various nature involving the basal ganglia, especially the striatum, to autoimmune causes (such as Sydenham's chorea in children, antiphospholipid syndrome in adults and autoimmune encephalitides)[26]. Genetic causes of chorea, both with childhood- and adult onset, represent an important proportion of cases. The most frequent type of genetic chorea with a progressive course, variably associated with cognitive decline and psychiatric disturbances, is Huntington's disease (HD), due to an abnormal CAG repeat expansion in the *HTT* gene that is transmitted as an autosomal dominant trait[27]. Classically, HD presents in adulthood, with an age at onset largely depending on the CAG repeat expansion size: the larger the expansion, the earlier the age at onset[28].

HD is a paradigmatic example of the importance of age at onset in the differential diagnosis of choreic syndromes. In fact, children affected by HD, who carry very large CAG triplet expansions, do not present with chorea, but with akinetic-rigid parkinsonism (so called "Westphal variant") accompanied by progressive cognitive deterioration often associated with drug-resistant epilepsy, thus HD is rarely considered among genetic causes of chorea in childhood and adolescence. The type of onset (acute/subacute vs slowly progressive)

and the disease course are also major determinants in dissecting the possible underlying etiologies of chorea.

For example, mutations in the *NKX2-1* gene cause generalized chorea during childhood with no progression into adulthood; the movement disorder is generally mild-to-moderate in severity, and frequent association with thyroid and pulmonary dysfunction is observed[29].

As for dystonia, the list of monogenic causes of chorea has hugely expanded thanks to the advent of NGS.

In particular, two genes causing a childhood onset hyperkinetic movement disorder dominated by generalized chorea have been discovered in the last years: *ADCY5* and *PDE10A*[9,10]. Among outpatients followed in our movement disorder clinic, we were able to individuate by means of NGS and subsequent Sanger sequencing two subjects carrying two different *de novo* missense mutations in *ADCY5* (see Chapter 8) and one patient carrying a *de novo* mutation in *PDE10A*, the latter being the twelfth case worldwide at the time of publication (see Chapter 10). For details on clinical features and disease course associated with *ADCY5* and *PDE10A* mutations, as well as a comprehensive list of monogenic causes of chorea, see Chapter 6.

SCOPE OF THE THESIS

The work I carried out during my PhD has contributed to the publication of 11 papers on movement disorders (9 original articles and 2 reviews), being the first author in 5.

In chapters 2 and 4, papers leading to the discovery of two new genes, *KCTD17* and *PDE10A*, are reported. Chapter 10 is a case report on a newly described *PDE10A* mutation carrier that we diagnosed in our Institute, being the twelfth case reported worldwide.

Chapter 3 is a screening of a large European cohort of patients with myoclonus dystonia for a specific missense mutation in *CACNA1B* to which we contributed.

Chapter 5 includes a paper in which I reported an Italian family with an autosomal dominant type of dystonia caused by a novel mutation in a very rare gene, *GNAL*, that was diagnosed by means of gene panels.

Chapter 6 and 11 are invited review on emerging genetic movement disorders that are focused on complex diseases.

Chapter 7 contains an article regarding the screening of a recently-discovered gene, *HPCA* (DYT2) in our cohort of childhood-onset dystonia patients, that yielded negative results.

Chapter 8 is a case report of a patient carrying a *de novo PSEN1* mutation presenting with an atypical motor phenotype later evolving to dementia.

Chapter 9 is a paper resulting from an international collaborative study that aimed at gathering patients affected by a recently-described genetic movement disorder caused by mutations in *ADCY5* gene to better understand its disease course and long-term outcome.

References

1. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chainterminating inhibitors. Proc Natl Acad Sci USA 1977;74:5463-5467.
2. Mamanova L, Coffey AJ, Scott CE, et al. Target-enrichment strategies for next-generation sequencing. Nat Methods 2010;7:111-118.
3. Morey M, Fernandez-Marmiesse A, Castineiras D, Fraga JM, Couce ML, Cocho JA. A glimpse into past, present, and future DNA sequencing. Mol Genet Metab 2013;110:3-24.
4. Metzker ML. Sequencing technologies—the next generation. Nat Rev Genet 2010;11:31-46.
5. Olgiati S, Quadri M, Bonifati V. Genetics of movement disorders in the next-generation sequencing era. Mov Disord. 2016;31:458-70.
6. Valsesia A, Mace A, Jacquemont S, Beckmann JS, Kutalik Z. The growing Importance of CNVs: New Insights for Detection and Clinical Interpretation. Front Genet 2013;4:92.
7. Fuchs T, Saunders-Pullman R, Masuho I, et al. Mutations in GNAL cause primary torsion dystonia. Nat Genet 2013;45:88-92.
8. Charlesworth G, Plagnol V, Holmstrom KM, et al. Mutations in ANO3 cause dominant craniocervical dystonia: ion channel implicated in pathogenesis. Am J Hum Genet 2012;91:1041-105.
9. 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.

10. Mencacci NE, Kamsteeg E-J, Nakashima K, et al. De Novo Mutations in PDE10A Cause Childhood-Onset Chorea with Bilateral Striatal Lesions. The American Journal of Human Genetics 2015; 98: 763-771.
11. Mastrangelo M. Novel Genes of Early-Onset Epileptic Encephalopathies: From Genotype to Phenotypes. Pediatr Neurol. 2015;53:119-29.
12. de Carvalho Aguiar P, Sweadner KJ, Penniston JT, et al. Mutations in the Na1/K1-ATPase alpha3 gene ATP1A3 are associated with rapid-onset dystonia parkinsonism. Neuron 2004;43:169-175.
13. Rosewich H, Thiele H, Ohlenbusch A, et al. Heterozygous de novo mutations in ATP1A3 in patients with alternating hemiplegia of childhood: a whole-exome sequencing gene-identification study. Lancet Neurol 2012;11:764-773.
14. Heinzen EL, Swoboda KJ, Hitomi Y, et al. De novo mutations in ATP1A3 cause alternating hemiplegia of childhood. Nat Genet 2012;44:1030-1034.
15. van Egmond ME, Lugtenberg CHA, Brouwer OF, et al. A post hoc study on gene panel analysis for the diagnosis of dystonia. Mov Disord. 2017;32:569-575.
16. Barzaghi C, Panteghini C, Carecchio M, et al. The relevance of movement disorders gene panels in clinical practice: How many patients are we sorting out? Mov Disord. 2016; 31 (suppl 2).

17. Edwards MJ, Stamelou M, Quinn N., et al. Parkinson's disease and other movement disorders.Second edition. Oxford Specialist Handbooks in Neurology; Oxford University Press 2016.
18. Ariani F, Hayek G, Rondinella D, et al. FOXG1 is responsible for the congenital variant of Rett syndrome. Am J Hum Genet 2008:83:89-93.
19. Steeves TD, Day L, Dykeman J, et al. The prevalence of primary dystonia: a systematic review and meta-analysis. Mov Disord. 2012;27:1789-96.
20. Albanese A, Bhatia K, Bressman SB, et al. Phenomenology and classification of dystonia: a consensus update. Mov Disord 2013;28:863-73.
21. Ozelius LJ, Hewett JW, Page, et al. The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein. Nat Genet 1997;17:40-8.
22. Bressman SB, Sabatti C, Raymond D, et al. The DYT1 phenotype and guidelines for diagnostic testing. Neurology 2000;54:1746-52.
23. Petrucci S, Valente EM. Genetic issues in the diagnosis of dystonias. Front Neurol. 2013;4:34.
24. Meyer E, Carss KJ, Rankin J, Nichols JM, Grozeva D, Joseph AP, et al. Mutations in the histone methyltransferase gene KMT2B cause complex early-onset dystonia. Nat Genet 2017:49:223-37.
25. Zech M, Boesch S, Maier EM, et al. Haploinsufficiency of KMT2B, Encoding the Lysine-Specific Histone Methyltransferase 2B, Results in Early-Onset Generalized Dystonia. Am J Hum Genet 2016:99:1377-87.
26. Cardoso F. Autoimmune Chorea. J Neurol Neurosurg Psychiatry

2017;88:412-417.
27. Ross CA, Tabrizi SJ. Huntington's disease: from molecular pathogenesis to clinical treatment. Lancet Neurol 2011;10:83-98.
28. Andrew SE, Goldberg YP, Kremer B, et al. The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. Nat Genet 1993;4:398-403.
29. Gras D, Jonard L, Roze E, et al. Benign hereditary chorea: phenotype, prognosis, therapeutic outcome and long term follow-up in a large series with new mutations in the TITF1/NKX2-1 gene. J Neurol Neurosurg Psychiatry 2012;83:956-62.

Chapter 2

A missense mutation in *KCTD17* causes autosomal dominant myoclonus-dystonia

Niccolo E. Mencacci,[1,2] Ignacio Rubio-Agusti,[3,4*] Anselm Zdebik,[5,6*] Friedrich Asmus,[7*] Marthe H.R. Ludtmann,[1] Mina Ryten,[1,8] Vincent Plagnol,[9] Ann-Kathrin Hauser,[7] Sara Bandres-Ciga,[10] Conceição Bettencourt,[1] Paola Forabosco,[11] Deborah Hughes,[1] Marc P. M. Soutar,[1] Kathryn Peall,[12] Huw R. Morris,[13] Daniah Trabzuni,[1,14] Mehmet Tekman,[6] Horia C. Stanescu,[6] Robert Kleta,[6] **Miryam Carecchio**,[15,16] Giovanna Zorzi,[15] Nardo Nardocci,[15] Barbara Garavaglia,[16] Ebba Lohmann,[7] Anne Weissbach,[17] Christine Klein,[17] John Hardy,[1,18] Alan M. Pittman,[1,18] Thomas Foltynie,[4] Andrey Y. Abramov,[1] Thomas Gasser,[7] Kailash P. Bhatia,[4#] and Nicholas W. Wood[1#].

[1]Department of Molecular Neuroscience, Institute of Neurology, University College London, WC1N 3BG London, United Kingdom

[2]IRCCS Istituto Auxologico Italiano, Department of Neurology and Laboratory of Neuroscience – Department of Pathophysiology and Transplantation, "Dino Ferrari" Centre, Universita` degli Studi di Milano, 20149 Milan, Italy

[3]Unidad de Trastornos del Movimiento, Hospital Universitario La Fe, 46026 Valencia, Spain

[4]Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, WC1N 3BG London, United Kingdom

[5]Department of Neuroscience, Physiology and Pharmacology, University College London, WC1E 6BT London, United Kingdom

[6]Centre for Nephrology, University College London, NW3 2PF London, United

Kingdom
[7]Department of Neurodegenerative Diseases, Hertie Institute for Clinical Brain Research, University of Tübingen, and German Center for Neurodegenerative Diseases (DZNE), 72076 Tübingen, Germany
[8]Department of Medical and Molecular Genetics, King's College London, Guy's Hospital, SE1 7EH London, UK
[9]UCL Genetics Institute, WC1E 6BT London, United Kingdom
[10]Department of Physiology and Institute of Neurosciences Federico-Olóriz, Centro de Investigaciones Biomedicas (CIBM), University of Granada, 18071 Granada, Spain
[11]Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche, 09042 Cagliari, Italy
[12]MRC Centre for Neuropsychiatric Genetics and Genomics, Institute of Psychological Medicine and Clinical Neurosciences, School of Medicine, Cardiff University, CF24 4HQ Cardiff, United Kingdom
[13]Department of Clinical Neuroscience, UCL Institute of Neurology, WC1N 3BG London, United Kingdom
[14]Department of Genetics, King Faisal Specialist Hospital and Research Centre, PO Box 3354, Riyadh 11211, Saudi Arabia
[15]Neuropediatrics Unit, Fondazione IRCCS Istituto Neurologico "Carlo Besta", 20133 Milan, Italy
[16]Molecular Neurogenetics Unit, IRCCS Foundation Carlo Besta, 20133 Milan, Italy
[17]Institute of Neurogenetics, University of Lübeck, 23538 Lübeck, Germany
[18]Reta Lila Weston Institute of Neurological Studies, UCL Institute of Neurology, WC1N 3BG London, United Kingdom
[*]These authors contributed equally to this work.
[#]Joint senior authors

Abstract

Myoclonus-dystonia (M-D) is a rare movement disorder characterized by a combination of non-epileptic myoclonic jerks and dystonia. *SGCE* mutations represent a major cause for familial M-D being responsible for 30-50% of cases. After excluding *SGCE* mutations, we identified through a combination of linkage analysis and whole-exome sequencing *KCTD17* c.434 G>A p.(Arg145His) as the only segregating variant in a dominant British pedigree with 7 subjects affected by M-D. A subsequent screening in a cohort of M-D cases without mutations in *SGCE* revealed the same *KCTD17* variant in a German family. The clinical presentation of the *KCTD17*-mutated cases was distinct from the phenotype usually observed in M-D due to *SGCE* mutations. All cases initially presented with mild myoclonus affecting the upper limbs. Dystonia showed a progressive course, with increasing severity of symptoms and spreading from the cranio-cervical region to other sites. *KCTD17* is abundantly expressed in all brain regions with the highest expression in the putamen. Weighted gene co-expression network analysis, based on mRNA expression profile of brain samples from neuropathologically healthy individuals, showed that *KCTD17* is part of a putamen gene network, which is significantly enriched for dystonia genes. Functional annotation of the network showed an over-representation of genes involved in post-synaptic dopaminergic transmission. Functional studies in mutation bearing fibroblasts demonstrated abnormalities in endoplasmic reticulum-dependent calcium signaling. In conclusion, we demonstrate that the *KCTD17* c.434 G>A p.(Arg145His) mutation causes autosomal dominant M-D. Further functional studies are warranted to further characterize the

nature of *KCTD17* contribution to the molecular pathogenesis of M-D.

Report

Dystonias are a clinically and genetically heterogeneous group of non-neurodegenerative movement disorders, mainly characterized by involuntary muscle contractions leading to abnormal postures or movements of body segments.[1]

Mutations in a growing number of genes (recently reviewed by our group)[2] are responsible for Mendelian forms of dystonia. The identification of these genes allowed the recognition of different cellular pathways involved in the molecular pathogenesis of dystonia, including perturbed synaptic transmission and plasticity, abnormal transcription and cell-cycle regulation and endoplasmic reticulum (ER) dysfunction.[3]

The association with additional movement disorders identifies a sub-group of dystonias, defined as combined dystonias.[4]

Myoclonus-dystonia (M-D [MIM 159900]), one of the combined dystonia syndromes, is a very rare condition with a suggested prevalence of about 2 per million in Europe.[5] M-D is clinically characterized by a variable combination of non-epileptic myoclonic jerks, mainly affecting the upper body, and mild to moderate dystonia, usually in the form of cervical dystonia or writer's cramp.[6] There is often a dramatic improvement of myoclonus after alcohol consumption.[7] Psychiatric co-morbidities (eg. depression, anxiety and obsessive-compulsive disorder) are frequently described.[8]

Mutations in *SCGE* [MIM 604149], coding for ε-sarcoglycan, represent a major cause of inherited autosomal dominant M-D.[9] *SGCE* mutations

are detected in 30-50% of familial M-D cases, suggesting genetic heterogeneity and the existence of mutations in other genes responsible for this condition.[10-14]

We used a combination of genome-wide linkage analysis and whole-exome sequencing to investigate a previously unpublished dominant British pedigree (shown in Figure 1A) with multiple individuals affected with M-D, in which *SCGE* mutations (both point mutations and copy number variants) had been excluded.

Out of 19 living family members from the index family, 14 were clinically assessed. Assessment included a detailed medical interview and a full-videotaped neurological examination, with focus on movement disorders. All videos were reviewed by two experts in movement disorders (TF and KPB), blinded to disease status.

The proband (III-2) developed involuntary jerky movements of her arms during childhood. In her late forties she developed constant head jerks and head deviation to the left. In her sixties her speech became involved. On examination at the age of 69 she had spasmodic dysphonia, facial myoclonus, blepharospasm, left torticollis and frequent irregular dystonic head jerks. There was dystonic hand posturing and low amplitude brief myoclonus. When she walked she presented trunk and bilateral foot dystonia (Video section 1).

Six other family members displayed signs of dystonia and/or myoclonus, and were accordingly categorized as affected (Table 1). Age of onset of movement disorder symptoms ranged from 5 to 20 years. All affected family members initially presented with jerks or a jerky tremor, with mild dystonic features presenting later in life. All cases fulfilled the currently proposed clinical criteria for a definite diagnosis

of M-D,[15] except individual IV-3, who upon examination displayed isolated cervical dystonia, although she reported intermittent jerky arm tremor. Myoclonus involved predominantly the arms (video section 2). Dystonia predominantly affected the cranio-cervical region and upper limbs. Older individuals (>60 years; III-2 and III-5) were more severely affected and also showed laryngeal involvement. None of the affected subjects reported improvement of symptoms with alcohol. Subject IV-3 had anxiety and social phobia and subject IV-14 had obsessive traits and suffered from depression. No other individuals presented with psychiatric symptoms.

Case IV-12 had strabismus and benign congenital nystagmus, but no signs of M-D. The remaining individuals were asymptomatic and had an entirely normal neurological examination.

Samples were collected with the written consent of participants and formal ethical approval by the relevant research ethics committee (UCLH Project ID number 06/N076). DNA of 13 family members and 4 spouses was extracted from blood lymphocytes.

A genome-wide linkage analysis was subsequently performed in 7 affected individuals (III-2, III-5, IV-1, IV-3, IV-14, V-1 and V-3), 5 unaffected (III-4, IV-6, IV-8, IV-12 and V-4) and 4 spouses using the HumanCytoSNP-12 DNA Analysis BeadChip Kit (Illumina, San Diego). The unaffected subject V-2 was not included as she was too young (17 when last examined) to exclude or confirm disease status.

Genome-wide multipoint parametric linkage analysis for an autosomal dominant model (estimated allele frequency 0.00001 and 90% penetrance) and haplotype reconstruction were performed with Simwalk2,[16] using 24,000 informative single nucleotide

polymorphisms (SNP), equally spaced 0.1cM apart as described before.[17]

One single locus with a LOD score > 2 was identified on chromosome 22q13 (LOD score 2.4, the maximal expected value given the pedigree size; see Figure 1C).

Fine mapping identified a segregating haplotype delimitated by SNP markers rs926543 and rs3213584 and spanning 6.7Mb (chr22:36989327-43716324; UCSC hg19 Genome Build), which contained 132 protein-coding genes. In addition, 5 other regions presented with uninformative multipoint LOD scores, ranging from -0.9 to +0.14, but haplotype analysis excluded segregation of these regions with the disease.

We subsequently performed whole-exome sequencing in the two most distantly related affected individuals (V-3 and IV-14). In short, paired-end sequence reads (TruSeq SBS chemistry sequenced on the Illumina HiSeq 2000) were aligned with Novoalign against the reference human genome (UCSC hg19). Duplicate read removal, format conversion, and indexing were performed with Picard. The Genome Analysis Toolkit (GATK) was used to recalibrate base quality scores, perform local realignments around possible indels, and to call and filter the variants. Annotated variant files were generated using ANNOVAR[18] and included a comparison to publicly available databases of sequence variations (dbSNP version 129, 1000 Genomes project, NHLBI Exome Variant Server and Complete Genomics 69). *In silico* prediction of pathogenicity was assessed using SIFT,[19] PolyPhen2,[20] MutationTaster,[21] Provean,[22] and CADD.[23] Conservation of nucleotides involved by variants was scored using Genomic Evolutionary Rate

Profiling (GERP). Interspecies alignment of protein sequences was generated using ClustalW2.[25]

In total, 83,572,847 (V-3) and 81,527,162 (IV-14) unique reads were generated. According to the Consensus Coding Sequences hg19 definition of the 'TruSeq exome', the average read depth of both exomes was > 70, > 95% of the target bases were covered at a read depth of 2x and > 90% at a depth of 10x. A total of 22,857 (V-3) and 22,946 (IV-14) exonic/splicing variants were detected. We filtered out all synonymous changes and those not shared by the two affected individuals. Then, under the assumption that the mutation causing this rare autosomal dominant disease is extremely rare and not present in the general population, we also excluded variants that are present in the databases of sequence variations listed above. Furthermore, we excluded variants found in our own in-house exomes (n=200) from individuals with unrelated diseases.

After applying filtering criteria, we were left with only 4 novel missense variants shared by the two affected individuals (see Table S1): c.10976 C>T p.(Ser3659Phe) in *FLG* (filaggrin; RefSeq NM_002016.1), c.1055 T>G p.(Phe352Cys) in *OBSCN* (obscurin; RefSeq NM_052843.3), c.1076 A>C p.(Lys359Thr) in *LRRC6* (leucine rich repeat containing 6; RefSeq NM_012472.4) and c.434 G>A p.(Arg145His) in *KCTD17* (potassium channel tetramerisation domain containing 17; RefSeq NM_001282684.1). Of these variants, only the missense change in *KCTD17* was located within the linked chromosomal locus on chromosome 22q. We did not detect any shared rare copy number variants in exome sequencing data using the Exome depth algorithm.[26] Sanger sequencing of the *KCTD17* variant in all available family

members confirmed perfect co-segregation of the variant with the disease-phenotype, being the nucleotide change present in all affected individuals and absent in all unaffected (including subject V-2, initially excluded from the linkage analysis). The variant is absent in over 3,700 individuals of European origin without movement disorders, who were exome sequenced by the UCL-exomes consortium, and in a further > 61,000 individuals listed in the Exome Aggregation Consortium database (last accessed in March 2015).

Although the KCTD17 p.(Arg145His) substitution falls in a functionally uncharacterized portion of the protein, it lies in an extremely conserved amino acid motif, not only completely conserved down to invertebrate species, but also identical in the KCTD17 human paralogs KCTD2 and KCTD5 (Figure 1E). All *in silico* tools consistently predicted a deleterious effect of the substitution (Table S1).

We subsequently sequenced the 9 coding exons of *KCTD17* (NM_001282684.1; primers available in table S2) in a further 87 unrelated probands with familial M-D of British, German and Italian origin. All cases did not carry mutations in *SGCE.* Mutational screening of *KCTD17* exon 4 (containing the c.434 G>A mutation) was performed in a further 358 sporadic M-D cases without mutations in *SCGE*.

This analysis revealed the presence of the same *KCTD17* mutation, c.434 G>A p.(Arg145His), in the index case of a German family with autosomal dominant M-D (Figure 1B). No further pathogenic mutations were identified.

The clinical presentation of this case closely resembled that of III-2 and III-5, the older affected subjects from the British family. He reported arm jerks and difficulty writing, starting in childhood. Right torticollis

and a jerky head tremor appeared around age 40, becoming progressively debilitating. There was no response to alcohol or psychiatric comorbidities. He underwent surgery for bilateral pallidal deep brain stimulation at age 58, which resulted in marked improvement of cervical dystonia and myoclonus of the upper limbs. Clinical examination at age 62 showed generalized dystonia, with prominent cranio-cervical involvement, and myoclonic jerks involving the upper limbs (Video section 3). His father was also affected with a movement disorder, presenting with perioral dyskinesia in his forties. The proband's brother had M-D, with similar clinical features, including generalized jerks, cervical dystonia and dysarthria. Unfortunately, DNA samples of the deceased father and brother were not available for segregation analysis. The 25-years old proband's only son, who had no signs upon examination, refused genetic testing.

Haplotype comparison between the 2 pedigrees with *KCTD17* c.434 G>A p.(Arg145His) was performed with SNP markers located 0.5 Mb up- and down-stream the mutation. This analysis showed that different alleles are located at markers rs5756477 and rs228924, delimitating a small region of ~100 Kb of a possibly shared haplotype (Table S3). A further analysis with a highly polymorphic microsatellite, located only 1.4 Kb upstream of the 5' end of *KCTD17*, revealed that the 2 pedigrees have different alleles, possibly suggesting the absence of a shared ancestral haplotype and that the variant may have arisen independently in the two pedigrees. Of relevance, the absence of a shared haplotype between the two families would make unlikely that the *KCTD17* c.434 G>A p.(Arg145His) mutation is in linkage disequilibrium with the actual causative mutation but not itself pathogenic.

We explored the regional distribution of *KCTD17* expression in the normal adult human brain. As previously described, we used microarray data (Affymetrix Exon 1.0 ST) from human post-mortem brain tissue collected by the UK Human Brain Expression Consortium (UKBEC).[27] *KCTD17* mRNA expression throughout the course of human brain development was assessed using the data available in the Human Brain Transcriptome (HBT) database.[28; 29]

KCTD17 mRNA expression was high across all brain regions, but it was highest in the putamen followed by the thalamus (Figure 2A). These findings are consistent with the data available in the HBT database, showing increasing *KCTD17* brain mRNA levels in the striatum and the thalamus from early midfetal development to adolescence (Figure 2B). In light of the current view of the neuroanatomical bases of dystonia, which is thought to be a network disorder of the basal ganglia connections,[30; 31] this pattern of expression is highly relevant and supports the pathogenic role of *KCTD17* in the pathogenesis of M-D.

KCTD17 encodes for a member of a recently identified family of 26 closely related and highly conserved proteins, the potassium channel tetramerisation domain (KCTD)-containing proteins. KCTD proteins are characterized by the presence of a N-terminal *b*ric-a-brack, *t*ram-track, *b*road complex/*po*xvirus *z*inc finger (BTB/POZ) domain, homologous to the cytoplasmic domain T1 of voltage-gated potassium channels.[32] The BTB/POZ domain is known to permit protein-protein interactions, either promoting self-oligomerisation or facilitating interaction with other biological partners.[33] KCTDs are small soluble proteins, which are not predicted by their structure to form

transmembrane domains. Despite the homology reflected in their names, a direct interaction with potassium channels has not been shown for most members of the family and was explicitly excluded for KCTD5, a paralog 85% identical to KCTD17.[34]

KCTD proteins, despite the high level of sequence similarity, are involved in a surprisingly wide spectrum of cell functions, including regulation of cellular proliferation, gene transcription, cytosketelon organization, protein degradation targeting via the ubiquitin-proteasome system, and regulation of G protein-coupled receptors.[35]

Several members of the KCTD family have a primary role in the central nervous system[36; 37] and a growing number of neurological diseases have been linked to mutations in KCTD genes. *KCTD7* [MIM 611725] mutations cause recessive progressive myoclonic epilepsy.[38-40] Copy number variants in *KCTD13* [MIM 608947] have been associated with size of the head, autism disorder and epilepsy.[41] More recently a homozygous missense mutation in *KCTD3* [MIM 613272] was identified as the likely cause in a pedigree with severe psychomotor retardation, seizure, and cerebellar hypoplasia.[42]

The precise cellular localization and function of the KCTD17 protein are largely unknown. Recent work has shown that KCTD17 contributes to the ubiquitin-proteasome machinery, acting as an adaptor for the CUL3-RING E3 ligase and targeting substrates for degradation through polyubiquitinylation.[43] Although most of the KCTD17-CUL3 substrates are currently unknown, CUL3 has been implicated in the elaboration of dendrite branching and neurite terminal morphogenesis in drosophila models.[44; 45]

Stably transfected SH-SY5 cells were generated by incorporating N- and C-terminally HA-tagged wild-type and mutant *KCTD17* cDNAs. KCTD17 staining with anti-HA primary monoclonal antibodies showed that the protein is diffusely distributed in the cytosol with fine reticular pattern and does not localize at the plasma membrane (Figure S1). Co-staining with ER, Golgi apparatus, mitochondria and lysosomal markers failed to show any co-localization with KCTD17 (data not shown). We did not observe significant changes in subcellular localization of mutant versus wild-type KCTD17, indicating that the amino acid substitution does not lead to cellular mislocalization of the protein.

To gain further insight into the functional role of KCTD17 and identify molecular pathways possibly dysregulated by mutant KCTD17, Weighted Gene Co-expression Network Analysis (WGCNA) was performed based on the UKBEC human brain mRNA expression data. In brief, this systems biology analytic approach uses brain regional whole-transcriptome gene expression data and establishes the degree of gene neighborhood sharing, as defined on the basis of co-expression relationships. This approach allows to identify in an unsupervised and unbiased manner modules of genes that are highly co-expressed and co-regulated and therefore likely to be functionally related.[46] Microarray data on 19152 transcripts (corresponding to 17247 genes), generated from 101 brains, were used to create weighted modules of co-expressed genes for each analyzed brain regions. A detailed description of the methods used to generate the dataset is available in the manuscript of Forabosco and colleagues.[47]

KCTD17, in common with other dystonia genes (e.g. *ANO3* [MIM

610110] and *GNAL* [MIM 139312]), shows the highest expression in the putamen and this brain structure has an established role in the pathogenesis of dystonia. We therefore focused the analysis on the putamen module including *KCTD17*. This module contains 179 transcripts (equating to 172 genes; Table S4; see Figure 3 for a graphic representation of the module).

We first assessed if the module was enriched for genes linked to Mendelian forms of dystonia. We focused the analysis on the 9 genes known to be associated with dystonia (*TOR1A* [MIM 605204], *THAP1* [MIM 609520], *SGCE*, *TUBB4A* [MIM 602662], *CIZ1* [MIM 611420], *ANO3*, *GNAL*, *ATP1A3* [MIM 182350], *PRKRA* [MIM 603424])[2] and *HPCA* [MIM 142622], a gene recently associated with autosomal recessive dystonia.[48] We did not include in the analysis genes causing DOPA-responsive dystonia (*GCH1* [MIM#600225], *TH* [MIM#191290], and *SPR* [MIM#182125]) as their established functional role in nigrostriatal dopamine synthesis, together with the specificity of the clinical presentation, clearly identifies them as a separate entity.

Importantly, the putamen *KCTD17*-module showed significant clustering of dystonia genes (*KCTD17* and *HPCA*; Fisher's exact test $P = 5x10^{-3}$), suggesting the relevance of this gene network to the molecular pathogenesis of dystonia. The module was poorly preserved across other brain regions, indicating its specificity to the putamen (Figure S2). The brain regional specificity of this module may suggest why mutations in *KCTD17* and *HPCA* manifest purely as a dysfunction of the basal ganglia (i.e. dystonia), in spite of the ubiquitous expression in the human brain.

To infer the biological and functional relevance of the putamen *KCTD17* gene network, functional annotation enrichment analysis was then carried out using the online tool g:Profiler.[49] This analysis allowed the identification of over-represented genes assigned to specific Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, namely "Circadian entrainment" (KEGG:04713; $P = 5.13x10^{-3}$) and "Dopaminergic synapse" (KEGG:04728; $P = 2.71x10^{-2}$). This suggests the involvement of the genes in the module with these molecular pathways.

Recent work in drosophila strongly reinforces the results of WGCNA and further suggests a relevant contribution of KCTD17 to regulation of dopaminergic transmission in the putamen. *Insomniac* (the *KCTD17* fly ortholog) is an essential regulator of sleep homeostasis through the control of the dopaminergic arousal pathways.[50; 51] More specifically, *insomniac* seems to regulate dopaminergic signaling at the post-synaptic level, possibly controlling the turnover of dopamine receptors or their downstream effectors.[51] Interestingly, abnormal post-synaptic dopaminergic signaling in the basal ganglia is one of the main themes in molecular dystonia pathogenesis, a concept recently strengthened by the identification of mutations in *GNAL* causing dystonia.[52] Fitting well with this model, all the genes in the putamen *KCTD17* module assigned to the KEGG "Dopaminergic synapse" pathway (*CACNA1C* [MIM 114205], *PPP1R1B* [MIM 604399], *PPP2R2C* [MIM 605997], *AKT1* [MIM 164730], *GNAO1* [MIM 139311], and *GNB2* [MIM 139390]) localize and act at the post-synaptic level.

Disruption of calcium (Ca^{2+}) homeostasis has been recently implicated in the pathogenesis of several genetic forms of dystonia (eg. *TOR1A*, *ANO3, HPCA*).[48; 53; 54]

As the putamen *KCTD17*-module included *HPCA*, a gene with an established role in intracellular Ca^{2+}-dependent signaling,[55] we hypothesized that the *KCTD17* p.(Arg145His) substitution may have a significant impact on intracellular Ca^{2+} homeostasis. For this purpose, fibroblasts were isolated from a skin biopsy taken from a subject with the *KCTD17* p.(Arg145His) mutation (index family, III-2). Two unrelated age- and passage-matched controls were selected from in-house fibroblast lines. The expression of *KCTD17* in fibroblasts was confirmed by RT-PCR (data not shown). Calcium homeostasis was assessed using the ratio-metric Ca^{2+} dye, Fura-2, AM (Molecular Probes, Paisley, UK), which indicates intracellular Ca^{2+} concentration and allows recordings of Ca^{2+} fluxes upon application of different pharmacological stimuli. We observed that stimulation with ATP (50µM), which stimulates P2Y receptors and releases Ca^{2+} from the ER via IP_3 receptors, resulted in significantly reduced and delayed cytosolic Ca^{2+} signal ($P < 0.01$; Figure 4A and 4B) in cells carrying the p.(Arg145His) mutation when compared to both control cells, indicating a smaller calcium pool within the ER. To further prove this finding, a second round of experiments using thapsigargin (1µM) in Ca^{2+}-free medium (plus 0.5 mM EGTA) was subsequently carried out. Thapsigargin is an inhibitor of the ER calcium ATPase (SERCA) and induces the release of calcium from the ER to the cytosol, allowing an estimation of the ER Ca^{2+}-pool. Ca^{2+} was then added at the end of the experiment to stimulate elevation of cytosolic Ca^{2+} through opening of

store operated calcium channels. Thapsigargin stimulation resulted in a significantly smaller Ca^{2+}signal in fibroblasts bearing the p.(Arg145His) mutation when compared to controls ($P < 0.01$; Figure 4C and 4D), confirming that the Ca^{2+} pool in the ER of mutation-carrying fibroblasts is reduced. Furthermore, stimulation of the store-operated Ca^{2+} channels induced a smaller Ca^{2+} influx in mutated fibroblasts (figure 4C), possibly suggesting an insufficient Ca^{2+} influx across the plasma membrane in response to the fall in Ca^{2+} concentration within the ER lumen. Interestingly, we recently showed very similar defects of ER Ca^{2+} storage in fibroblasts bearing a pathogenic mutation in *ANO3*.[53] This indicates that defective ER calcium signaling may represent a converging pathogenic mechanism in genetically unrelated forms of dystonia.

In conclusion, we demonstrate that a missense mutation in *KCTD17*, c.434 G>A p.(Arg145His), represents a rare genetic cause for inherited autosomal dominant M-D.

The clinical features of the *KCTD17*-mutated cases, although fully consistent with a clinical diagnosis of M-D, were distinct in many ways from the usual phenotype of subjects with *SGCE* mutations. Dystonia dominated the clinical picture and showed a progressive course, worsening over time and spreading to other sites (including speech involvement), a course unusual for *SGCE*-related M-D. Myoclonus, despite being the presenting symptom in most cases, was overall mild and not as disabling as in *SGCE*-mutated subjects.

These phenotypic differences may be explained by the different functions of the two genes, but also by the clearly distinct patterns of brain regional expression. *SGCE* is highly expressed in the cerebellum,

whereas its expression is low to moderate in putamen and globus pallidus.[56] On the other hand, *KCTD17* expression is high in the putamen and thalamus but relatively low in the cerebellum. Intriguingly, this could be the explanation for the scarce response to alcohol consumption in *KCTD17*-mutated cases, as alcohol probably exerts its beneficial effect in M-D secondary to *SGCE* mutations by modulating cerebellar activity.[56]

Preliminary data suggest an involvement of KCTD17 in dopamine synaptic transmission regulation and an effect of the p.(Arg145His) substitution on ER-derived Ca^{2+} signaling. Further insight into the physiological role of KCTD17 and a better understanding of the pathogenic effect of the p.(Arg145His) substitution will shed light onto the mechanisms leading to abnormal neuronal activity underlying M-D. Furthermore, the identification of KCTD17 interactors will possibly highlight new potential pharmacological targets for the treatment of dystonia.

Mutational screening of additional cohorts of M-D cases will help to define the frequency and the spectrum of *KCTD17* mutations. *KCTD17* mutations should be considered in cases without mutations in *SGCE* presenting with myoclonus, dystonia or a combination of both, particularly if there is predominant cranio-cervical and laryngeal involvement.

Description of Supplementary Data

Table S1, S2, S3, S4 and Figures S1, S2.

Acknowledgements

We would like to extend our thanks to the individuals whose participation made this research possible. This work was supported financially by a Medical Research Council/Wellcome Trust Strategic Award (WT089698/Z/09/Z) and a grant from the Bachman-Strauss Dystonia Parkinsonism Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The work was undertaken at University College London Hospitals (UCLH) and University College London (UCL), who receive support from the Department of Health's NIHR Biomedical Research Centers funding streams. E.L. and T.G. are supported by a grant from the Dystonia Medical Research Foundation (DMRF). C.K. is the recipient of a career development award from the Herman and Lilly Schilling Foundation. A.M.P. is funded by the Reta Lila Weston Trust. S.B. held a FPU fellowship from the Spanish Ministry of Education and Science jointly with a short-term stay grant by Cei-BioTic and University of Granada. Next Generation Sequencing was performed at the UCL Institute of Neurology Sequencing Facility. We thank the UCL-exomes consortium for providing the exome sequencing data of UK population controls. Expression data was provided by the UK Human Brain Expression Consortium (UKBEC), which comprises John A. Hardy, Mina Ryten, Michael Weale, Daniah Trabzuni, Adaikalavan Ramasamy, Colin Smith and Robert Walker. UKBEC members are affiliated with UCL Institute of Neurology (J.H., M.R., D.T.), King's College London (M.R., M.W., A.R.) and the University of Edinburgh (C.S., R.W.). We thank Miss Elisavet Preza for providing help with the skin biopsy preparation. All authors report no conflict of interest relevant to this work.

Web Resources

The URLs for data presented herein are as follows:

1000 Genomes project: www.1000genomes.org

CADD: http://cadd.gs.washington.edu/home

ClustalW2: http://www.ebi.ac.uk/Tools/msa/clustalw2/

Complete Genomics cg69 database:

www.completegenomics.com/public-data/69-Genomes

dbSNP version 130: www.ncbi.nlm.nih.gov/projects/SNP

Exome Aggregation Consortium database:

http://exac.broadinstitute.org/

G-profiler: http://biit.cs.ut.ee/gprofiler/index.cgi

Human Brain Transcritptome database: http://hbatlas.org/

Kyoto Encyclopedia of Genes and Genomes (KEGG):

http://www.genome.jp/kegg/

Merlin: http://www.sph.umich.edu/csg/abecasis/merlin/index.html

MutationTaster: http://www.mutationtaster.org/

Online Mendelian Inheritance in Man (OMIM),

http://www.omim.org/.

PolyPhen2: http://genetics.bwh.harvard.edu/pph2/

NHLBI Exome Variant Server EVS: evs.gs.washington.edu

Simwalk2: http://www.genetics.ucla.edu/software/simwalk

Provean: http://provean.jcvi.org/

SIFT: http://sift.jcvi.org/

References

1. Albanese, A., Bhatia, K., Bressman, S.B., Delong, M.R., Fahn, S., Fung, V.S., Hallett, M., Jankovic, J., Jinnah, H.A., Klein, C., et al. (2013). Phenomenology and classification of dystonia: a consensus update. Mov Disord 28, 863-873.
2. Charlesworth, G., Bhatia, K.P., and Wood, N.W. (2013). The genetics of dystonia: new twists in an old tale. Brain 136, 2017-2037.
3. Ledoux, M.S., Dauer, W.T., and Warner, T.T. (2013). Emerging common molecular pathways for primary dystonia. Mov Disord 28, 968-981.
4. Fung, V.S., Jinnah, H.A., Bhatia, K., and Vidailhet, M. (2013). Assessment of patients with isolated or combined dystonia: an update on dystonia syndromes. Mov Disord 28, 889-898.
5. Asmus, F., and Gasser, T. (2004). Inherited myoclonus-dystonia. Adv Neurol 94, 113-119.
6. Asmus, F., Zimprich, A., Tezenas Du Montcel, S., Kabus, C., Deuschl, G., Kupsch, A., Ziemann, U., Castro, M., Kuhn, A.A., Strom, T.M., et al. (2002). Myoclonus-dystonia syndrome: epsilon-sarcoglycan mutations and phenotype. Ann Neurol 52, 489-492.
7. Nardocci, N. (2011). Myoclonus-dystonia syndrome. Handbook of clinical neurology / edited by PJ Vinken and GW Bruyn 100, 563-575.
8. Peall, K.J., Smith, D.J., Kurian, M.A., Wardle, M., Waite, A.J., Hedderly, T., Lin, J.P., Smith, M., Whone, A., Pall, H., et al. (2013). SGCE mutations cause psychiatric disorders: clinical and genetic characterization. Brain 136, 294-303.

9. Zimprich, A., Grabowski, M., Asmus, F., Naumann, M., Berg, D., Bertram, M., Scheidtmann, K., Kern, P., Winkelmann, J., Muller-Myhsok, B., et al. (2001). Mutations in the gene encoding epsilon-sarcoglycan cause myoclonus-dystonia syndrome. Nat Genet 29, 66-69.
10. Schule, B., Kock, N., Svetel, M., Dragasevic, N., Hedrich, K., De Carvalho Aguiar, P., Liu, L., Kabakci, K., Garrels, J., Meyer, E.M., et al. (2004). Genetic heterogeneity in ten families with myoclonus-dystonia. J Neurol Neurosurg Psychiatry 75, 1181-1185.
11. Tezenas du Montcel, S., Clot, F., Vidailhet, M., Roze, E., Damier, P., Jedynak, C.P., Camuzat, A., Lagueny, A., Vercueil, L., Doummar, D., et al. (2006). Epsilon sarcoglycan mutations and phenotype in French patients with myoclonic syndromes. J Med Genet 43, 394-400.
12. Carecchio, M., Magliozzi, M., Copetti, M., Ferraris, A., Bernardini, L., Bonetti, M., Defazio, G., Edwards, M.J., Torrente, I., Pellegrini, F., et al. (2013). Defining the epsilon-sarcoglycan (SGCE) gene phenotypic signature in myoclonus-dystonia: a reappraisal of genetic testing criteria. Mov Disord 28, 787-794.
13. Peall, K.J., Kurian, M.A., Wardle, M., Waite, A.J., Hedderly, T., Lin, J.P., Smith, M., Whone, A., Pall, H., White, C., et al. (2014). SGCE and myoclonus dystonia: motor characteristics, diagnostic criteria and clinical predictors of genotype. J Neurol.
14. Grunewald, A., Djarmati, A., Lohmann-Hedrich, K., Farrell, K., Zeller, J.A., Allert, N., Papengut, F., Petersen, B., Fung, V., Sue, C.M., et al. (2008). Myoclonus-dystonia: significance of large

SGCE deletions. Hum Mutat 29, 331-332.

15. Kinugawa, K., Vidailhet, M., Clot, F., Apartis, E., Grabli, D., and Roze, E. (2009). Myoclonus-dystonia: an update. Mov Disord 24, 479-489.
16. Sobel, E., Sengul, H., and Weeks, D.E. (2001). Multipoint estimation of identity-by-descent probabilities at arbitrary positions among marker loci on general pedigrees. Hum Hered 52, 121-131.
17. Hersheson, J., Mencacci, N.E., Davis, M., MacDonald, N., Trabzuni, D., Ryten, M., Pittman, A., Paudel, R., Kara, E., Fawcett, K., et al. (2013). Mutations in the autoregulatory domain of beta-tubulin 4a cause hereditary dystonia. Ann Neurol 73, 546-553.
18. Wang, K., Li, M., and Hakonarson, H. (2010). ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38, e164.
19. Kumar, P., Henikoff, S., and Ng, P.C. (2009). Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 4, 1073-1081.
20. Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A., Bork, P., Kondrashov, A.S., and Sunyaev, S.R. (2010). A method and server for predicting damaging missense mutations. Nat Methods 7, 248-249.
21. Schwarz, J.M., Cooper, D.N., Schuelke, M., and Seelow, D. (2014). MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods 11, 361-362.
22. Choi, Y., Sims, G.E., Murphy, S., Miller, J.R., and Chan, A.P.

(2012). Predicting the functional effect of amino acid substitutions and indels. PLoS One 7, e46688.

23. Kircher, M., Witten, D.M., Jain, P., O'Roak, B.J., Cooper, G.M., and Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet 46, 310-315.

24. Davydov, E.V., Goode, D.L., Sirota, M., Cooper, G.M., Sidow, A., and Batzoglou, S. (2010). Identifying a high fraction of the human genome to be under selective constraint using GERP++. PLoS Comput Biol 6, e1001025.

25. Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., et al. (2007). Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947-2948.

26. Plagnol, V., Curtis, J., Epstein, M., Mok, K.Y., Stebbings, E., Grigoriadou, S., Wood, N.W., Hambleton, S., Burns, S.O., Thrasher, A.J., et al. (2012). A robust model for read count data in exome sequencing experiments and implications for copy number variant calling. Bioinformatics 28, 2747-2754.

27. Trabzuni, D., Ryten, M., Walker, R., Smith, C., Imran, S., Ramasamy, A., Weale, M.E., and Hardy, J. (2011). Quality control parameters on a large dataset of regionally dissected human control brains for whole genome expression studies. J Neurochem 119, 275-282.

28. Johnson, M.B., Kawasawa, Y.I., Mason, C.E., Krsnik, Z., Coppola, G., Bogdanovic, D., Geschwind, D.H., Mane, S.M., State, M.W., and Sestan, N. (2009). Functional and evolutionary

insights into human brain development through global transcriptome analysis. Neuron 62, 494-509.

29. Kang, H.J., Kawasawa, Y.I., Cheng, F., Zhu, Y., Xu, X., Li, M., Sousa, A.M., Pletikos, M., Meyer, K.A., Sedmak, G., et al. (2011). Spatio-temporal transcriptome of the human brain. Nature 478, 483-489.
30. Kimmich, O., Molloy, A., Whelan, R., Williams, L., Bradley, D., Balsters, J., Molloy, F., Lynch, T., Healy, D.G., Walsh, C., et al. (2014). Temporal discrimination, a cervical dystonia endophenotype: penetrance and functional correlates. Mov Disord 29, 804-811.
31. Neychev, V.K., Gross, R.E., Lehericy, S., Hess, E.J., and Jinnah, H.A. (2011). The functional neuroanatomy of dystonia. Neurobiol Dis 42, 185-201.
32. Liu, Z., Xiang, Y., and Sun, G. (2013). The KCTD family of proteins: structure, function, disease relevance. Cell Biosci 3, 45.
33. Stogios, P.J., Downs, G.S., Jauhal, J.J., Nandra, S.K., and Prive, G.G. (2005). Sequence and structural analysis of BTB domain proteins. Genome Biol 6, R82.
34. Dementieva, I.S., Tereshko, V., McCrossan, Z.A., Solomaha, E., Araki, D., Xu, C., Grigorieff, N., and Goldstein, S.A. (2009). Pentameric assembly of potassium channel tetramerization domain-containing protein 5. J Mol Biol 387, 175-191.
35. Skoblov, M., Marakhonov, A., Marakasova, E., Guskova, A., Chandhoke, V., Birerdinc, A., and Baranova, A. (2013). Protein partners of KCTD proteins provide insights about their

functional roles in cell differentiation and vertebrate development. Bioessays 35, 586-596.

36. Matsui, A., Tran, M., Yoshida, A.C., Kikuchi, S.S., U, M., Ogawa, M., and Shimogori, T. (2013). BTBD3 controls dendrite orientation toward active axons in mammalian neocortex. Science 342, 1114-1118.
37. Schwenk, J., Metz, M., Zolles, G., Turecek, R., Fritzius, T., Bildl, W., Tarusawa, E., Kulik, A., Unger, A., Ivankova, K., et al. (2010). Native GABA(B) receptors are heteromultimers with a family of auxiliary subunits. Nature 465, 231-235.
38. Kousi, M., Anttila, V., Schulz, A., Calafato, S., Jakkula, E., Riesch, E., Myllykangas, L., Kalimo, H., Topcu, M., Gokben, S., et al. (2012). Novel mutations consolidate KCTD7 as a progressive myoclonus epilepsy gene. J Med Genet 49, 391-399.
39. Krabichler, B., Rostasy, K., Baumann, M., Karall, D., Scholl-Burgi, S., Schwarzer, C., Gautsch, K., Spreiz, A., Kotzot, D., Zschocke, J., et al. (2012). Novel mutation in potassium channel related gene KCTD7 and progressive myoclonic epilepsy. Ann Hum Genet 76, 326-331.
40. Van Bogaert, P., Azizieh, R., Desir, J., Aeby, A., De Meirleir, L., Laes, J.F., Christiaens, F., and Abramowicz, M.J. (2007). Mutation of a potassium channel-related gene in progressive myoclonic epilepsy. Ann Neurol 61, 579-586.
41. Golzio, C., Willer, J., Talkowski, M.E., Oh, E.C., Taniguchi, Y., Jacquemont, S., Reymond, A., Sun, M., Sawa, A., Gusella, J.F., et al. (2012). KCTD13 is a major driver of mirrored neuroanatomical phenotypes of the 16p11.2 copy number

variant. Nature 485, 363-367.

42. Alazami, A.M., Patel, N., Shamseldin, H.E., Anazi, S., Al-Dosari, M.S., Alzahrani, F., Hijazi, H., Alshammari, M., Aldahmesh, M.A., Salih, M.A., et al. (2015). Accelerating Novel Candidate Gene Discovery in Neurogenetic Disorders via Whole-Exome Sequencing of Prescreened Multiplex Consanguineous Families. Cell Rep 10, 148-161.

43. Kasahara, K., Kawakami, Y., Kiyono, T., Yonemura, S., Kawamura, Y., Era, S., Matsuzaki, F., Goshima, N., and Inagaki, M. (2014). Ubiquitin-proteasome system controls ciliogenesis at the initial step of axoneme extension. Nat Commun 5, 5081.

44. Zhu, S., Perez, R., Pan, M., and Lee, T. (2005). Requirement of Cul3 for axonal arborization and dendritic elaboration in Drosophila mushroom body neurons. J Neurosci 25, 4189-4197.

45. Djagaeva, I., and Doronkin, S. (2009). COP9 limits dendritic branching via Cullin3-dependent degradation of the actin-crosslinking BTB-domain protein Kelch. PLoS One 4, e7598.

46. Oldham, M.C., Horvath, S., and Geschwind, D.H. (2006). Conservation and evolution of gene coexpression networks in human and chimpanzee brains. Proc Natl Acad Sci U S A 103, 17973-17978.

47. Forabosco, P., Ramasamy, A., Trabzuni, D., Walker, R., Smith, C., Bras, J., Levine, A.P., Hardy, J., Pocock, J.M., Guerreiro, R., et al. (2013). Insights into TREM2 biology by network analysis of human brain gene expression data. Neurobiol Aging 34, 2699-2714.

48. Charlesworth, G., Angelova, P.R., Bartolome-Robledo, F., Ryten,

M., Trabzuni, D., Stamelou, M., Abramov, A.Y., Bhatia, K.P., and Wood, N.W. (2015). Mutations in HPCA Cause Autosomal-Recessive Primary Isolated Dystonia. Am J Hum Genet. http://dx.doi.org/10.1016/j.ajhg.2015.02.007.

49. Reimand, J., Arak, T., and Vilo, J. (2011). g:Profiler--a web server for functional interpretation of gene lists (2011 update). Nucleic Acids Res 39, W307-315.
50. Stavropoulos, N., and Young, M.W. (2011). insomniac and Cullin-3 regulate sleep and wakefulness in Drosophila. Neuron 72, 964-976.
51. Pfeiffenberger, C., and Allada, R. (2012). Cul3 and the BTB adaptor insomniac are key regulators of sleep homeostasis and a dopamine arousal pathway in Drosophila. PLoS Genet 8, e1003003.
52. Fuchs, T., Saunders-Pullman, R., Masuho, I., Luciano, M.S., Raymond, D., Factor, S., Lang, A.E., Liang, T.W., Trosch, R.M., White, S., et al. (2013). Mutations in GNAL cause primary torsion dystonia. Nat Genet 45, 88-92.
53. Charlesworth, G., Plagnol, V., Holmstrom, K.M., Bras, J., Sheerin, U.M., Preza, E., Rubio-Agusti, I., Ryten, M., Schneider, S.A., Stamelou, M., et al. (2012). Mutations in ANO3 cause dominant craniocervical dystonia: ion channel implicated in pathogenesis. Am J Hum Genet 91, 1041-1050.
54. Iwabuchi, S., Kakazu, Y., Koh, J.Y., and Harata, N.C. (2013). Abnormal cytoplasmic calcium dynamics in central neurons of a dystonia mouse model. Neurosci Lett 548, 61-66.
55. Palmer, C.L., Lim, W., Hastie, P.G., Toward, M., Korolchuk, V.I.,

Burbidge, S.A., Banting, G., Collingridge, G.L., Isaac, J.T., and Henley, J.M. (2005). Hippocalcin functions as a calcium sensor in hippocampal LTD. Neuron 47, 487-494.

56. Ritz, K., van Schaik, B.D., Jakobs, M.E., van Kampen, A.H., Aronica, E., Tijssen, M.A., and Baas, F. (2011). SGCE isoform characterization and expression in human brain: implications for myoclonus-dystonia pathogenesis? Eur J Hum Genet 19, 438-444.

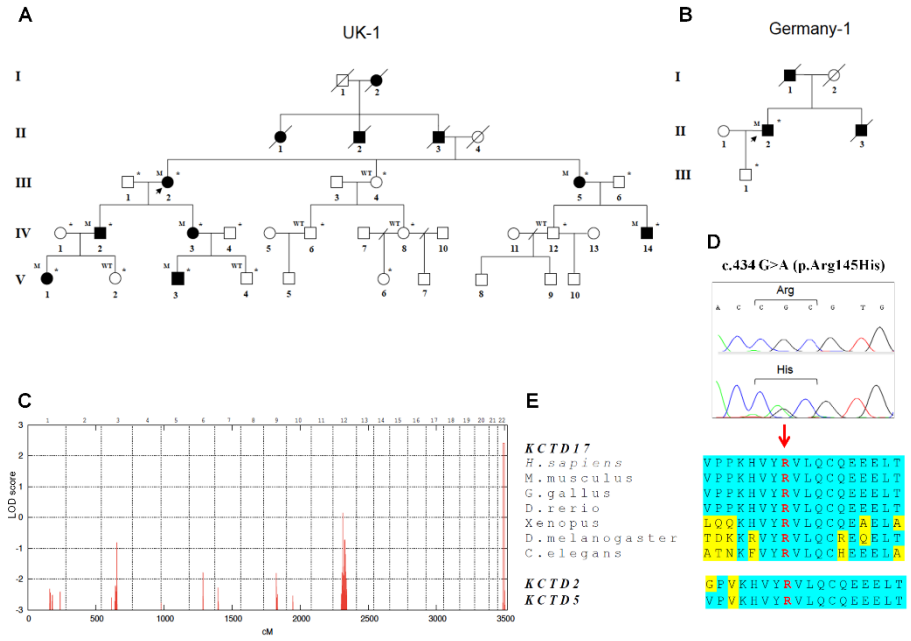


Figure 1. Family trees, linkage analysis, and *KCTD17* mutation analysis.

Pedigree of the British (A) and German (B) families with the *KCTD17* c.434 G>A p.(Arg145His) mutation. Open symbols indicate unaffected family members, and solid black symbols indicate affected members. Individuals marked with an asterisk were clinically examined. The following abbreviations are used: WT, homozygous wild-type alleles; and M, heterozygous mutation carrier. (C) LOD score plot for genome-wide linkage analysis in the British index pedigree showing a single linkage peak on chromosome 22q13 with a maximum LOD score of 2.4. An autosomal dominant model was specified with an estimated allele frequency of 0.00001 and 90% penetrance. (D) Sanger sequencing confirmation of the *KCTD17* c.434G>A p.(Arg145His) mutation. (E) Multiple-sequence alignment showing complete conservation of protein sequence across species and human paralogs (*KCTD2* and *KCTD5*) in the region of exon 4 of *KCTD17*, in which the disease-segregating mutation p.(Arg145His) was found.

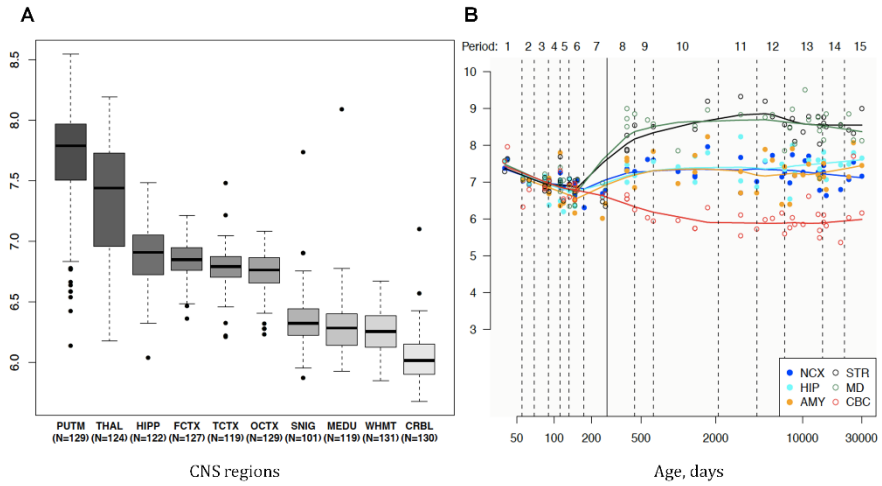


Figure 2. Summary of brain regional mRNA expression data.

(A) Box plot of mRNA expression levels for KCTD17 in 10 adult brain regions, based on exon array experiments and plotted on a log2 scale (y axis). This dataset was generated using Affymetrix Exon 1.0 ST Arrays and brain and CNS tissue originating from 134 control individuals, collected by the Medical Research Council (MRC) Sudden Death Brain and Tissue Bank, Edinburgh, UK, and the Sun Health Research Institute (SHRI), an affiliate of Sun Health Corporation, USA.27 The plot shows significant variation in KCTD17 transcript expression across the 10 CNS regions analyzed: putamen (PUTM), frontal cortex (FCTX), temporal cortex (TCTX), hippocampus (HIPP), substantia nigra (SNIG), medulla (specifically inferior olivary nucleus, MEDU), intralobular white matter (WHMT), thalamus (THAL), and cerebellar cortex (CRBL). "N" indicates the number of brain samples analyzed to generate the results for each CNS region. KCT17 expression is higher in the putamen, followed by the thalamus. Whiskers extend from the box to 1.53 the interquartile range.

(B) Graph to show mRNA expression levels for KCTD17 in 6 brain regions during the course of human brain development, based on exon array experiments and plotted on a log2 scale (y axis).28; 29 The brain regions analyzed are the striatum (STR), amygdala (AMY), neocortex (NCX), hippocampus (HIP), mediodorsal nucleus of the thalamus (MD), and cerebellar cortex (CBC). This shows increasing expression

of KCTD17 mRNA during human brain development, particularly in the striatum and thalamus, from the early midfetal period to adolescence.

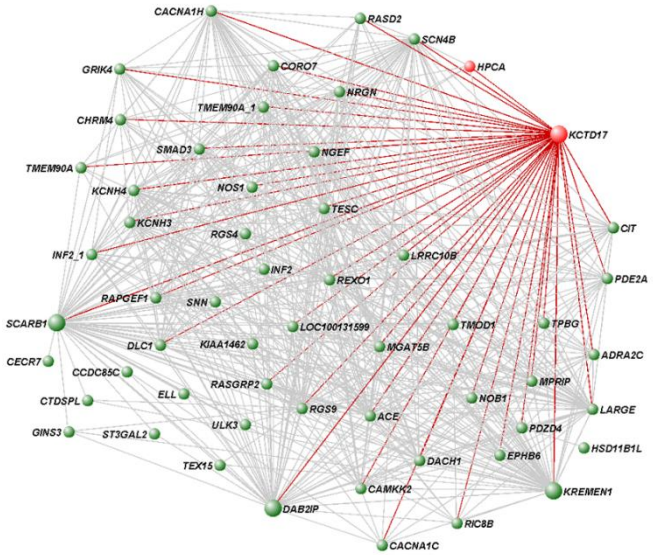


Figure 3. Network representation of the putamen *KCTD17*-containing gene module.

Array expression profiling of 788 brain samples obtained from 101 neuropathologically healthy individuals was performed and used for weighted gene co-expression network analysis (WGCNA). The WGCNA network was constructed for each brain region using a scale-free topology, as previously described.[47] A dissimilarity matrix based on topological overlap measure was used to identify gene modules (i.e., densely interconnected and co-expressed genes) through a dynamic tree-cutting algorithm. Shown are all genes in the putamen *KCTD17*-containing module connected with a topological overlap measure exceeding 0.03. The dystonia genes in the module (*KCDT17* and *HPCA*) and all the direct connections of *KCTD17*, based on topological overlap values, are highlighted in red. Larger circles represent the most interconnected genes in the module, including *KCTD17*.

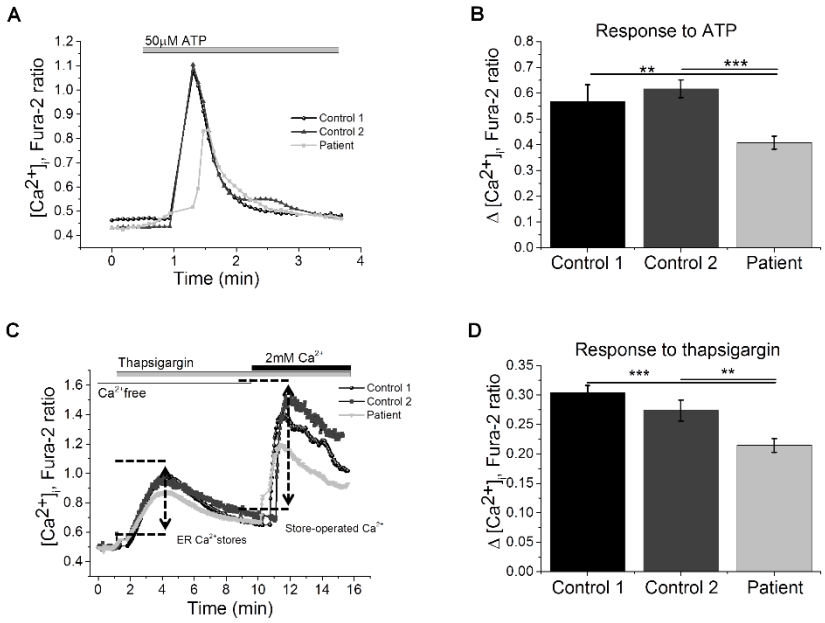


Figure 4. Functional studies showing abnormalities of endoplasmic reticulum calcium signaling in KCTD17 p.(Arg145His) substitution bearing fibroblasts.

After obtaining informed consent, fibroblasts were isolated from a skin biopsy taken from a subject with the KCTD17 p.(Arg145His) substitution (index family, III-2). Two unrelated age- and passage-matched controls (ctrl 1 and 2) were selected from in-house cell lines. The fibroblasts were cultured in Dulbecco's modified Eagle's medium supplemented with GlutaMAX, 10% heat-inactivated fetal bovine serum and 1% penicillin-streptomycin. Cytosolic calcium ($[Ca^{2+}]_c$) was measured with Fura-2, AM. Fibroblasts were loaded at room temperature for 30 minutes with 5M Fura-2 AM and 0.005% Pluronic in HBSS. Fluorescence measurements were obtained on an epifluorescence inverted microscope equipped with a 20x fluorite objective. $[Ca^{2+}]_c$ was monitored in single cells using excitation light provided by a Xenon arc lamp, with the beam passing monochromator centered at 340 and 380 nm (Cairn Research, Kent, UK). Emitted fluorescence light was reflected through a 515 nm long-pass filter to a cooled CCD camera (Retiga, QImaging, Surrey, BC, Canada). All

experiments were carried out in triplicate. Data are represented as the mean ± SEM. ‘‘n’’ indicates the total number of cells analyzed. The asterisks indicate $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***). (A) Typical trace of $[Ca^{2+}]_c$ in control and *KCTD17*-mutant fibroblasts in response to the application of 50 mM ATP. (B) Histograms showing a significantly decreased $[Ca^{2+}]_c$ response upon ATP stimulation in mutation-bearing fibroblasts (n=56) versus controls (control 1 n=41; control 2 n=35), as measured by changes in Fura-2 fluorescence intensity. (C) Typical trace of $[Ca^{2+}]_c$ in control and *KCTD17*-mutant fibroblasts in response to the application of thapsigargin (1 μM), and subsequent Ca^{2+} challenge (2 mM). (D) Histograms demonstrating a significant reduction in ER calcium pool in response to thapsigargin in mutation-bearing fibroblasts (n=53) versus controls (control 1 n=51; control 2 n=65), as measured by changes in Fura-2 fluorescence intensity.

Legend to videos

Index case (III.2): This segment shows the index case of the British family. Note the generalized dystonia with severe cranial and cervical involvement, including spasmodic dysphonia. Trunk and leg involvement are more evident while walking. Superimposed brief myoclonus are seen in the face and arms. Case V.3: This segment shows another member of the British family. He has frequent brief myoclonus involving the head and the arms. Myoclonus becomes more intense and frequent while talking. Index case German family: This segment shows the index case of the German family. He has generalized dystonia. There is marked cranial involvement, including dysarthria and tongue dystonia. Trunk involvement is more evident while walking. He also displays arm myoclonus.

Supplementary material

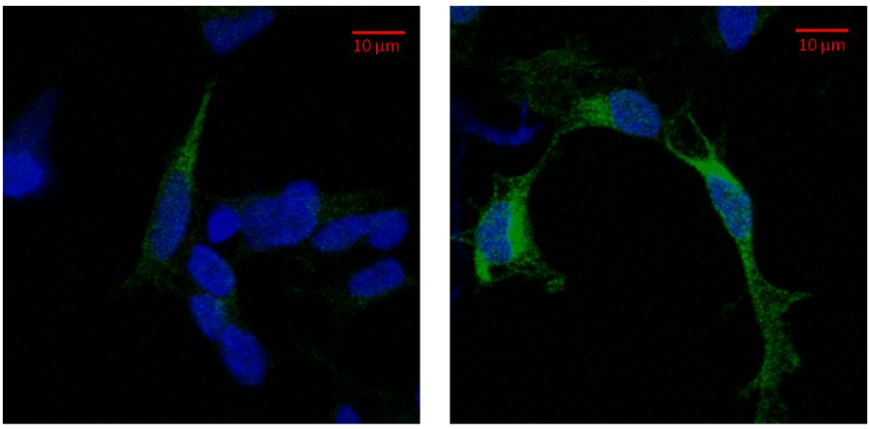


Figure S1. Immunocytochemistry in stably transfected SH-SY5 cells showing no difference between wild-type and mutant KCTD17 subcellular localization.

The mutation c.434 G>A p.(Arg145His) was inserted by recombinant PCR. Both N- and C-terminal HA tagged wild-type and mutant cDNAs were inserted with a 1-step recombinant PCR into pcDNA3.1 constructs for expression in mammalian cells. Stable SH-SY5 cells were generated by electroporating 5 µg linearized tagged WT and mutant plasmids into ~1 million cells and G418 (InvivoGen) selection at 250 mg/l over at least 4 weeks and at least 6 passages. A control cell line expressing the empty vector was obtained in parallel. After fixation with either 4% PFA in PBS or ice-cold 50% methanol/50% acetone, cells were blocked in PBS+2% BSA%, 3% normal goat serum, 1% NP-40, 0.5% sodiumdesoxycholate for 30 minutes and primary antibodies added in block diluted 1:1 with PBS at 4°C overnight. The Roche 3F10 monoclonal antibodies were used for detection of the HA tag, at 1:1000 dilution. After washing, secondary detection used Alexa-dye labelled, highly cross-absorbed anti-rat (Invitrogen, UK) in 0.5x block, with 1 mg/l DAPI. Microscopy was performed on a Zeiss confocal microscope. HA-tagged KCTD17 is shown in green.

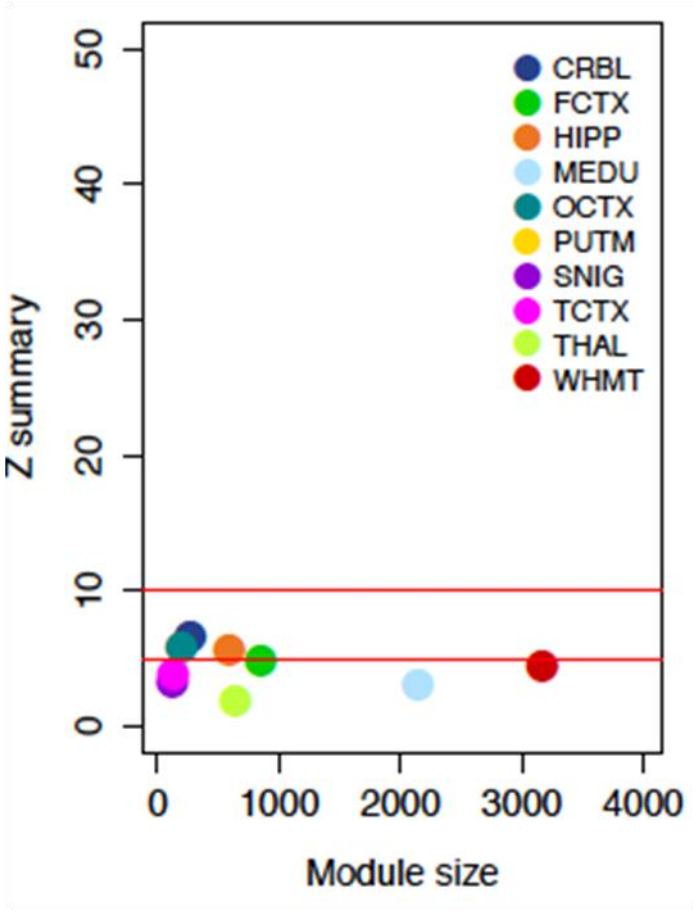


Figure S2. Putamen *KCTD17*-containing module preservation across other brain regions.

Module preservation statistics were calculated (z score) to assess how well modules from one tissue are reproducible (or preserved) in another brain region.[1] Previously proposed thresholds were considered (z score of <2 indicates no evidence of module preservation, z score between 2 and <10 indicates weak to moderate evidence, and z score of ≥10 indicates strong evidence). The module is poorly conserved across other brain regions, indicating its specificity to the putamen.

Chr	Position (hg19)	Gene (Transcript)	Variant	GERP score[a]	CADD C-score[b]	SIFT	Provean	PolyPhen-2 HumVar	Mutation Taster	Gene previously associated with disease?	Linkage analysis (LOD score)
1	152276386	*FLG* (NM_002016.1)	c.10976 C>T p.(Ser3659Phe)	2.48	11.15	T (0.06)	N (-0.8)	D (0.78)	P (0.99)	Yes, skin diseases (e.g. ichthyosis vulgaris, and/or eczema)[2]	< -2
1	228401208	*OBSCN* (NM_052843.3)	c.1055 T>G; p.(Phe352Cys)	5.58	21	D (0)	D (-3.8)	D (0.94)	D (0.99)	Hypertrophic cardiomyopathy[3]	< -2
8	133622476	*LRRC6* (NM_012472.4)	c.1076 A>C; p.(Lys359Thr)	3.5	15.74	D (0.01)	D (-3.1)	B (0.39)	D (0.99)	Recessive primary ciliary dyskinesia[4]	< -3
22	37453460	*KCTD17* (NM_001282684.1)	c.434 G>A; p.(Arg145His)	4.46	28.8	D (0)	D (-4.8)	D (0.53)	D (0.99)	No	2.4

Table S1 – Summary of novel variants detected by whole-exome sequencing and shared by individuals V-3 and IV-14. B=benign; D=deleterious/damaging/disease-causing; N=neutral; P=polymorphism; T=tolerated. [a]Positive scores represent a substitution deficit and indicate that a site may be under evolutionary constraint. Negative scores indicate that a site is probably evolving neutrally. Positive scores scale with the level of constraint, such that the greater the score, the greater the level of evolutionary constraint inferred to be acting on that site. [b]C-scores greater or equal 10 indicates that the variant is predicted to be the among the 10% most deleterious substitutions that you can do to the human genome; a score of greater or equal 20 indicates the 1% most deleterious.

Exon 1 FOR	AGGCGCGGACTACAGCTC
Exon 1 REV	CCACGGCAATGGGTACATC
Exon 2 FOR	TCTCCCTCCACTCTCCTTC
Exon 2 REV	TCCTGGTTGTCCAAATGG
Exon 3 FOR	GGAGGGAACAAGAGGAGAATG
Exon 3 REV	TCCCAACCTCCTCTGCTTC
Exon 4 FOR	TCTTCTTTGGGTATGTTGCG
Exon 4 REV	TGGTCAGAGGCTAGGAGGTC
Exon 5 FOR	GAGGTCTGTCGTATCCTGCC
Exon 5 REV	AGAGGTGGAGGGATGGTG
Exon 6 FOR	CTTTCACCTTGCCTGAGACC
Exon 6 REV	AGGCAAGTGGCTGAGCTAAC
Exon 7 FOR	CAGGGTTAGCTCAGCCACTT
Exon 7 REV	AGGCAGGGTGCAGATGAGAT
Exon 8 FOR	TCTGTGCCCACTAACCCTG
Exon 8 REV	TCAAGAGATGAGCACCCTCC
Exon 9 FOR	CACCCGTCAATCTCCTCTC
Exon 9 REV	AGGCAGGAGTAAGTCACAGC

Table S2. *KCTD17* primers used for Sanger sequencing

Marker	Chromosomal position	Genotype UK family	Genotype German family
rs5756370	37242476	A	A
rs6000449	37251377	A	A
rs4821542	37252918	G	G
rs909483	37260474	A	A
rs2413429	37289869	G	A
rs4821558	37308785	G	A
rs11705394	37329676	A	A
rs9622506	37338286	A	G
rs8137446	37347959	G	G
rs9622521	37350881	G	G
rs4821576	37357169	G	G
rs8142593	37363121	A	A
rs877166	37369148	C	C
rs5756437	37375668	G	G
rs1157557	37381674	G	G
rs5756477	37407527	G	A
rs5756492	37424991	G	G
Microsatellite 19xAG	37446300	17[a]	18/14[a]
***KCTD17* c.434G>A**	**37453460**	**A**	**A**
rs2160906	37493178	G	G
rs228924	37507250	A	G
rs11914132	37509087	G	G
rs228942	37524619	C	C
rs3218258	37544245	A	G
rs229483	37553619	G	A
rs12167757	37567490	G	G
rs229518	37577872	A	A
rs11913300	37580627	A	A
rs5756540	37582205	G	G
rs5756546	37589805	G	G
rs64547	37592504	A	A
rs9610680	37621951	A	G
rs8137698	37624236	G	A
rs739042	37625419	G	G
rs2285110	37628145	G	G
rs9607431	37629938	C	A
rs5995404	37632938	C	C

Table S3. Disease haplotype of the families with the *KCTD17* c.434 G>A p.(Arg145His)

SNP markers on chromosome 22 located ~0.5 Mb up- and down-stream the *KCTD17* C.434 G>A mutation were analysed and compared. In the British family, the haplotype of the identified *KCTD17* mutation was determined using MERLIN.[5] The German case was genotyped using the same array, HumanCytoSNP-12 DNA Analysis BeadChip Kit (Illumina, San Diego). In the German case SNP phasing was possible only for homozygous alleles. The *KCTD17* c.434 G>A mutation is marked in red. All alleles where the haplotype of the UK family differs from that of the German family are highlighted in yellow. The physical position of the markers refers to the human genome assembly hg19.

[a]These values indicate the number of AG repeats

Supplemental references

1. Langfelder, P., Luo, R., Oldham, M.C., and Horvath, S. (2011). Is my network module preserved and reproducible? PLoS Comput Biol 7, e1001057.

2. Irvine, A.D., McLean, W.H., and Leung, D.Y. (2011). Filaggrin mutations associated with skin and allergic diseases. N Engl J Med 365, 1315-1327.

3. Arimura, T., Matsumoto, Y., Okazaki, O., Hayashi, T., Takahashi, M., Inagaki, N., Hinohara, K., Ashizawa, N., Yano, K., and Kimura, A. (2007). Structural analysis of obscurin gene in hypertrophic cardiomyopathy. Biochem Biophys Res Commun 362, 281-287.

4. Kott, E., Duquesnoy, P., Copin, B., Legendre, M., Dastot-Le Moal, F., Montantin, G., Jeanson, L., Tamalet, A., Papon, J.F., Siffroi, J.P., et al. (2012). Loss-of-function mutations in LRRC6, a gene essential for proper axonemal assembly of inner and outer dynein arms, cause primary ciliary dyskinesia. Am J Hum Genet 91, 958-964.

5. Abecasis, G.R., Cherny, S.S., Cookson, W.O., and Cardon, L.R. (2002). Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet 30, 97-101.

Chapter 3

The *CACNA1B* R1389H variant is not associated with myoclonus-dystonia in a large European multicentric cohort

Niccolo E. Mencacci,[1] Léa R'Bibo,[1] Sara Bandres Ciga,[1,2] **Miryam Carecchio**,[3,4] Giovanna Zorzi,[3] Nardo Nardocci,[3] Barbara Garavaglia,[4] Amit Batla,[5] Kailash P. Bhatia,[5] Alan M. Pittman[1] John Hardy,[1] Anne Weissbach,[6] Christine Klein,[6] Thomas Gasser,[7] Ebba Lohmann,[7] and Nicholas W. Wood[1].

[1]Department of Molecular Neuroscience, Institute of Neurology, University College London, London WC1N 3BG, United Kingdom
[2]Department of Physiology and Institute of Neurosciences Federico-Olóriz, Centro de Investigaciones Biomedicas (CIBM), University of Granada, 18071 Granada, Spain
[3]Neuropediatrics Unit, IRCCS Istituto Neurologico Carlo Besta, 20133 Milan, Italy
[4]Molecular Neurogenetics Unit, IRCCS Istituto Neurologico Carlo Besta, 20133 Milan, Italy
[5]Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, London WC1N 3BG, United Kingdom
[6]Institute of Neurogenetics, University of Lübeck, 23538 Lübeck, Germany
[7]Department of Neurodegenerative Diseases, Hertie Institute for Clinical Brain Research, University of Tübingen, and German Center for Neurodegenerative Diseases (DZNE), 72076 Tübingen, Germany

Hum Mol Genet. 2015 Sep 15;24(18):5326-9. doi: 10.1093/hmg/ddv255.

Abstract

Myoclonus-dystonia (M-D) is a very rare movement disorder, caused in approximately 30-50% of cases by mutations in *SGCE*. The *CACNA1B* variant c.4166G>A; (p.R1389H) was recently reported as the likely causative mutation in a single 3-generation Dutch pedigree with 5 subjects affected by a unique dominant M-D syndrome and cardiac arrhythmias. In an attempt to replicate this finding, we assessed by direct sequencing the frequency of *CACNA1B* c.4166G>A; (p.R1389H) in a cohort of 520 M-D cases, in which *SGCE* mutations had been previously excluded. 146 cases (28%) had a positive family history of M-D. The frequency of the variant was also assessed in 489 neurologically healthy controls and in publicly available datasets of genetic variation (1000 Genomes, Exome Variant Server and Exome Aggregation Consortium). The variant was detected in a single sporadic case with M-D, but in none of the 146 probands with familial M-D. Overall, the variant was present at comparable frequencies in M-D cases (1/520; 0.19%) and healthy controls (1/489; 0.2%). A similar frequency of the variant was also reported in all publicly available databases. These results do not support a causal association between the *CACNA1B* c.4166G>A; (p.R1389H) variant and M-D.

Introduction

Myoclonus-dystonia (M-D [MIM 159900]) is a rare familial movement disorder, which classically features a variable combination of non-epileptic myoclonic jerks and dystonia (1). Heterozygous loss-of-function mutations in the maternally imprinted ε-sarcoglycan gene (*SCGE*, DYT11; [MIM 604149]) represent a major cause of autosomal dominant M-D (2). However up to 50-70% of familial cases with M-D lack mutations in *SGCE* (3-5), suggesting that disease-causing mutations in other genes are responsible for this syndrome.

Recently Groen and colleagues identified the missense variant c.4166G>A; (p.R1389H) (rs184841813) in *CACNA1B* [MIM 601012] as the likely causative mutation in a Dutch pedigree with five subjects affected by autosomal dominant M-D lacking mutations in *SGCE* (6). Unique features in the pedigree were lower limb orthostatic high-frequency myoclonus, attacks of limb painful cramps and cardiac arrhythmias in 3 of the affected subjects (7). Sanger sequencing of the *CACNA1B* exons coding for the protein portion spanning from III-S5 to III-S6 failed to reveal other mutations in a further 47 M-D cases.

CACNA1B encodes neuronal voltage-gated calcium channels CaV2.2, which have a key role in controlling synaptic neurotransmitter release (8). Furthermore *CACNA1A* [MIM 601011] mutations in the homologous region of the gene cause familial hemiplegic migraine [MIM 141500] (9) and episodic ataxia type 2 [MIM 108500] (10).

The *CACNA1B* p.(R1389H) substitution represents therefore an excellent candidate as a disease-causing mutation for M-D. However, in the absence of identification of *CACNA1B* mutations in other unrelated pedigrees, the implication of mutations in this gene as a cause for M-D

is not confirmed.

In this study, we assessed the frequency of the *CACNA1B* c.4166G>A; (p.R1389H) variant in a large multicentric cohort of M-D cases without mutations in *SGCE* (both point mutations and copy number variants).

Results

A total of 520 M-D cases (28% were familial) were screened for the presence of the c.4166G>A; (p.R1389H) variant. Additionally, we assessed the frequency of the variant in whole-exome sequencing data from 489 white healthy controls of UK and US origin and in European cases listed in publicly available datasets of genetic variation (1000 Genomes, Exome Variant Server and Exome Aggregation Consortium). None of the 146 probands with familial M-D carried the *CACNA1B* c.4166G>A; (p.R1389H) variant. The variant was detected only in a single female case of UK origin with sporadic M-D (see chromatogram of the mutation in the Supplementary Material, **Figure S1**). This case presented in her mid 30s with tremulous cervical dystonia and myoclonic jerks in the upper limbs. She had no family history for M-D or any other movement disorder. No other family members were available for segregation analysis of the variant.

The total carrier frequency in our M-D cohort, including familial and sporadic cases, is 0.19% (1/520 cases). The variant is present at a similar frequency in our healthy controls (0.2%; 1/489 individuals). The control carrier of the variant is a 38-year old male without any neurological disease and with no relevant family history of movement disorders.

The *CACNA1B* c.4166G>A; (p.R1389H) variant is reported at comparable frequencies in the 1000 genome project (0.26%; 1/379

individuals) and Exome Variant Server (0.28%; 12/4,203 individuals) databases. In the Exome Aggregation Consortium database, c.4166G>A; (p.R1389H) is present in 0.11% (38/33,367) of the European subjects (difference to M-D cases not significant; Fisher's exact test $p = 0.4$).

Discussion

The advent of next generation sequencing has led to an extraordinary acceleration in the discovery rate of rare genetic variants, the majority of which are of uncertain clinical significance. Hence, a close scrutiny is necessary before causally linking a candidate variant to a disease. To avoid false assignment of pathogenicity, MacArthur and colleagues have recently proposed guidelines for implicating causality of rare variants in human disease (11).

In family-based studies, assessment of co-inheritance of a candidate variant with the disease status within family members represents the first requirement to prove causality.

The c.4166G>A; (p.R1389H) variant was identified by Groen and colleagues through a combination of whole-exome sequencing and linkage analysis (13 chromosomal regions identified, with a maximum LOD score of 1.2) in a single dominant M-D pedigree. Notably, two other rare missense changes, c.10355A>G; (p.Q3452R) in *VPS13D* [MIM 608877] and c.5308C>T; (p.R1770C) in *SPTAN1* [MIM 182810], were found to perfectly co-segregate with the disease in the family. *De novo* mutations in *SPTAN1* have been shown to cause a neurological phenotype (West syndrome with severe cerebral hypomyelination,

spastic quadriplegia, and developmental delay) (12) and more recently a microdeletion encompassing *SPTAN1* was detected in a child with epileptic encephalopathy and severe dystonia (13).

Given the clinical presentation pointing towards a possible channelopathy, the authors assumed that the causative variant was the one in *CACNA1B* (6).

However, co-segregation of a variant with disease in a single pedigree does not establish with certainty its pathogenic role, especially if other co-segregating coding variants and the possibility of a separate undetected pathogenic variant in linkage disequilibrium cannot be convincingly ruled out.

In addition, a candidate variant responsible for a rare disease should be found at a low frequency in population controls, consistent with the proposed model of inheritance and disease prevalence.

M-D is a very rare disorder with a suggested prevalence of around 2 per million in Europe (14). We would therefore anticipate highly penetrant mutations causing dominant forms of M-D to be absent or extremely rare in the general population. Yet, this is not the case for p.(R1389H), which is present at a considerable frequency in our healthy controls and all publicly available databases (~0.1-0.3%). According to the Exome Aggregation Consortium database, the carrier frequency of this variant in Europeans is ~4 times higher than the *TOR1A* [MIM 605204] c.904_906delGAG deletion (0.026%), which is by far the most common single mutation responsible for dystonia described to date (15). Given this frequency, if c.4166G>A; (p.R1389H) were a pathogenic variant, we would expect it to be responsible for a large proportion of familial

M-D cases. However, in our cohort not only was the variant not identified in any of the probands with familial M-D, but the overall frequency of the variant did not differ between M-D cases and healthy controls. This does not support a pathogenic effect of the variant even assuming a reduced penetrance.

In conclusion, our study suggests that the role of the *CACNA1B* variant c.4166G>A; (p.R1389H) as a cause for M-D is questionable. Further genetic evidence is needed before designating *CACNA1B* mutations as a cause for dominant M-D.

Materials and methods

A total of 520 M-D cases of British, German and Italian origin were recruited in four tertiary movement disorders centers (London, Lübeck, Tübingen and Milan). All selected cases fulfilled the proposed diagnostic criteria for M-D (2). 146 cases (28%) had a positive family history of M-D. All participants provided written informed consent.

M-D cases were screened by direct Sanger sequencing for mutations in exon 28 of *CACNA1B* (RefSeq NM_000718.3), which contains the c.4166G>A; (p.R1389H) variant. Each reaction was performed in a 20 µl volume containing 10 µl of FastStart PCR master mix (Roche), 5 µl of water, 2 µl of each primer (5pmol/µL), and 30 ng of genomic DNA. After purification PCR products sequenced in both forward and reverse directions using BigDye Terminator v3.1 sequencing chemistry and then were loaded on the ABI3730xl genetic analyzer (Applied Biosystems, Foster City, CA). The sequences were analyzed with Sequencher software (version 4.9; Gene Codes).

Whole-exome sequencing data from 489 white healthy controls of UK and US origin were provided by the International Parkinson's Disease Genomic Consortium (IPDGC). In short, prior to sequencing, DNA templates were bridge amplified to form clonal clusters inside a flowcell via the cBot cluster generation process. The flowcells were then loaded into the next-generation sequencer Illumina HiSeq 2000. Paired end sequence reads were aligned with Burrows-Wheeler Aligner (BWA) against the reference human genome (UCSC hg19). Duplicate read removal, format conversion, and indexing were performed with Picard (http://picard.sourceforge.net/). The Genome Analysis Toolkit (GATK) was used to recalibrate base quality scores, perform local realignments around possible indels, and to call and filter the variants.

Web resources

1000 Genomes project (URL: http://www.1000genomes.org/) [last accessed: April 2015].

Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/) [last accessed: April 2015]

Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: http://exac.broadinstitute.org) [last accessed: April 2015].

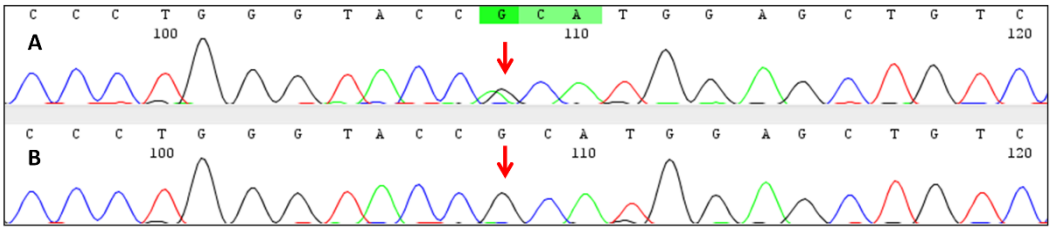


Figure S1. Chromatograms showing the *CACNA1B* c.4166G>A; (p.R1389H) variant (**A**) and a control sequence (**B**).

Acknowledgements

We are grateful to all participants in this study. This work was supported by a Medical Research Council/Wellcome Trust Strategic Award [grant number WT089698/Z/09/Z] and a grant from the Bachman-Strauss Dystonia Parkinsonism Foundation. The work was undertaken at University College London Hospitals (UCLH) and University College London (UCL), who receive support from the Department of Health's NIHR Biomedical Research Centers funding streams. We thank the International Parkinson Disease Genomic Consortium (IPDGC) for providing the exome-sequencing data of healthy population controls. We would also like to thank the Exome Aggregation Consortium and the groups that provided exome variant data for comparison. A full list of contributing groups can be found at http://exac.broadinstitute.org/about. The authors would also like to thank the NHLBI GO Exome Sequencing Project and its ongoing studies which produced and provided exome variant calls for comparison: the Lung GO Sequencing Project (HL-102923), the WHI Sequencing Project (HL-102924), the Broad GO Sequencing Project (HL-102925), the Seattle GO Sequencing Project (HL-102926) and the Heart GO Sequencing Project (HL-103010). NEM is funded by a MRC-Wellcome Trust grant. SB holds a FPU fellowship from the Spanish Ministry of Education and Science jointly with a short-term stay grant by Cei-BioTic and University of Granada. AMP is funded by the Reta Lila Weston Trust. CK is the recipient of a career development award from the Herman and Lilly Schilling Foundation. EL and TG are supported by a grant from the Dystonia Medical Research Foundation (DMRF). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest Statement

All authors declare no conflict of interest concerning this research.

References

1 Nardocci, N. (2011) Myoclonus-dystonia syndrome. *Hand. Clin. Neurol.* **100**, 563-575.

2 Kinugawa, K., Vidailhet, M., Clot, F., Apartis, E., Grabli, D. and Roze, E. (2009) Myoclonus-dystonia: an update. *Mov. Disord.*, **24**, 479-489.

3 Ritz, K., Gerrits, M.C., Foncke, E.M., van Ruissen, F., van der Linden, C., Vergouwen, M.D., Bloem, B.R., Vandenberghe, W., Crols, R., Speelman, J.D. *et al.* (2009) Myoclonus-dystonia: clinical and genetic evaluation of a large cohort. *J. Neurol. Neurosurg. Psychiatry*, **80**, 653-658.

4 Carecchio, M., Magliozzi, M., Copetti, M., Ferraris, A., Bernardini, L., Bonetti, M., Defazio, G., Edwards, M.J., Torrente, I., Pellegrini, F. *et al.* (2013) Defining the epsilon-sarcoglycan (SGCE) gene phenotypic signature in myoclonus-dystonia: a reappraisal of genetic testing criteria. *Mov. Disord.*, **28**, 787-794.

5 Grunewald, A., Djarmati, A., Lohmann-Hedrich, K., Farrell, K., Zeller, J.A., Allert, N., Papengut, F., Petersen, B., Fung, V., Sue, C.M. *et al.* (2008) Myoclonus-dystonia: significance of large SGCE deletions. *Hum. Mutat.*, **29**, 331-332.

6 Groen, J.L., Andrade, A., Ritz, K., Jalalzadeh, H., Haagmans, M., Bradley, T.E., Jongejan, A., Verbeek, D.S., Nurnberg, P., Denome, S. *et al.* (2015) CACNA1B mutation is linked to unique myoclonus-dystonia syndrome. *Hum. Mol. Genet.*, **24**, 987-993.

7 Groen, J., van Rootselaar, A.F., van der Salm, S.M., Bloem, B.R. and Tijssen, M. (2011) A new familial syndrome with dystonia and lower limb action myoclonus. *Mov. Disord.*, **26**, 896-900.

8 Beuckmann, C.T., Sinton, C.M., Miyamoto, N., Ino, M. and Yanagisawa, M. (2003) N-type calcium channel alpha1B subunit (Cav2.2) knock-out mice display hyperactivity and vigilance state differences. *J. Neurosci.*, **23**, 6793-6797.

9 Carrera, P., Piatti, M., Stenirri, S., Grimaldi, L.M., Marchioni, E., Curcio, M., Righetti, P.G., Ferrari, M. and Gelfi, C. (1999) Genetic heterogeneity in Italian families with familial hemiplegic migraine. *Neurology*, **53**, 26-33.

10 Jen, J., Wan, J., Graves, M., Yu, H., Mock, A.F., Coulin, C.J., Kim, G., Yue, Q., Papazian, D.M. and Baloh, R.W. (2001) Loss-of-function EA2 mutations are associated with impaired neuromuscular transmission. *Neurology*, **57**, 1843-1848.

11 MacArthur, D.G., Manolio, T.A., Dimmock, D.P., Rehm, H.L., Shendure, J., Abecasis, G.R., Adams, D.R., Altman, R.B., Antonarakis, S.E., Ashley, E.A. *et al.* (2014) Guidelines for investigating causality of sequence variants in human disease. *Nature*, **508**, 469-476.

12 Saitsu, H., Tohyama, J., Kumada, T., Egawa, K., Hamada, K., Okada, I., Mizuguchi, T., Osaka, H., Miyata, R., Furukawa, T. *et al.* (2010) Dominant-negative mutations in alpha-II spectrin cause West syndrome with severe cerebral hypomyelination, spastic quadriplegia, and developmental delay. *Am. J. Hum. Genet.*, **86**, 881-891.

13 Matsumoto, H., Zaha, K., Nakamura, Y., Hayashi, S., Inazawa, J. and Nonoyama, S. (2014) Chromosome 9q33q34 microdeletion with early infantile epileptic encephalopathy, severe dystonia, abnormal eye movements, and nephroureteral malformations. *Pediatr. Neurol.*, **51**, 170-175.

14 Asmus, F. and Gasser, T. (2010) Dystonia-plus syndromes. *Eur.*

J. Neurol., **17 Suppl 1**, 37-45.

15 Valente, E.M., Warner, T.T., Jarman, P.R., Mathen, D., Fletcher, N.A., Marsden, C.D., Bhatia, K.P. and Wood, N.W. (1998) The role of DYT1 in primary torsion dystonia in Europe. *Brain*, **121**, 2335-2339.

Chapter 4

De novo mutations in PDE10A cause childhood-onset chorea with bilateral striatal lesions

Niccolò E. Mencacci,[1,2,17] Erik-Jan Kamsteeg,[3,17] Kosuke Nakashima,[4,17] Lea R'Bibo,[1] David S. Lynch,[1] Bettina Balint,[5,6] Michèl A.A.P. Willemsen,[7] Matthew E. Adams,[8] Sarah Wiethoff,[1,9] Kazunori Suzuki,[4] Ceri H. Davies,[4] Joanne Ng,[10,11] Esther Meyer,[10] Liana Veneziano,[12] Paola Giunti,[1] Deborah Hughes,[1] F. Lucy Raymond,[13] **Miryam Carecchio**,[14,15] Giovanna Zorzi,[14] Nardo Nardocci,[14] Chiara Barzaghi,[15] Barbara Garavaglia,[15] Vincenzo Salpietro,[1] John Hardy,[1,16] Alan M. Pittman,[1,16] Henry Houlden,[1] Manju A. Kurian,[10,11] Haruhide Kimura,[4,18] Lisenka E.L.M. Vissers,[3,18] Nicholas W. Wood,[1,18] and Kailash P. Bhatia.[5,18]

[1]Department of Molecular Neuroscience, UCL Institute of Neurology, WC1N 3BG London, United Kingdom

[2]IRCCS Istituto Auxologico Italiano, Department of Neurology and Laboratory of Neuroscience – Department of Pathophysiology and Transplantation, "Dino Ferrari" Centre, Universita` degli Studi di Milano, 20149 Milan, Italy

[3]Department of Human Genetics, Donders Centre for Brain, Cognition and Behavior, Radboud University Medical Center, Geert Grooteplein 10, 6525 GA, Nijmegen, the Netherlands

[4]CNS Drug Discovery Unit, Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited, 251-8555 Fujisawa, Japan.

[5]Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, WC1N 3BG London, United Kingdom
[6]Department of Neurology, University Hospital Heidelberg, 69120 Heidelberg, Germany
[7]Department of Paediatric Neurology, Donders Centre for Brain, Cognition and Behavior, Radboud University Medical Center, Geert Grooteplein 10, 6525 GA, Nijmegen, the Netherlands
[8]Lysholm Department of Neuroradiology, National hospital for neurology and neurosurgery, WC1N 3BG London, United Kingdom
[9] Center for Neurology and Hertie Institute for Clinical Brain Research, Eberhard-Karls-University, 72076 Tübingen, Germany
[10]Developmental Neurosciences, UCL Institute of Child Health, London WC1N 1EH, United Kingdom.
[11]Department of Neurology, Great Ormond Street Hospital, WC1N 3JH London, United Kingdom
[12]Institute of Translational Pharmacology, National Research Council, 00133 Rome, Italy.
[13]Department of Medical Genetics, University of Cambridge, CB2 0XY Cambridge, United Kingdom
[14]Neuropediatrics Unit, IRCCS Istituto Neurologico Carlo Besta, 20133 Milan, Italy
[15]Molecular Neurogenetics Unit, IRCCS Istituto Neurologico Carlo Besta, 20133 Milan, Italy
[16]Reta Lila Weston Institute of Neurological Studies, UCL Institute of Neurology, WC1N 3BG London, United Kingdom
[17] Shared first authors
[18]Joint senior authors

Am J Hum Genet. 2016 Apr 7;98(4):763-71. doi: 10.1016/j.ajhg.2016.02.015.

Abstract

Chorea is a hyperkinetic movement disorder resulting from dysfunction of striatal medium spiny neurons (MSNs), which form the main basal ganglia output projections. Here we used whole exome sequencing to unravel the underlying genetic cause in three unrelated individuals with a very similar and unique clinical presentation of childhood-onset chorea and characteristic brain MRI showing symmetrical bilateral striatal lesions. All cases were identified to carry a de novo heterozygous mutation in PDE10A (c.898T>C; p.Phe300Leu in two cases and c.1000T>C; p.Phe334Leu in one case), encoding a phosphodiesterase highly and selectively expressed in MSNs. PDE10A contributes to the regulation of the intracellular levels of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Both substitutions affect highly conserved amino acids located in the regulatory GAF-B domain, which, by binding to cAMP, stimulates the activity of the PDE10A catalytic domain. In silico modeling shows that the mutated residues are located deep into the binding pocket, where they are likely to alter cAMP binding properties. In vitro functional studies showed that both substitutions do not affect the basal PDE10A activity, but severely disrupt the stimulatory effect mediated by cAMP binding to the GAF-B domain. The identification of PDE10A mutations as a cause of chorea further motivates the study of cAMP signaling in MSNs, and highlights the crucial role of striatal cAMP signaling in the regulation of basal ganglia circuitry. Pharmacological modulation of this pathway may offer promising aetiologically-targeted treatments for chorea and other hyperkinetic movement disorders.

Report

Movement disorders comprise a large clinically and genetically heterogeneous group of disorders, which can be subdivided in various clinical entities, including dystonia and chorea. Although monogenic causes are overall rare, >200 genes are known to cause either an isolated movement disorder or a syndromic form of movement disorders when mutated.[1-3] However, in total, mutations in these genes only explain a small proportion of cases, suggesting that mutations in more genes await discovery.

Chorea is a hyperkinetic movement disorder clinically characterized by continuous and brief involuntary movements, flowing from one body part to another, being unpredictable in terms of timing, speed and direction. Chorea is a major feature of several inherited neurological disorders.[4] Functional dysregulation of striatal GABAergic medium spiny neurons (MSNs), which form the main basal ganglia output projections, is considered to underlie the pathophysiology of the choreic movements.[5]

We have identified three subjects of European descent who presented with a similar childhood-onset movement disorder predominantly characterized by chorea and bilateral striatal abnormalities on cerebral magnetic resonance imaging (MRI). The main clinical and radiological features of the three cases are presented in Table 1. In brief, all three cases presented in childhood (age of onset between 5 and 10 years) with a scarcely progressive movement disorder dominated by chorea. Developmental milestones were normal and there were no other major

neurological features, in particular intellectual disability or cognitive decline. Given these clinical features and the absence of a significant progression of symptoms, a diagnosis of benign hereditary chorea (BHC; MIM 118700)[6] was initially considered. However, brain MRI consistently showed bilateral T2 hyperintensity within the striatum in all three cases (Figure 1), which is an atypical finding for BHC.

It is noteworthy that the MRI images of case 1 (subject II-1 in figure 2A; aged 11 when scanned) showed slight swelling of the striata (Figure 1A), together with restricted diffusion (Figure 1B and 1C), suggesting an active disease process. Conversely, MRI of case 2 (subject II-1 in figure 2A; aged 22 when scanned) demonstrated modest atrophy of the putamina (Figure 1D) and normal diffusion (Figure 1E and 1F), suggesting a more advanced stage of disease. The MRI of case 3 (subject II-8 in figure 2A; aged 53 when scanned) was markedly degraded by movement artefacts, but also showed T2 hyperintensity within the posterolateral putamina (figure S1A), albeit less dramatic than in the two younger cases.

Interestingly, case 3, who is currently 60-year-old, developed levodopa-responsive parkinsonism with freezing and falls in the fifth decade. Striatal dopamine reuptake transporter density imaging (i.e. DatSCAN) was bilaterally abnormal, consistent with nigrostriatal degeneration (figure S1B).

The homogeneous clinical and radiological appearance of these cases was suggestive of a common genetic entity. Yet, extensive genetic and biochemical diagnostic work-up, focused on a wide spectrum of genetic diseases - including BHC, metabolic disorders, and

mitochondrial diseases, was unrevealing.

Next, whole exome sequencing (WES) was performed in all three cases, as well as in the unaffected parents of case 1 and 2. The study was approved by the local ethics committees (CMO Arnhem/Nijmegen for case 1 under the realm of diagnostic exome sequencing and UCLH project 06/N076 for cases 2 and 3). Written informed consent was obtained for all individuals, after which DNA was extracted from peripheral lymphocytes following standard protocols. WES was performed as previously described.[7; 8] Briefly, exomes were enriched using either Agilent SureSelectXT Human All Exon 50 Mb Kit (Case 1) or Illumina's Nextera Rapid Capture (Case 2 and 3) and sequenced on SOLiD 5500XL (Case 1) or a Hiseq3000 (Case 2 and 3) to an average sequence depth of 91 fold, with on average 89% of targets covered at least 20-fold. Subsequently, variant calling was performed, followed by variant annotation using a custom in-house diagnostic pipeline[7] (Case 1) or ANNOVAR[9] (Case 2 and 3). Given the sporadic occurrence of the phenotypes, filtering of variants focused on *de novo* dominant or recessive mutations (Figure 2A). Under the assumption that all three cases would carry a mutation in the same gene, we determined the overlap for putatively damaging mutations (defined as nonsense, frameshift, canonical splice site, predicted damaging missense mutations based on CADD scores[10] >20) with a minor allele frequency <1% in Exome Aggregation Consortium (ExAC)[11] and in an in-house database containing >10,000 individuals.

We identified only a single gene, *PDE10A* (MIM 610652; transcript NM_001130690.2), containing a variant in all three individuals. In

case 1, the heterozygous variant c.1000T>C was identified and predicted to result in p.Phe334Leu. Case 2 and 3 showed the same heterozygous variant, c.898T>C, which is predicted to result in p.Phe300Leu. Notably, the family-based sequencing approach of cases 1 and 2 directly indicated that both *PDE10A* mutations had occurred *de novo* (Figure 2A). The parents of case 3 are deceased, but the DNA of six unaffected siblings was available for testing, and none of them carried the mutation. Further haplotype analysis using three microsatellites spanning the *PDE10A* locus identified the four parental haplotypes and revealed that the individual carrying the mutation shares one of the haplotypes with two siblings and the other with three other siblings, strongly suggestive for the *de novo* occurrence of the mutation also in this case (Figure 2A and Figure S2). Analysis of the same three microsatellites in the family of case 2, who carries the same *de novo PDE10A* change, indicates the mutation has arisen on a different background haplotype (Figure S2). *De novo* mutations in *PDE10A* have not been observed in control individuals,[12-16] and neither p.Phe300Leu nor p.Phe334Leu are listed in ExAC (last accessed in November 2015) or in-house databases, together containing ~75,000 individuals. *PDE10A* has a Residual Variation Intolerance Score (RVIS)[17] of -0.98, indicating it belongs to the top 8.8% (<10%) of the human genes most intolerant to genetic variation. Furthermore, constraint metrics reported in ExAC indicate that *PDE10A* is intolerant to both loss-of-function (pLI=1.00) and missense mutations (z-score=3.78).[18] Interspecies alignment of protein sequences generated using Clustal Omega[19] revealed that the substitutions affect amino acid residues that are completely conserved down to invertebrate species

(Figure 2B).

Next, we explored the regional expression of these genes in the normal adult human brain. To this end, we used microarray data (Affymetrix Exon 1.0 ST) from human post-mortem brain tissue collected by the UK Human Brain Expression Consortium (UKBEC) as previously described.[20] This analysis shows exceptionally high expression in the putamen (Figure 3A), which is consistent with the data available on the Allen Mouse Brain Atlas[21] (Figure 3B and 3C) and previous work in the literature, demonstrating high and selective *PDE10A* expression in human striatum, both at the RNA and protein level.[22; 23]

PDE10A encodes a member of the cyclic nucleotide (cNMP) phosphodiesterase (PDE) family, consisting of 21 different genes, grouped into 11 sub-families based on their affinity for the type of cNMP (cyclic adenosine monophosphate [cAMP] and/or cyclic guanosine monophosphate [cGMP]), cellular regulation, expression and tissue distribution.[24] cNMPs are ubiquitously expressed intracellular second messengers, which modulate a broad range of cellular functions and pathways.[25] The intracellular concentration of cNMPs is tightly regulated through a fine balance between their synthesis, controlled by the activity of adenylyl/guanylyl cyclases,[26; 27] and degradation, mediated by PDEs which hydrolyze the cNMPs into their corresponding monophosphate nucleoside.[28] PDEs function as homodimers, with the dimer interface extending over the entire length of the molecule, and all share a highly similar catalytic domain located in the C-terminal portion of the protein. Conversely, the N-terminal

portion, which contains the regulatory domains, is variable and differs between different PDE families.[29] PDE10A contains two N-terminal domains, GAF-A and GAF-B, of which the latter binds to cAMP (Figure 2C).[30; 31] cAMP binding increases the enzyme activity of the PDE10A catalytic domain.[32] Although details of the GAF-B dependent modulation of PDE10A enzyme activity are currently unclear, a general mechanism for the regulation of all PDEs has been postulated. In the non-activated state the dimerized catalytic domains are packed against each other at the dimer interface, occluding the catalytic pockets. The binding of cAMP to the GAF-B domain induces a rotating movement of the catalytic domains, enabling substrate access to the catalytic pockets and a consequent increase of cNMP hydrolysis.[33]

The crystal structure of the PDE10A-GAF-B domain and its interaction with cAMP has been elucidated and consists of six stranded anti-parallel β-sheet (β3, β2, β1, β6, β5, β4), sandwiched between a three-helix bundle (α1, α2, and α5) on one side and three short helices (α3, α4, 3_{10}) on the other side.[34] The cAMP molecule is almost completely buried deep into a tight binding pocket, the floor of which is formed by the β-sheets and the roof by two α-helices (α3 and α4). Importantly, the amino acids Phe300 and Phe334 are located in the β1 and β3 sheets, positioned deep into the cAMP binding pocket of GAF-B and in very close proximity to the cAMP molecule (Figure 2D). It is therefore postulated that the substitutions severely affect the morphology of the GAF-B binding pocket and/or alter its affinity for cAMP.

To assess the functional effect of the identified PDE10A substitutions *in vitro*, we investigated whether they affect (i) PDE basal enzyme

activity and/or (ii) the stimulatory effect on PDE catalytic activity mediated by cAMP binding to the GAF-B domain. cDNA for human PDE10A (transcript NM_001130690.2) was used as a template and mutant constructs (c.898T>C; p.Phe300Leu and c.1000T>C; p.Phe334Leu) were inserted by site-directed mutagenesis. Wild-type (WT) and mutant constructs were cloned into the pcDNA3.1(+)neo vector (Thermo Fisher Scientific, Inc., Waltham, MA, US) and transfected into COS-7 cells (ECACC, Salisbury, UK). *In vitro* PDE enzyme activity was measured using scintillation proximity assay (SPA)-based method.[35] In this assay, the product of the PDE reaction, either [^{3}H]-labeled AMP or GMP, binds directly to yttrium silicate PDE SPA beads (GE Healthcare Ltd. UK), resulting in light emission. Reactions for kinetic studies were conducted using a mixture of [^{3}H]-labeled and unlabeled cAMP or cGMP together with either WT or mutant PDE10A-expressing COS-7 cell membrane fractions.
These experiments showed no significant difference between WT and mutant PDE10As (Figure S3), suggesting that both p.Phe300Leu and p.Phe334Leu do not affect substantially basal PDE10A enzyme activity.

We then explored whether the identified substitutions affect the stimulatory properties of cAMP binding to the GAF-B domain. Experiments were conducted using only [^{3}H]cGMP as a substrate (to avoid the binding of [^{3}H]cAMP substrate to the GAF-B domain) and the cAMP analogue 1-NO-cAMP (Biolog Life Science Institute, Bremen, Germany), which has a higher selectivity for the GAF-B domain over the catalytic site compared to cAMP (247-fold for 1-NO-cAMP vs. 8.7-fold for cAMP).[35] These experimental conditions were

chosen as, on the one hand, cAMP activates PDE10A enzyme activity via its binding to GAF-B and, on the other hand, competes at the catalytic domain with radio-labelled substrates and thus inhibits their degradation.[35] 1-NO-cAMP markedly increased (approximately 2.7-fold over the basal levels) the enzyme activity of WT PDE10A, whereas this effect was almost completely lost for both mutant PDE10As (Figure 2E). These experiments demonstrate that p.Phe300Leu and p.Phe334Leu severely affect the positive regulatory mechanism of cAMP binding to the GAF-B domain on PDE catalytic activity.

PDEs have previously been implicated in the pathogenesis of neurodegenerative disorders, such as Parkinson disease and Huntington disease.[36] Mutations in *PDE8B* (MIM 603390), a gene highly expressed in the brain and especially in the putamen, causes autosomal dominant striatal degeneration (ADSD, MIM 609161), a disease that clinically presents with adult-onset parkinsonism.[37; 38] Although the reported MRI abnormalities observed in subjects with ADSD are slightly different from those observed in our cases, it is striking that both diseases are caused by mutations in PDEs leading to clearly visible, largely symmetric, striatal MRI signal abnormalities. Furthermore, the fact that two PDEs are now directly linked to a basal ganglia disease may point towards a crucial role of PDEs in these types of disorders. The latter is of great interest given the pharmacological potential to manipulate PDE activity. Given its high and selective expression in striatal MSNs, PDE10A is a primary target in pharmacological research for diseases where dysregulation of striatal circuits is believed to be crucial (e.g. psychosis, Huntington disease, substance abuse and Parkinson

disease).[39]

According to the classic model of basal ganglia motor circuits, chorea mainly results from dysregulation of MSN activity.[40] Importantly, modulation of MSN activity is largely dependent on cAMP signaling.[41] cAMP synthesis, and thus indirectly its signaling, is promoted by the stimulation of the G protein-coupled receptors D1 dopamine receptors (D1DR) and adenosine 2 receptors (A2AR), whereas synthesis is inhibited by dopamine stimulation of D2 dopamine receptors (D2DR).[42] The G protein $G\alpha_{olf}$ positively couples D1DR and A2AR to the activation of adenyl cyclase 5 (AC5), the main molecule responsible for cAMP production in MSNs.[43] Interestingly, the genes encoding $G\alpha_{olf}$ (*GNAL* [MIM 139312]) and AC5 (*ADCY5*) have both been identified as cause of primary dystonia[44] and chorea[45; 46] respectively.

Mechanistically, *ADCY5* mutations seem to increase the AC5 activity with consequent raised intracellular cAMP levels in cellular models.[47] As both *PDE10A* and *ADCY5* pathogenic mutations cause chorea, but with PDE10A exerting an opposite effect to AC5 on cAMP levels, one would expect that the p.Phe300Leu and p.Phe334Leu variants exert a deleterious effect on the PDE enzyme activity. Recent studies suggest that PDE10A has two functional states; `active` and `super-active`.[32; 48] In presence of high intracellular levels of cAMP, its binding to the GAF-B domain would stimulate the PDE catalytic activity, switching PDE10A from the 'active' to the 'super-active' state. In light of this, PDE10A may function as a 'brake' for MSN activation. Our functional studies show that pathogenic *PDE10A* mutations located in the GAF-B

domain severely disrupt this positive regulatory mechanism without affecting the basal PDE enzyme activity. These mutations may therefore have a strong impact on the *in vivo* regulation of MSN activity, especially when MSNs are activated by high levels of cAMP. Given the homodimerized structure of PDE10A, the mutant proteins could exert a dominant negative effect on the activity of the WT protein.

In conclusion, we demonstrate that *de novo* dominant mutations in *PDE10A* are the cause of a unique movement disorder characterized by benign childhood-onset chorea and typical MRI abnormalities of the striatum. Of note, screening of a cohort of ~60 individuals with a BHC-like syndrome and lacking mutations in *NKX2-1* – clinically resembling subjects with *PDE10A* mutations, but with normal brain MRI - did not reveal any additional mutations in *PDE10A*. The latter suggests that *PDE10A*-related chorea may represent a distinct genetic clinico-radiological entity. Mutational screening of additional cohorts of cases with such MRI abnormalities is warranted to further define the clinical spectrum associated with *PDE10A* mutations. Furthermore, it will be important to establish whether the observation of parkinsonism with nigrostriatal degeneration in case 3 is coincidental or whether individuals with *de novo PDE10A* mutations are also at an increased risk of developing degeneration of nigral neurons. In this regard, recent work has demonstrated that striatal loss of PDE10A expression is associated with Parkinson's disease duration and severity.[49] With the previous discoveries of mutations in *GNAL*, *PDE8B*, and *ADCY5*, and now *PDE10A*, there is accumulating evidence that striatal MSNs intracellular cAMP signaling is crucial for normal activity of basal

ganglia circuitry, and that disruptions thereof play an important role in the pathophysiology of movement disorders. Our results highlight pharmacological manipulation of cAMP levels in MSNs as a promising therapeutic strategy for the treatment of chorea and other movement disorders.

Description of Supplementary Data

Figure S1, S2, and S3.

Acknowledgements

We would like to extend our thanks to the individuals whose participation made this research possible. This work was supported financially by a Medical Research Council/Wellcome Trust Strategic Award (WT089698/Z/09/Z), the Netherlands Organization of Scientific Research (ZonMW grant 40-41200-98-9131) and grants from the Bachman-Strauss Dystonia Parkinsonism Foundation, NIHR Bioresource Rare Diseases, and UK10K. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The work was undertaken at University College London Hospitals (UCLH), Great Ormond Street Hospital (GOSH) for Children NHS Foundation Trust and University College London (UCL), who receive support from the Department of Health's National Institute for Health Research (NIHR) Biomedical Research Centers funding streams. We acknowledge the "Cell lines and DNA Bank of Movement Disorders and Mitochondrial Diseases" of the Telethon Network of Genetic Biobanks (grant GTB12001J) and the Eurobiobank Network which provided the Italian samples. N.E.M. is funded by a NIHR funding scheme. A.M.P. is funded by the Reta Lila Weston Trust. M.A.K. is funded by a Wellcome Intermediate Fellowship. Next Generation Sequencing was performed at the UCL Institute of Neurology Sequencing Facility and the Genome Technology Center at the Radboudumc. Expression data was provided by the UK Human Brain Expression Consortium (UKBEC), which comprises John A. Hardy, Mina Ryten, Michael Weale, Daniah

Trabzuni, Adaikalavan Ramasamy, Colin Smith and Robert Walker. UKBEC members are affiliated with UCL Institute of Neurology (J.H., M.R., D.T.), King's College London (M.R., M.W., A.R.) and the University of Edinburgh (C.S., R.W.). The work was partly funded by Takeda Pharmaceutical Company Limited who provided support in the form of salaries for some of the authors (K.N., K.S., C.H.D., H.K.), but did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. All other authors report no potential conflict of interest relevant to this work.

Web Resources

The URLs for data presented herein are as follows:

Allen Mouse Brain Atlas: http://mouse.brain-map.org/

CADD: http://cadd.gs.washington.edu/home

Clustal Omega: http://www.ebi.ac.uk/Tools/msa/clustalo/

Exome Aggregation Consortium database:

http://exac.broadinstitute.org/

Genic Intolerance http://genic-intolerance.org/

Online Mendelian Inheritance in Man (OMIM), http://www.omim.org/.

UK Human Brain Expression Consortium: http://www.braineac.org/

		Case 1	Case 2	Case 3
Age at most recent clinical examination (*years*)		11	22	60
Gender		Male	Female	Female
Descent		European (Dutch)	European (British)	European (British)
***PDE10A* mutation**	Genomic[a]	Chr6:165829768 A>G	Chr6:165832223 A>G	Chr6:165832223 A>G
	cDNA[b]	c.1000T>C	c.898T>C	c.898T>C
	Protein	p.Phe334Leu	p.Phe300Leu	p.Phe300Leu
	Inheritance	*de novo*	*de novo*	*de novo*[d]
CADD score[c]		31.0	28.7	28.7
Neurology				
Developmental milestones		Normal	Normal	Normal
Cognition		Normal	Normal	Normal
Chorea (age of onset)		+ (5)	+ (8)	+ (5)

	Other	No	Anxiety	Adult-onset parkinsonism
MRI				
	Bilateral striatal hyperintensities	+	+	+
	Bilateral striatal swelling	+	-	-
	Restriction of diffusion	+	-	N.A.
	Bilateral striatal atrophy	-	+	+

Table 1: Genetic, clinical and radiological findings of individuals with *PDE10A* mutations

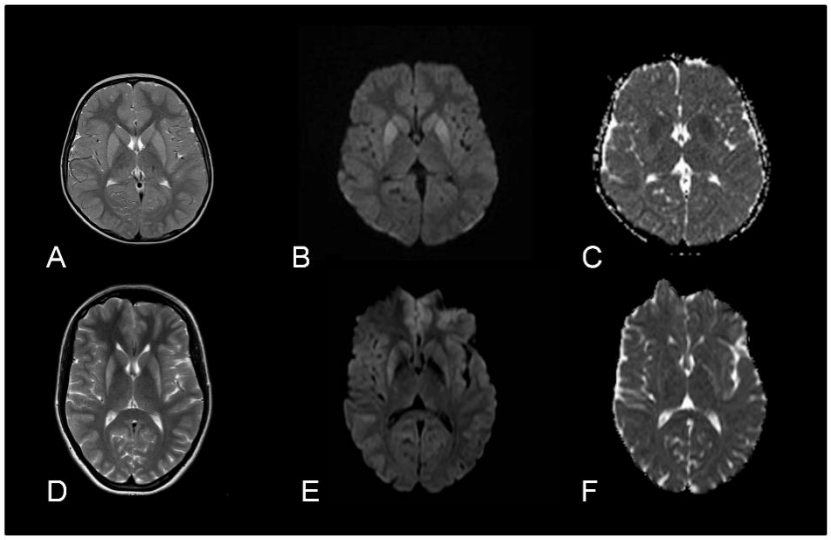


Figure 1. MRI features associated with dominant PDE10A mutations. Axial MR images of case 1 (**A-C**) and 2 **(D-F**). There is increased signal intensity within the striatum on T2-weighted images (**A, D**) and diffusion-weighted images (DWI) (**B, E**). In case 1, the putamen and caudate nucleus appear slightly swollen (**A**) and high signal on DWI (**B**) is confirmed to represent abnormal restricted diffusion on the ADC map (**C**). In case 2, the abnormal signal is principally located in the postero-lateral putamina, which also appear atrophic (**D**). There is no corresponding restriction of diffusion on the ADC map (**F**), and appearances suggest a more chronic disease stage.

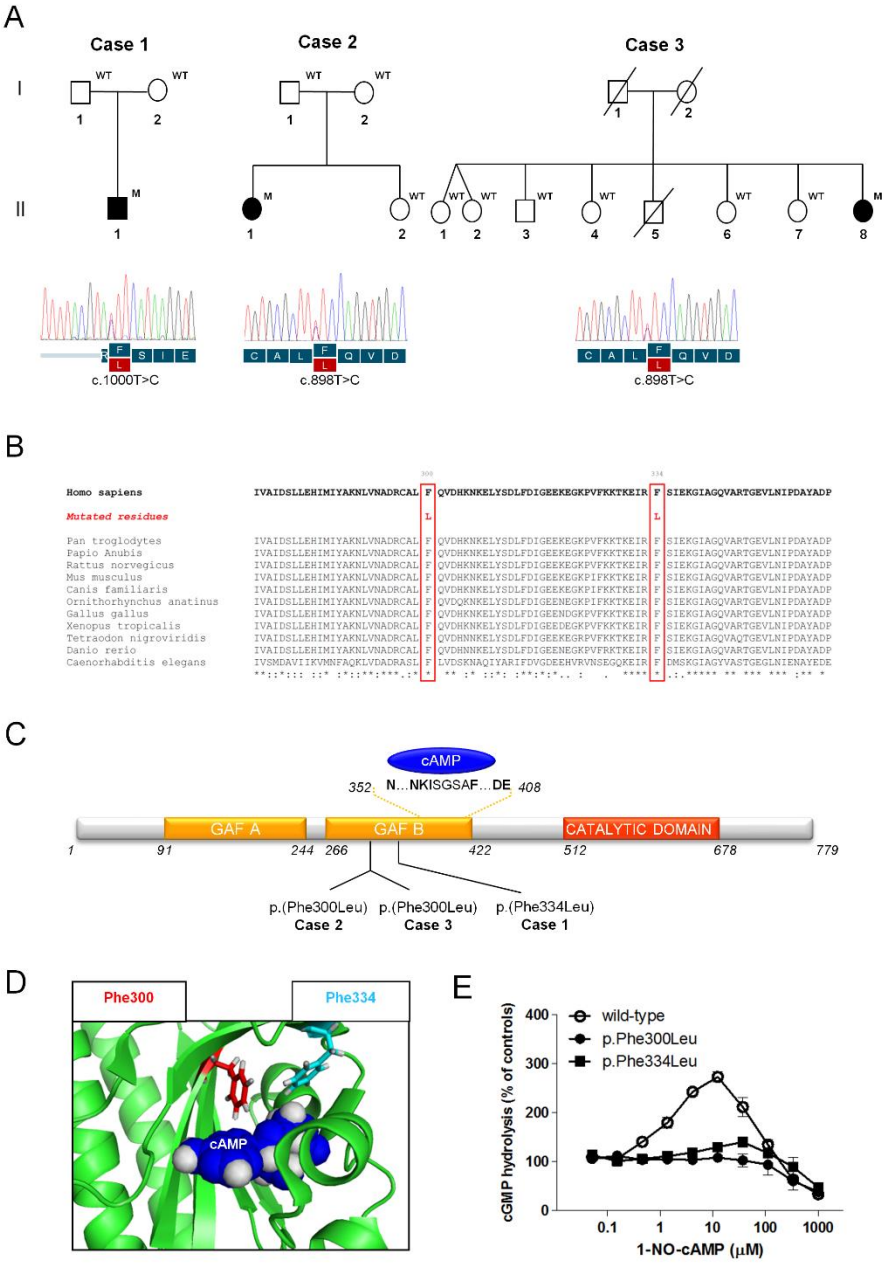


Figure 2. Family trees, PDE10A mutation analysis, interspecies alignment, schematic representation of the PDE10A protein, in silico modeling of the 3D structure of the PDE10A GAF-B domain, and functional studies of the identified PDE10A substitutions.

(**A**) Pedigrees of the three cases carrying the de novo PDE10A c.898T>C; p.Phe300Leu and c.1000T>C; p.Phe334Leu mutations and Sanger sequencing confirmation of the mutations. The following abbreviations are used: WT for homozygous wild-type alleles; and M for subjects carrying heterozygous PDE10A mutations. (**B**) Interspecies alignment performed with Clustal Omega showing the complete conservation down to invertebrates of the amino acid residues involved by the substitutions. Asterisks indicate invariant residues (full conservation), whereas a colon (:) and period (.) represent strong and moderate similarities, respectively. (**C**) A schematic representation of the PDE10A protein showing its organization in three domains, the regulatory GAF-A and GAF-B domains located in the N-terminal portion of the protein and the catalytic domain located in the C-terminus. The p.Phe300Leu and p.Phe334Leu substitutions are both located in the GAF-B domain which binds to cAMP. (**D**) In silico modeling of the 3-D structure of the GAF-B domain binding pocket and its interaction with the cyclic adenosine monophosphate (cAMP; shown in blue), generated using the PDB-file 2ZMF. The mutated residues Phe300 and Phe334 and their aromatic side chains, located in the β1 and β3 sheets forming the floor of the cAMP binding pocket, are shown in red and cyan respectively. Both residues are located in very close proximity to the cAMP molecule and are therefore likely to play an essential role in nucleotide binding. (**E**) Loss of stimulatory effect of GAF-B domain on PDE10A catalytic activity determined by the p.Phe300Leu and p.Phe334Leu substitutions. Effect of cyclic nucleotides binding to the GAF-B domain on PDE activity was evaluated measuring the enzyme activity after incubating wild-type and mutant PDE10As in the presence of various concentrations of 1-NO-cAMP and 70 nM [3H]cGMP. Each data point represents the mean ± S.E.M. of three independent experiments.

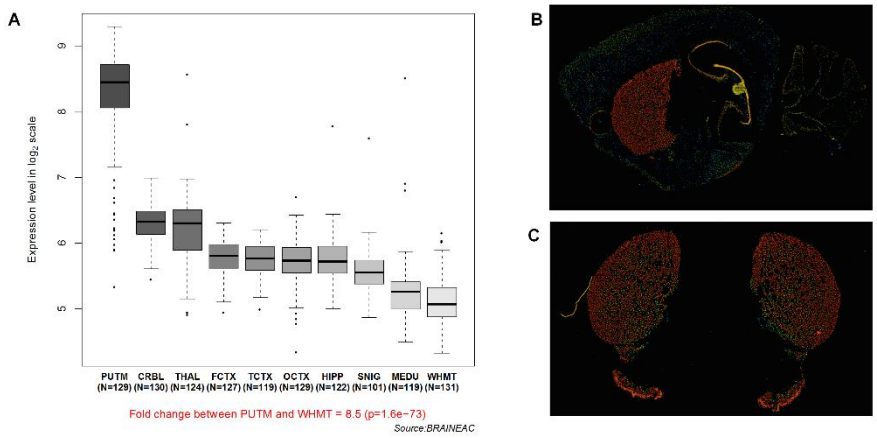


Figure 3. Summary of human and mouse brain PDE10A mRNA expression data.

(**A**) Box plots of PDE10A mRNA expression levels in 10 adult brain regions (Source: BRAINEAC; http://www.braineac.org/). The expression levels are based on exon array experiments and are plotted on a log2 scale (y axis). This dataset was generated using Affymetrix Exon 1.0 ST Arrays and brain tissue originating from 134 control individuals, collected by the Medical Research Council (MRC) Sudden Death Brain and Tissue Bank, Edinburgh, UK, and the Sun Health Research Institute (SHRI), an affiliate of Sun Health Corporation, USA.20 This plot shows significant variation in PDE10A expression across the 10 brain regions analyzed, with expression higher in the putamen than in any other region: putamen (PUTM), frontal cortex (FCTX), temporal cortex (TCTX), occipital cortex (OCTX). hippocampus (HIPP), substantia nigra (SNIG), medulla (specifically inferior olivary nucleus, MEDU), intralobular white matter (WHMT), thalamus (THAL), and cerebellar cortex (CRBL). “N” indicates the number of brain samples analyzed to generate the results for each brain region. PDE10A expression in mouse brain in (**B**) sagittal and (**C**) coronal sections. PDE10A is very highly and selectively expressed in the striata and in the olfactory tubercula. Images were obtained from the Allen Mouse Brain Atlas website (© 2015 Allen Institute for Brain Science). Expression intensity is color-coded, ranging from blue (low intensity) through green and yellow to red (high intensity).

References

1. Klein, C. (2014). Genetics in dystonia. Parkinsonism Relat Disord 20 Suppl 1, S137-142.

2. Spatola, M., and Wider, C. (2014). Genetics of Parkinson's disease: the yield. Parkinsonism Relat Disord 20 Suppl 1, S35-38.

3. Gardiner, A.R., Jaffer, F., Dale, R.C., Labrum, R., Erro, R., Meyer, E., Xiromerisiou, G., Stamelou, M., Walker, M., Kullmann, D., et al. (2015). The clinical and genetic heterogeneity of paroxysmal dyskinesias. Brain 138, 3567-3580.

4. Hermann, A., and Walker, R.H. (2015). Diagnosis and treatment of chorea syndromes. Curr Neurol Neurosci Rep 15, 514.

5. Gittis, A.H., and Kreitzer, A.C. (2012). Striatal microcircuitry and movement disorders. Trends Neurosci 35, 557-564.

6. Peall, K.J., and Kurian, M.A. (2015). Benign Hereditary Chorea: An Update. Tremor Other Hyperkinet Mov (N Y) 5, 314.

7. de Ligt, J., Willemsen, M.H., van Bon, B.W., Kleefstra, T., Yntema, H.G., Kroes, T., Vulto-van Silfhout, A.T., Koolen, D.A., de Vries, P., Gilissen, C., et al. (2012). Diagnostic exome sequencing in persons with severe intellectual disability. N Engl J Med 367, 1921-1929.

8. Mencacci, N.E., Rubio-Agusti, I., Zdebik, A., Asmus, F., Ludtmann, M.H., Ryten, M., Plagnol, V., Hauser, A.K., Bandres-Ciga, S., Bettencourt, C., et al. (2015). A missense mutation in KCTD17 causes autosomal dominant myoclonus-dystonia. Am J Hum Genet 96, 938-947.

9. Wang, K., Li, M., and Hakonarson, H. (2010). ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38, e164.

10. Kircher, M., Witten, D.M., Jain, P., O'Roak, B.J., Cooper, G.M., and Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet 46, 310-315.

11. Exome Aggregation Consortium. (2015). Analysis of protein-coding genetic variation in 60,706 humans. Biorxiv doi: http://dxdoiorg/101101/030338.

12. Xu, B., Ionita-Laza, I., Roos, J.L., Boone, B., Woodrick, S., Sun, Y., Levy, S., Gogos, J.A., and Karayiorgou, M. (2012). De novo gene mutations highlight patterns of genetic and neural complexity in schizophrenia. Nat Genet 44, 1365-1369.

13. Gulsuner, S., Walsh, T., Watts, A.C., Lee, M.K., Thornton, A.M., Casadei, S., Rippey, C., Shahin, H., Consortium on the Genetics of, S., Group, P.S., et al. (2013). Spatial and temporal mapping of de novo mutations in schizophrenia to a fetal prefrontal cortical network. Cell 154, 518-529.

14. Iossifov, I., O'Roak, B.J., Sanders, S.J., Ronemus, M., Krumm, N., Levy, D., Stessman, H.A., Witherspoon, K.T., Vives, L., Patterson, K.E., et al. (2014). The contribution of de novo coding mutations to autism spectrum disorder. Nature 515, 216-221.

15. Genome of the Netherlands Consortium. (2014). Whole-genome sequence variation, population structure and demographic history of the Dutch population. Nat Genet 46, 818-825.

16. Rauch, A., Wieczorek, D., Graf, E., Wieland, T., Endele, S., Schwarzmayr, T., Albrecht, B., Bartholdi, D., Beygo, J., Di Donato, N., et al. (2012). Range of genetic mutations associated with severe non-

syndromic sporadic intellectual disability: an exome sequencing study. Lancet 380, 1674-1682.

17. Petrovski, S., Wang, Q., Heinzen, E.L., Allen, A.S., and Goldstein, D.B. (2013). Genic intolerance to functional variation and the interpretation of personal genomes. PLoS Genet 9, e1003709.

18. Samocha, K.E., Robinson, E.B., Sanders, S.J., Stevens, C., Sabo, A., McGrath, L.M., Kosmicki, J.A., Rehnstrom, K., Mallick, S., Kirby, A., et al. (2014). A framework for the interpretation of de novo mutation in human disease. Nat Genet 46, 944-950.

19. Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Soding, J., et al. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 7, 539.

20. Trabzuni, D., Ryten, M., Walker, R., Smith, C., Imran, S., Ramasamy, A., Weale, M.E., and Hardy, J. (2011). Quality control parameters on a large dataset of regionally dissected human control brains for whole genome expression studies. J Neurochem 119, 275-282.

21. Lein, E.S., Hawrylycz, M.J., Ao, N., Ayres, M., Bensinger, A., Bernard, A., Boe, A.F., Boguski, M.S., Brockway, K.S., Byrnes, E.J., et al. (2007). Genome-wide atlas of gene expression in the adult mouse brain. Nature 445, 168-176.S

22. Fujishige, K., Kotera, J., and Omori, K. (1999). Striatum- and testis-specific phosphodiesterase PDE10A isolation and characterization of a rat PDE10A. Eur J Biochem 266, 1118-1127.

23. Coskran, T.M., Morton, D., Menniti, F.S., Adamowicz, W.O., Kleiman, R.J., Ryan, A.M., Strick, C.A., Schmidt, C.J., and

Stephenson, D.T. (2006). Immunohistochemical localization of phosphodiesterase 10A in multiple mammalian species. J Histochem Cytochem 54, 1205-1213.

24. Lakics, V., Karran, E.H., and Boess, F.G. (2010). Quantitative comparison of phosphodiesterase mRNA distribution in human brain and peripheral tissues. Neuropharmacology 59, 367-374.

25. Beavo, J.A., and Brunton, L.L. (2002). Cyclic nucleotide research -- still expanding after half a century. Nat Rev Mol Cell Biol 3, 710-718.

26. Steegborn, C. (2014). Structure, mechanism, and regulation of soluble adenylyl cyclases - similarities and differences to transmembrane adenylyl cyclases. Biochim Biophys Acta 1842, 2535-2547.

27. Koesling, D., Bohme, E., and Schultz, G. (1991). Guanylyl cyclases, a growing family of signal-transducing enzymes. FASEB J 5, 2785-2791.

28. Soderling, S.H., and Beavo, J.A. (2000). Regulation of cAMP and cGMP signaling: new phosphodiesterases and new functions. Curr Opin Cell Biol 12, 174-179.

29. Bender, A.T., and Beavo, J.A. (2006). Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. Pharmacol Rev 58, 488-520.

30. Heikaus, C.C., Pandit, J., and Klevit, R.E. (2009). Cyclic nucleotide binding GAF domains from phosphodiesterases: structural and mechanistic insights. Structure 17, 1551-1557.

31. Gross-Langenhoff, M., Hofbauer, K., Weber, J., Schultz, A., and Schultz, J.E. (2006). cAMP is a ligand for the tandem GAF domain of

human phosphodiesterase 10 and cGMP for the tandem GAF domain of phosphodiesterase 11. J Biol Chem 281, 2841-2846.
32. Jäger, R., Russwurm, C., Schwede, F., Genieser, H.G., Koesling, D., and Russwurm, M. (2012). Activation of PDE10 and PDE11 phosphodiesterases. J Biol Chem 287, 1210-1219.
33. Pandit, J., Forman, M.D., Fennell, K.F., Dillman, K.S., and Menniti, F.S. (2009). Mechanism for the allosteric regulation of phosphodiesterase 2A deduced from the X-ray structure of a near full-length construct. Proc Natl Acad Sci U S A 106, 18225-18230.
34. Handa, N., Mizohata, E., Kishishita, S., Toyama, M., Morita, S., Uchikubo-Kamo, T., Akasaka, R., Omori, K., Kotera, J., Terada, T., et al. (2008). Crystal structure of the GAF-B domain from human phosphodiesterase 10A complexed with its ligand, cAMP. J Biol Chem 283, 19657-19664.
35. Matthiesen, K., and Nielsen, J. (2009). Binding of cyclic nucleotides to phosphodiesterase 10A and 11A GAF domains does not stimulate catalytic activity. Biochem J 423, 401-409.
36. Bollen, E., and Prickaerts, J. (2012). Phosphodiesterases in neurodegenerative disorders. IUBMB Life 64, 965-970.
37. Appenzeller, S., Schirmacher, A., Halfter, H., Baumer, S., Pendziwiat, M., Timmerman, V., De Jonghe, P., Fekete, K., Stogbauer, F., Ludemann, P., et al. (2010). Autosomal-dominant striatal degeneration is caused by a mutation in the phosphodiesterase 8B gene. Am J Hum Genet 86, 83-87.
38. Barsottini, O.G., Martins, P.M., Chien, H.F., Raskin, S., Nunes, R.H., da Rocha, A.J., and Pedroso, J.L. (2015). Familial striatal degeneration: New mutation and neuroimaging clues. Neurology.

39. Chappie, T.A., Helal, C.J., and Hou, X. (2012). Current landscape of phosphodiesterase 10A (PDE10A) inhibition. J Med Chem 55, 7299-7331.
40. Marsden, C.D. (1984). The pathophysiology of movement disorders. Neurol Clin 2, 435-459.
41. Threlfell, S., and West, A.R. (2013). Review: Modulation of striatal neuron activity by cyclic nucleotide signaling and phosphodiesterase inhibition. Basal Ganglia 3, 137-146.
42. Herve, D. (2011). Identification of a specific assembly of the g protein golf as a critical and regulated module of dopamine and adenosine-activated cAMP pathways in the striatum. Front Neuroanat 5, 48.
43. Lee, K.W., Hong, J.H., Choi, I.Y., Che, Y., Lee, J.K., Yang, S.D., Song, C.W., Kang, H.S., Lee, J.H., Noh, J.S., et al. (2002). Impaired D2 dopamine receptor function in mice lacking type 5 adenylyl cyclase. J Neurosci 22, 7931-7940.
44. Fuchs, T., Saunders-Pullman, R., Masuho, I., Luciano, M.S., Raymond, D., Factor, S., Lang, A.E., Liang, T.W., Trosch, R.M., White, S., et al. (2013). Mutations in GNAL cause primary torsion dystonia. Nat Genet 45, 88-92.
45. Chen, Y.Z., Matsushita, M.M., Robertson, P., Rieder, M., Girirajan, S., Antonacci, F., Lipe, H., Eichler, E.E., Nickerson, D.A., Bird, T.D., et al. (2012). Autosomal dominant familial dyskinesia and facial myokymia: single exome sequencing identifies a mutation in adenylyl cyclase 5. Arch Neurol 69, 630-635.
46. Mencacci, N.E., Erro, R., Wiethoff, S., Hersheson, J., Ryten, M., Balint, B., Ganos, C., Stamelou, M., Quinn, N., Houlden, H., et al.

(2015). ADCY5 mutations are another cause of benign hereditary chorea. Neurology 85, 80-88.

47. Chen, Y.Z., Friedman, J.R., Chen, D.H., Chan, G.C., Bloss, C.S., Hisama, F.M., Topol, S.E., Carson, A.R., Pham, P.H., Bonkowski, E.S., et al. (2014). Gain-of-function ADCY5 mutations in familial dyskinesia with facial myokymia. Ann Neurol 75, 542-549.

48. Russwurm, C., Koesling, D., and Russwurm, M. (2015). Phosphodiesterase 10A Is Tethered to a Synaptic Signaling Complex in Striatum. J Biol Chem 290, 11936-11947.

49. Niccolini, F., Foltynie, T., Reis Marques, T., Muhlert, N., Tziortzi, A.C., Searle, G.E., Natesan, S., Kapur, S., Rabiner, E.A., Gunn, R.N., et al. (2015). Loss of phosphodiesterase 10A expression is associated with progression and severity in Parkinson's disease. Brain 138, 3003-3015.

Supplementary material

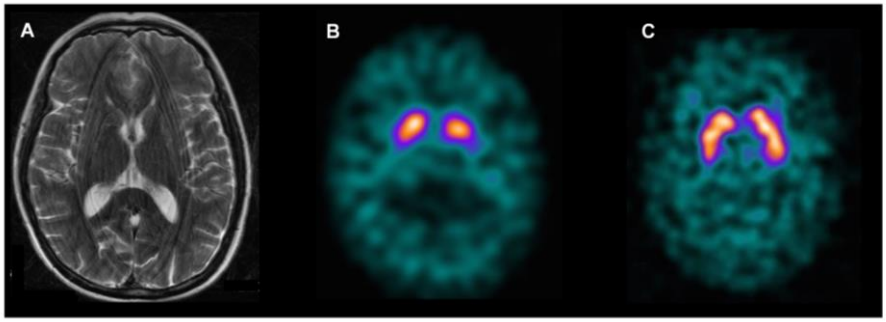


Figure S1. Brain MRI and single photon emission computed tomography (SPECT) dopamine reuptake transporter (DAT)-scan images in case 3. (A) Albeit markedly degraded by movement artefacts, axial MR images showed bilateral T2 hyperintensity within the posterolateral putamina. (B) Dopaminergic striatal innervation was evaluated as DAT density by means of ^{123}I-FP-CIT SPECT. The scan shows marked bilateral reduction of tracer uptake in the striatum, consistent with bilateral nigrostriatal dopaminergic denervation. (C) Normal DAT-scan from an age-and sex matched subject.

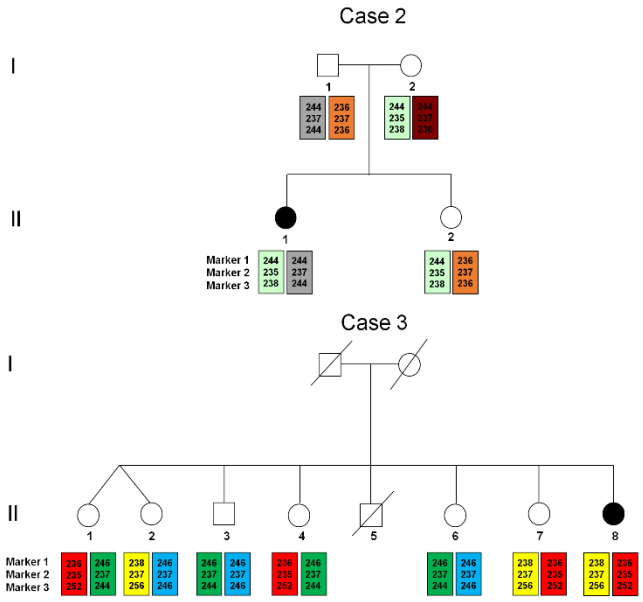


Figure S2. Haplotype analysis in the families of cases 2 and 3. Haplotype analysis was performed to test whether the c.898C>T mutation had arisen on the same genetic background in cases 2 and 3 and to unveil the *de novo* occurrence of the c.898C>T mutation identified in case 3. Three microsatellites (di-nucleotide repeats) surrounding the *PDE10A* locus (Marker 1 – chr6:166069747-166069785; Marker 2 – chr6:165862198-165862227; Marker 3 - chr6:165839259-165839288; primers available upon request) were sized up in all available relatives of the two cases. The four parental haplotypes were reconstructed in both families (each haplotype defined by a different color). Cases 2 and 3 did not share the haplotype encompassing the c.898C>T variant, suggesting the mutations arose on different haplotype backgrounds. Furthermore, haplotype analysis indicates that case 3, who carries the *PDE10A* c.898T>C variant, shares one of the allele (marked in yellow) with two unaffected siblings (II-2, II-7) whereas the other allele (marked in red) is shared with three unaffected siblings (II-1, II-4 and II-7). Of note, Sanger sequencing showed that all unaffected siblings are homozygous for the wild-type

allele. These data strongly support the *de novo* occurrence of the mutation in case 3.

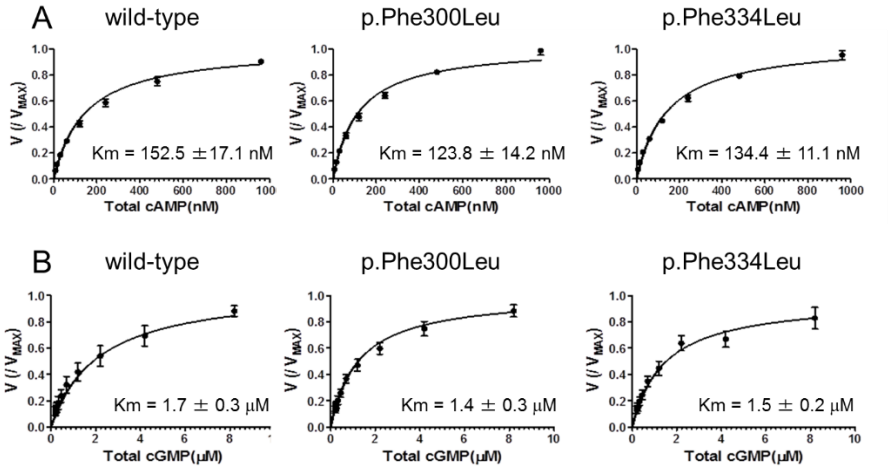


Figure S3. Enzyme Kinetics of Wild-Type and Mutant PDE10As. PDE10A enzymes was incubated at presence of mixture of unlabeled cAMP and [^{3}H]cAMP (A) or unlabeled cGMP and [^{3}H]cGMP (B) with the total concentration as indicated. To obtain the Michaelis–Menten constants (Km), the initial rates of the reaction were fitted to the following equations using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, US): V = Vmax [S] / (Km + [S]); where V is the initial velocity of the enzyme-catalyzed reaction, [S] is the substrate concentration, Vmax is the limiting reaction velocity at saturating substrate concentrations, and Km is the Michaelis–Menten constant (concentration of substrate at 1/2 of Vmax). Each data point represents the mean ± S.E.M. of five (for cAMP) and four (for cGMP) independent experiments. There was no statistically significant difference in Km values among the wild-type and the mutant PDE10As ($p > 0.05$ by Dunnett's test compared with wild-type).

Chapter 5

Novel *GNAL* mutation with intra-familial clinical heterogeneity: expanding the phenotype

Miryam Carecchio[a,b], Celeste Panteghini[a], Chiara Reale[a], Chiara Barzaghi[a], Valentina Monti[a], Luigi Romito[c], Francesco Sasanelli[d], Barbara Garavaglia[a,*]

[a] *Molecular Neurogenetics Unit, IRCCS Neurological Institute C. Besta, Via L. Temolo 4, 20126 Milan, Italy*

[b] *Department of Pediatric Neurology, IRCCS Neurological Institute C. Besta, Via Celoria 11, 20133 Milan, Italy*

[c] *Department of Neurology, IRCCS Neurological Institute C. Besta, Via Celoria 11, 20133 Milan, Italy*

[d] *Department of Neurology, AO Ospedale di Circolo di Melegnano, Strada Pandina 1, 20070 Vizzolo Predabissi (MI), Italy*

[*]Corresponding author

Keywords: GNAL, dystonia, tremor, phenotype.

Parkinsonism Relat Disord. 2016 Feb;23:66-71. doi: 10.1016/j.parkreldis.2015.12.012. Epub 2015 Dec 18.

Abstract

Introduction. Mutations in *GNAL* have been associated with adult-onset cranio-cervical dystonia, but a limited number of cases have been reported so far and the clinical spectrum associated with this gene still needs to be fully characterized.

Methods. We identified an Italian family with adult-onset, dominantly-inherited dystonia whose members presented with different combinations of dystonia affecting the cervical, oro-mandibular and laryngeal regions associated with prominent tremor in some cases. Pure asymmetric upper limb dystonic tremor was present in one of the members and jerky cervical dystonia was also observed. A dedicate dystonia gene panel (Illumina) was used to screen for dystonia-associated genes and Sanger sequencing was performed to confirm results obtained and to perform segregation analysis.

Results. A novel single-base mutation in *GNAL* exon 9 (c.628G>A; p.Asp210Asn) leading to an aminoacidic substitution was identified and confirmed by Sanger sequencing. *In silico* prediction programmes as well as segregation analysis confirmed its pathogenicity. Clinically, no generalization of dystonia was observed after onset and DBS lead to an excellent motor outcome in two cases.

Conclusion. We report a novel *GNAL* mutation and expand the clinical spectrum associated with mutations in this gene to comprise pure asymmetric dystonic tremor and a jerky cervical phenotype partially mimicking DYT11 positive cases.

1. Introduction

In 2013, mutations in *GNAL* (DYT25) were identified in eight unrelated kindred with familial dystonia mainly affecting the cervical and cranial regions, with a tendency to spread to contiguous sites but with a low rate of generalization (11%) [1]. Subsequently, other studies in patients with familial and sporadic cervical dystonia individuated 14 additional pathogenic mutations, which are dominantly-inherited and show reduced penetrance [2-6]. The frequency of *GNAL* mutations ranges from 0.007% in a cohort of sporadic patients with adult-onset cervical dystonia to 15% in selected families with multiplex dystonia [1]. Moreover, segregation analysis of some *GNAL* mutations was not performed in a proportion of reported cases, raising doubts about the actual pathogenicity of some of these variants [7].

GNAL encodes guanine nucleotide-binding protein G(olf), subunit alpha [Gα(olf)], first identified as a G protein (guanine nucleotide-binding protein) that mediates odorant signaling in the olfactory epithelium. Gα(olf) couples dopamine type 1 receptors (D1Rs) of the direct pathway and adenosine A2A receptors (A2ARs) of the indirect pathway to the activation of adenylate cyclase type 5 and plays a key role in signal transduction within the olfactory neuroepithelium and basal ganglia, being predominantly expressed in striatal medium spiny neurons [1].

To date, adult-onset cervical dystonia, with or without superimposed tremor, seems to be the most common clinical phenotype associated with *GNAL* mutations. However, the full clinical spectrum of *GNAL* mutations is still largely to be explored as a limited number of cases

have been published so far. Here we report a novel *GNAL* mutation in an Italian kindred showing phenotypic variability, including pure asymmetric upper limb dystonic tremor and a good response to DBS stimulation for cervical dystonia.

2. Materials and methods

2.1 Family description

The family reported herein is of Southern Italian origin and no consanguinity was documented (**Figure 1**).

The index case (III:6) is a 59-year old male with cervical dystonia who first noticed a head turning to the left at age 36. Over the following years, a superimposed head tremor also appeared. Both cervical dystonia and tremor reached their peak within 4-5 years from the onset and then remained stable. Botulinum toxin injections were only partially beneficial and neither tetrabenazine nor levodopa were effective. At age 55 he underwent bilateral stereotactic Deep Brain Stimulation (DBS) targeting at the posteroventrolateral portion of the GPi using quadripolar electrodes (Medtronic, Minneapolis, MN, USA). Intraoperative macrostimulation and postoperative TC imaging verified correct placement of the electrode. DBS leads were connected to a battery-operated programmable pulse generator (Activa PC, Medtronic). Stimulation parameters at last follow-up were: Right GPi = 2.0 V, 90 μs, 130 Hz, - 8 +9 (= - 0 +1); Left GPi = 2.5 V, 90 μs, 130 Hz, - 1 case +. A substantial clinical improvement rated in 80-90% was referred by the patient. Burke-Fahn-Marsden Dystonia Scale (BFMDS) improved from 16/120 to 5/120 after surgery (67% improvement). Examination at age 59 (23 years after the onset) showed a mild cervical

tilt to the left with some residual degrees of retrocollis and no head tremor at rest. A mild tremor was only detectable on extreme lateral rotation of the head. There was some dystonic posturing in the left arm when keeping it outstretched and arm swings were reduced on same side on walking (**Video 1**).

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2015.12.012.

The patient's younger sister (III:4) presented with isolated head tremor at age 42, followed by an abnormal head posture (left torticollis and retrocollis) after some years and by laryngeal dystonia (tremulous high-pitched voice). On examination at age 62, she showed left jerky torticollis with superimposed head tremor and a limited range of movements to the right along with tremor in the upper limbs, which was mainly visible when keeping arms flexed at the elbow; in this position, some dystonic posturing at the wrist junction was also detectable (BFMDS 12/120). The patient described the tremor as fluctuating and strictly asymmetrical, being more marked on the right side, although this feature was not visible when we examined her; the tremor intermittently impaired hand-writing. She reported she could ameliorate her head position by touching her chin with the hand. This patient was initially diagnosed with myoclonus dystonia due to DYT11 mutation, as she was found to carry a splicing variant (IVS3-3T>C) initially reported as a pathogenic mutation [8] but later classified as a polymorphism (rs17166384) with a minor allele frequency of 24% in the African population [9].

At age 62 the patient underwent a bilateral stereotactic DBS targeting at the posteroventrolateral portion of the GPi using quadripolar

electrodes (Medtronic, Minneapolis, MN, USA). Intraoperative macrostimulation and postoperative TC imaging verified correct placement of the electrode. DBS leads were connected to two battery-operated programmable pulse generators (Activa SC, Medtronic). For both sides, a interleaving deep brain stimulation setting was programmed, according to the following parameters: Right GPi # 1 = 2.15 V, 60 μs, 125 Hz, - 1 case +; Right GPi # 2 = 2.20 V, 90 μs, 125 Hz, - 2 case +; Left GPi # 1 = 1.25 V, 60 μs, 125 Hz, - 0 case +; Left GPi # 2 = 2.00 V, 60 μs, 125 Hz, - 1 case +. After the implant, a rapid improvement (in two weeks) of cervical and laryngeal dystonia was obtained (**Video 2**). No parkinsonian or akinetic signs were present on examination.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2015.12.012.

Subject III:5 is a 60-year-old man affected by Chronic Inflammatory Demyelinating Polyneuropathy (CIDP) since age 48 and treated with Ig ev administration and steroids with improvement of motor weakness in the lower limbs. At age 43, he had a traumatic intracranial bleeding of the head of the left caudate nucleus and the anterior arm of internal capsula (24x14 mm on CT scan), which presented with a mild right hemiparesis that resolved throughout one week and left no neurological sequelae. At age 58, he noticed an intermittent rest tremor in the right arm; DAT-Scan showed a selective absence of tracer uptake in the head of the left caudate nucleus, thus tremor was initially interpreted as post-traumatic in nature, although it had not been present for the previous 15 years. No response to Levodopa was observed. On examination at age 60, the patients showed a high-amplitude, irregular, markedly

asymmetric rest tremor that was brought out only by specific positions of the arm and by certain degrees of pronation of the forearm. It was also present on posture and on action and was not associated with bradykinesia on finger tapping (**Video 3**). Examination also showed absent tendon reflexes in the lower limbs and reduced power of the right tibialis anterioris muscle with mild steppage.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2015.12.012.

Subjects III:2, aged 66, was not available for examination and blood sampling but he is reported to be affected by involuntary movements of the oro-mandibular region that are absent at rest and brought out only by talking, possibly being consistent with a focal, speech-induced dystonia.

Among the remainder proband's siblings, subject III:3, III:7 and III:8 (aged 65, 57 and 53, respectively) all showed normal neurological examination.

The mother of the affected subjects (II:8) died at age 90 and was referred to be affected by involuntary movements of the oro-mandibular region, but head or upper limb tremor or abnormal postures were not reported. Her sister (II:9, deceased) was affected by history by severe head and trunk tremor with onset in the fourth decade, and so is her daughter (III:9) now aged 45, who complains of severe head tremor.

All patients' brain MRI scan was normal, with the exception of subject III:5, who showed a gliotic area consistent with the previous intracranial haemorrhage in the head of the left caudate nucleus.

Tremor EMG study was performed in subjects III:4 and III:5. In III:4, findings were consistent with dystonic tremor of the right upper limb

(co-contraction of agonist and antagonist muscles with arrhythmic EMG tremor activity activated by wrist and elbow flexion, and absent at rest); EMG recordings from cervical muscles showed spontaneous asynchronous EMG myoclonic bursts of the right sternocleidomastoid muscle and both splenii capitis lasting between 50 and 300 ms, with a frequency of 7 Hertz at rest and no EEG correlates on back-averaging. In subject III:5, although clear dystonic postures were not detectable on examination, surface EMG recordings showed a dystonic pattern of the tremor, with irregular EMG co-contraction bursts of forearm flexor and extensor muscles that were brought about only by specific positions, while electric silence was present at rest. Muscular reciprocal inhibition was not present. Nerve conduction studies were unremarkable in the upper limbs, with no signs of demyelinating neuropathy, while in the lower limbs motor conduction velocities were remarkably reduced.

The offspring of the three affected subjects are reported to be in good health, their age ranging from 25 to 35 years.

2.2 Genetic analysis

After obtaining informed consent, the subjects included in this study were blood sampled and DNA was extracted from peripheral blood lymphocytes according to standard procedures.

Patients' DNA was tested by targeted re-sequencing using customized gene panels including dystonia-associated genes selected on the basis of a systematic literature review.

The design of the panels is based on the TruSeq Custom Amplicon assay for target resequencing (Illumina). The regions of interest (coding sequence + UTR) of the target genes were amplified and the amplicons

generated were sequenced through the MiSeq platform (Illumina). The reads generated were aligned to the most recent version of the human genome assembly (GRCh37/hg19). The variants identified were annotated and filtered, focusing on those rare (minimum allele frequency < 1% in 1000 Genome Project, www.1000genomes.org, and Exome Sequencing Project, http://evs.gs.washington.edu/EVS) and potentially damaging for the protein function by Illumina variant Studio 2.2.

3. Results

Through this analysis, we identified a single base substitution in *GNAL* (NM_001142339) exon 9 (c.628G>A; p.Asp210Asn) in the proband (III:6; **Figure 2**). Sanger sequencing was performed to confirm this variant in affected and unaffected family members (primer sequences and conditions used are available upon request - disturbimovimento@istituto-besta.it).

In silico analysis with Polyphen-2 and SIFT indicated that this variant is disease-causing as it alters a highly conserved aminoacid which falls within one of the GTP-binding domains of GNAL (G3) (**Figure 3**) and is therefore expected to have a significant impact on the protein function.

To provide further evidence in support of the pathogenicity of this variant, we Sanger sequenced 100 healthy Italian controls (200 alleles) and could not find it in any subject; moreover, the p.Asp210Asn variant is not present the ExAC (http://exac.broadinstitute.org/) database. Accordingly, the variant segregated with the disease-status in adult individuals tested. In fact, clinically affected subjects (III:4 and III:5)

harboured the same *GNAL* mutation of the proband. Unaffected subjects genotyped (III:7 and III:8) resulted negative.

Subject II:8 deceased but her DNA was banked in our lab for previous segregation analysis of the above-mentioned IVS3-3T>C DYT11 variant, thus we were able to demonstrate that she carried the same *GNAL* mutation harboured by subjects III:4, III:5 and III:6.

4. Discussion

We here report a new *GNAL* positive family from Southern Italy, with a dominantly-inherited familial dystonia, with onset around the fourth decade and presenting in most cases with tremulous cervical dystonia. So far, 20 *GNAL* mutations have been reported, of which 10 are of a missense type (**Table 1**). Here we report a novel missense variant, c.628G>A, leading to an aminoacidic substitution in exon 9 (p.Asp210Asn), that segregated with the disease-status in subjects tested.

According to previously-published series, cervical dystonia is the most common clinical presentation in *GNAL* mutation carriers, mainly with onset in adulthood, although paediatric onset (age 7 and 11) was also reported in two *GNAL* mutations (c.409G>A and c.283_284insT), but with early involvement of legs and tongue, respectively [1]. Of all the genetically-defined published cases (n=49), belonging to 21 different families, 38 (77.5%) presented with cervical dystonia or developed it in the course of the disease. In line with these findings, two of our *GNAL*-positive patients presented with adult-onset cervical dystonia. Two additional family members were reported to have head and trunk tremor

and other two affected subjects (II:8 and III:1) were probably affected by oro-mandibular dystonia. In these subjects, previous neuroleptic intake was ruled out by enquiring relatives. Tremor as part of the dystonic phenotype is frequently observed in adult-onset cervical dystonia [10] (Defazio *et al*., 2015), and has been suggested to be the most consistent feature in *ANO-3* positive patients [11]. Similarly, some DYT1 positive patients can present with isolated dystonic tremor with no or very mild signs of dystonia. In all our patients, tremor was the most disabling feature and was observed in all *GNAL*-positive subjects and referred in those affected by history, involving the neck, but also larynx, trunk and upper limbs. *GNAL* mutations may therefore be present in cases of dystonia with severe tremor. In one subject, asymmetric upper limb dystonic tremor was the only clinical manifestation. Although brachial onset was not observed in the original report by Fuchs *et al*. [1], Saunders-Paullman reported one patient carrying *GNAL* c.514G>A mutation who was affected by isolated tremor of the upper limbs [4]. Electrophysiology was not performed to define the dystonic nature of tremor in this subject, and no segregation analysis was available in this family, making it impossible to ascertain whether c.514G>A *GNAL* mutation was present in all patients with tremor.

The presence of pure dystonic tremor in the upper limbs or in association with cervical and laryngeal dystonia expands the clinical phenotype of *GNAL* mutations. Tremor as the sole manifestation of a *GNAL* mutation opens the question as to whether the so-called SWEDDs (Scans Without Evidence of Dopaminergic Deficit), may be at least in part due to mutations in the most-recently discovered isolated

dystonia genes, including *GNAL*, although at present we can only speculate about it. Subject III:5 was initially diagnosed with by post-traumatic parkinsonism on the basis of a previous contralateral caudate nucleus haemorrhage with a consistent tracer uptake reduction on DAT-Scan. However, the time of onset of tremor together with its phenomenology and electrophysiology lead us to reconsider it as dystonic. Accordingly, the patient was found to carry c.628G>A *GNAL* mutation. Also subject III:4 showed upper limb dystonic tremor as part of the phenotype that also included jerky cervical dystonia and laryngeal dystonia. The jerky phenotype of cervical dystonia in this subject initially lead to a wrong diagnosis of myoclonus dystonia on the basis of a presumptive pathogenic DYT11 mutation which later turned out to be a polymorphism and that did not segregate with disease-status in the family. Moreover, the disease was clearly inherited from the affected mother, a feature inconsistent with maternal imprinting observed in DYT11 myoclonus dystonia, which typically has an early onset [12]. In this regard, jerky dystonia has also been described in 18p-syndrome, a rare genetic disease with complete deletion of the short arm of chromosome 18 [13], where *GNAL* maps, and in one subject of a large non-Jewish North American family initially reported by Bressman in 1994 and later found to carry a mutation in *GNAL* [14]. Mutations in this gene may therefore be responsible of a proportion of cases of jerky dystonia that do not completely fit with the classical SGCE-related phenotype.

Including the present family, 53 subjects harboring *GNAL* mutations have been reported (**Table 1**) with onset ranging from infancy to the sixth decade. In our family, cervical dystonia presented between the end

of the third and the beginning of the fourth decade, while dystonic tremor in subject III:5 appeared at age 58 and tremor in other affected subjects appeared in the fourth decade. Two patients were possibly affected by oro-mandibular dystonia as the only manifestation of *GNAL* mutation, which potentially widens the differential diagnosis of this type of focal-dystonia, which is often observed in heredodegenerative dystonia such as in PANK-2 mutated patients or in tardive cases [15, 16].

Two mutated subjects underwent DBS with substantial improvement of cervical dystonia, that almost completely disappeared in the index case (III:6), with a beneficial effect lasting at 4 year-follow up. Dystonia-associated genes have been suggested to be a potential factor to predict motor outcome after DBS, with DYT1 cases showing the best outcome in a 10-year follow up study as compared to DYT6 cases. *GNAL* mutations may be a positive prognostic genetic factor to predict motor outcome after DBS surgery, but more patients will need to be treated and followed to draw definite conclusions about the effectiveness of DBS in *GNAL* dystonia.

5. Conclusions

Exome sequencing has recently allowed the discovery of several new genes responsible for a wide range of neurological diseases, including movement disorders. *GNAL*, *ANO-3*, *CIZ*, *TUBB-4A* and *COL6A3* have been mapped over the last three years expanding the known genetic causes of isolated dystonia. The full clinical spectrum associated with these genes is still largely unknown as a limited number of families and sporadic cases have been reported and fully characterized from a

clinical and electrophysiological point of view. Moreover, the natural history of these rare forms of dystonia is still to be determined by long-term follow-up studies, although, from the available data in the literature, generalization of dystonia do not seem to be frequent, unlike DYT1 cases. Our *GNAL* positive patients had a disease history up to 23 years and no generalization of dystonia was observed, while contiguous sites were affected after onset in subject III:4 (upper limb tremor after cervical dystonia) and III:6 (mild upper limb dystonia). *GNAL*-associated clinical phenotype is mainly characterized by adult-onset cranio-cervical dystonia, but in our kindred tremor was the most common clinical feature, either in isolation at onset or as the most disabling feature 23 years after the onset. This feature expands the clinical spectrum of movement disorders associated with *GNAL* mutations together with a prominent jerky phenotype partially resembling DYT11 positive cases.

Further studies are needed to individuate larger cohorts of *GNAL* positive patients and to ascertain which clinical or electrophysiological features, if any, may be predictive of *GNAL* positive status.

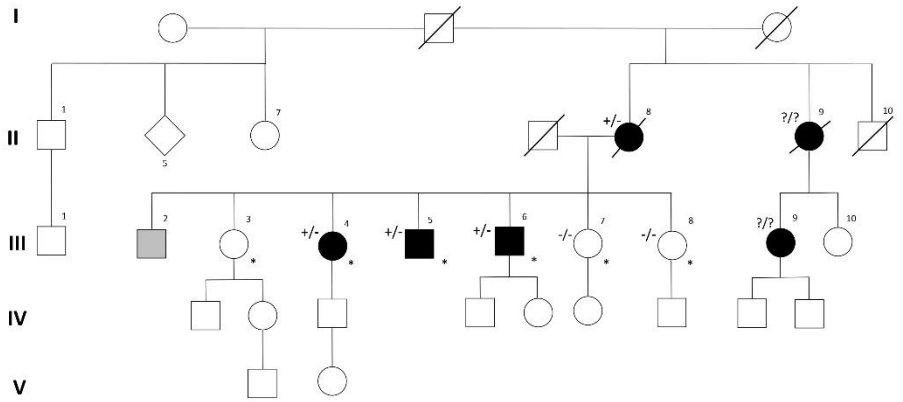


Figure 1. Family pedigree. Filled symbols indicate affected individuals. Gray symbols with question mark indicate possibly affected individuals. Sequencing findings for the *GNAL* c.628G>A (p.Asp210Asn) mutation are indicated above and to the left of each symbol. Individuals marked with an asterisk were evaluated clinically.

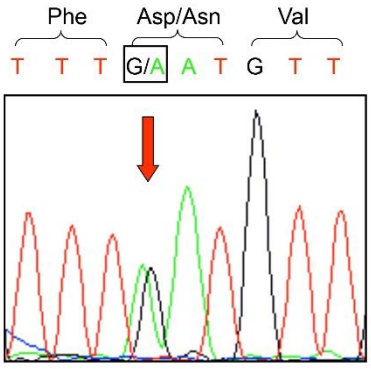


Figure 2. GNAL c.628G>A (p.Asp210Asn).

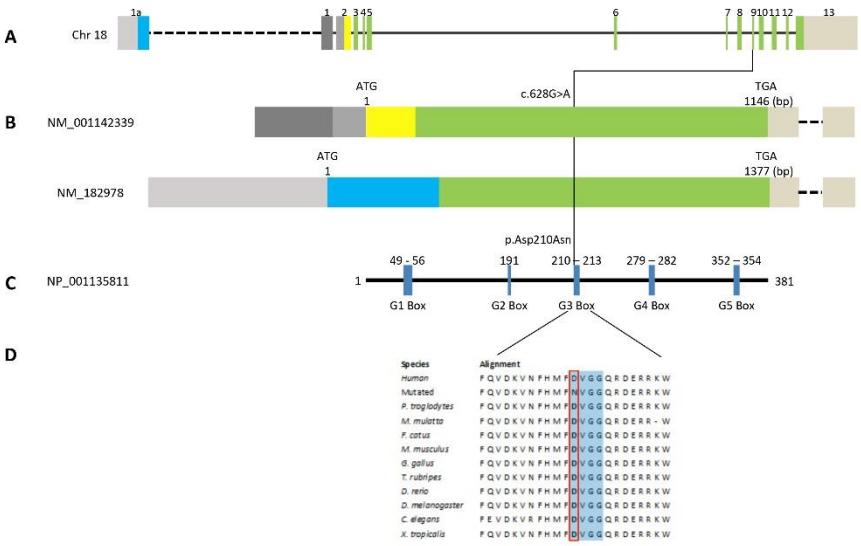


Figure 3. (A) Structure of GNAL on Chr 18p presented in the 5′ to 3′ direction showing the location of p.Asp210Asn mutation. **(B)** The long and major isoforms of GNAL differ at exon 1 (non-coding exons are represented in grey). **(C)** The p.Asp210Asn missense mutation is located in a highly conserved region of Ga(olf) and is shown in relationship to GTP binding domains (G1–G5). **(D)** The Ga(olf) amino acid altered by GNAL p.Asp210Asn mutation shows conservation in mammals (chimpanzees, mice, cats) non-mammalian vertebrates (chickens, fishes, and frogs) and invertebrates (roundworms and fruit flies).

DNA Variant	Protein variant	Exon	Mutation type	No of carriers reported	Age of onset	Site of onset	Ref.
c.1057G>A	p.Ala353Thr	13	missense	1	44	cervical dystonia	Kumar et al. 2014
c.1061T>C	p.Val354Ala	13	missense	1	40	cervical dystonia	Dobricic et al. 2014
c.166_167insA	p.Ser56-Lysfs*16	3	frameshift	1	35	cervical dystonia	Ziegan et al. 2014
c.274-5T>C	---	upstream ex 5	splice site mutation	2	3th and 5th decade	cervical dystonia	Fuchs et al. 2012
c.283_284insT	p.Ser95fs*110	5	frameshift	4	11-33	cranio-cervical dystonia	Fuchs et al. 2012
c.284C>T	p.Ser95*X	5	nonsense	1	41	cervical dystonia with early generalization	Miao et al. 2013
c.289A>G	p.Met97Val	5	missense	1	54	cervical dystonia	Ziegan et al. 2014

c.304_312delCCTCCAGTT	p.Pro102_Val104del	5	in frame deletion	1	20	cervical dystonia	Fuchs et al. 2012
c.3G>A	p.Met1?	1	start codon disruption	2	4th decade	cervical dystonia	Vemula et al. 2013
c.409G>A	p.Val137Met	6	missense	7	7-50	cervical dystonia; legs in one case	Fuchs et al. 2012
c.436G>A	p.Val146Met	6	missense	1	63	cervical dystonia	Zech et al. 2014
c.463G>A	p.Glu155Lys	6	missense	2	17-18	cervical dystonia	Fuchs et al. 2012
c.514G>A	p.Val72Iso	7	missense	2	21	larynx	Saunders-Pullman et al. 2014
c.591dupA	p.Arg198Tfs*13	8	frameshift	6	3th-4th decade	cervical dystonia	Fuchs et al. 2012, Vemula et al. 2013
c.61C>T	p.Arg21*	1	nonsense	3	2nd and 5th	cervical dystonia; laryngeal dystonia	Fuchs et al. 2012

					decade		
c.637G>A	p.Gly213Ser	9	missense	1	40	cervical dystonia	Kumar et al. 2014
c.682G>T	p.Val228Phe	10	missense	5	4^{th}-6^{th} decade	cervical dystonia	Vemula et al. 2013
c.733C>T	p.Arg245*	10	missense	1	45	cervical dystonia	Vemula et al. 2013
c.628G>A	p.Asp210Asn	9	missense	4	37	cervical dystonia/pure dystonic tremor	Present kindred
c.878C>A	p.Ser293*	11	nonsense	6	25-48	cervical dystonia	Fuchs et al. 2012
c.932-7T>G	---	upstream exon 12	tentative splice site mutation	1	26	cervical dystonia	Miao et al. 2013
Total 21				**53**			

Table 1. Previously-reported *GNAL* mutations and carriers.

Legend to videos

Video 1. Patient III:6 after DBS (age 59 years). Mild left laterocollis and dystonic posturing of the left arm. Reduced arm swings are detectable on the left when walking.

Video 2. Patient III:2 (age 62). Before DBS: tremulous jerky torticollis to the left with reduced range of rotation to the right and retrocollis; dystonic posturing at the wrist junction bilaterally on keeping arms outstretched; laryngeal dystonia. After DBS: normal head position at rest, mild tremulous cervical dystonia (left torticollis) on walking. Amelioration of laryngeal dystonia.

Video 3. Patient III:5 (age 60 years). Dystonic tremor of the right upper limb. Note the striking position-specificity of the tremor, without bradykinesia on finger tapping. Absence of cervical or laryngeal dystonia.

Author roles

Miryam Carecchio: concept and design, data collection, drafting and editing of manuscript; Celeste Panteghini: concept and design, data collection, data analysis; Chiara Reale: data collection, data analysis; Chiara Barzaghi: data collection, data analysis; Valentina Monti: data collection, data analysis; Luigi Romito: data collection, data analysis, data interpretation, revising of manuscript; Francesco Sasanelli: concept and design, data collection, revising of manuscript; Barbara Garavaglia: concept and design, data collection, editing and revising of manuscript.

Fundings

This work received financial support from the Fondazione Pierfranco e Luisa Mariani.

Acknowledgements

We acknowledge the "Cell lines and DNA Bank of Paediatric Movement Disorders and Mitochondrial diseases" of the Telethon Network of Genetic Biobanks (grant GTB12001J) and the Eurobiobank Network.

References

[1] T. Fuchs, R. Saunders-Pullman, I. Masuho, M.S. Luciano, D. Raymond, S. Factor, A.E. Lang, T.W. Liang, R.M. Trosch, S. White, E. Ainehsazan, D. Hervé, N. Sharma, M.E. Ehrlich, K.A. Martemyanov, S.B. Bressman, L.J. Ozelius, Mutations in GNAL cause primary torsion dystonia, Nat Genet 45 (2013) 88-92

[2] S.R. Vemula, A. Puschmann, J. Xiao, Y. Zhao, M. Rudzińska, K.P. Frei, D.D. Truong,

Z.K. Wszolek, M.S. LeDoux, Role of Gα(olf) in familial and sporadic adult-onset primary dystonia, Hum Mol Genet 22 (2013) 2510-2519

[3] J. Miao, X.H. Wan, Y. Sun, J.C. Feng, F.B. Cheng, Mutation screening of GNAL gene in patients with primary dystonia from Northeast China, Parkinsonism Relat Disord 19 (2013) 910-912

[4] R. Saunders-Pullman, T. Fuchs, M. San Luciano, D. Raymond, A. Brashear, R. Ortega, Heterogeneity in primary dystonia: Lessons from THAP1, GNAL, and TOR1A in Amish-Mennonites, Mov Disord. 29 (2014) 812-818

[5] J. Ziegan, M. Wittstock, A. Westenberger, V. Dobričić, A. Wolters, R. Benecke, C. Klein, C. Kamm, Novel GNAL mutations in two German patients with sporadic dystonia, Mov Disord. 29 (2014) 1833-1834

[6] K.R. Kumar, K. Lohmann, I. Masuho, R. Miyamoto, A. Ferbert, T. Lohnau, M. Kasten,

J. Hagenah, N. Brüggemann, J. Graf, A. Münchau, V.S. Kostic, C.M. Sue, A.R. Domingo,

R.L. Rosales, L.V. Lee, K. Freimann, A. Westenberger, Y. Mukai, T. Kawarai, R. Kaji, C. Klein, K.A. Martemyanov, A. Schmidt, Mutations

in GNAL: a novel cause of craniocervical dystonia, JAMA Neurol 71 (2014) 490-494

[7] R. Erro, K.P. Bhatia, J. Hardy, GNAL mutations and dystonia, JAMA Neurol. 71 (2014) 1052-1053

[8] E.M. Valente, M.J. Edwards, P. Mir, A. DiGiorgio, S. Salvi, M. Davis, N. Russo, M. Bozi,

H.T. Kim, G. Pennisi, N. Quinn, B. Dallapiccola, K.P. Bhatia, The epsilon-sarcoglycan

gene in myoclonic syndromes, Neurology 22(2005) 737-739

[9] M. Carecchio, M. Magliozzi, M. Copetti, A. Ferraris, L. Bernardini, M. Bonetti, G. Defazio, M.J. Edwards, I. Torrente, F. Pellegrini, C. Comi, K.P. Bhatia, E.M. Valente, Defining the epsilon-sarcoglycan (SGCE) gene phenotypic signature in myoclonus-dystonia: a reappraisal of genetic testing criteria, Mov Disord. 28 (2013):787-7

[10] G. Defazio, A. Conte, A.F. Gigante, G. Fabbrini, A. Berardelli, Is tremor in dystonia a phenotypic feature of dystonia?, Neurology 84 (2015) 1053-1059

[11] M. Stamelou, G. Charlesworth, C. Cordivari, S.A. Schneider, G. Kägi, U.M. Sheerin, I. Rubio-Agusti, A. Batla, H. Houlden, N.W. Wood, K.P. Bhatia, The phenotypic spectrum of DYT24 due to ANO3 mutations, Mov Disord. 29 (2014) 928-934

[12] K. Kinugawa, M. Vidailhet, F. Clot, E. Apartis, D. Grabli, E. Roze, Myoclonus-dystonia: an update, Mov Disord. 24 (2009) 479-489

[13] M.C. Kowarik, S. Langer, C. Keri, B. Hemmer, K. Oexle, J. Winkelmann, Myoclonus-dystonia in 18p deletion syndrome, Mov Disor. 26 (2011) 560-561

[14] S.B. Bressman, G.A. Heiman, T.G. Nygaard, L.J. Ozelius, A.L. Hunt, M.F. Brin, M.F. Gordon,
C.B. Moskowitz, D. de Leon, R.E. Burke, S. Fahn, N.J. Risch, X.O. Breakfield, P.L. Kramer, A study of idiopathic torsion dystonia in a non-Jewish family: evidence for genetic heterogeneity, Neurology 44 (1994) 283-287
[15] S.A. Schneider, K.P. Bhatia, Secondary dystonia-clinical clues and syndromic associations,
J Mov Disord 2 (2009) 58-63
[16] A. Tomić, I. Petrović, M. Svetel, V. Dobričić, N. Dragašević Mišković, V.S. Kostić, Pattern of disease progression in atypical form of pantothenate-kinase-associated neurodegeneration (PKAN) - Prospective study, Parkinsonism Relat Disord. 21 (2015) 521-524

Chapter 6

Recent advances in genetics of chorea

Niccolò E. Mencacci[1] and **Miryam Carecchio**[2,3,4]

[1] Department of Molecular Neuroscience, UCL Institute of Neurology, WC1N 3BG London, United Kingdom

[2] Molecular Neurogenetics Unit, IRCCS Foundation Carlo Besta Neurological Institute, Via Celoria 11, 20131 Milan, Italy

[3] Department of Pediatric Neurology, IRCCS Foundation Carlo Besta Neurological Institute, Via Celoria 11, 20131 Milan, Italy

[4] Department of Molecular and Translational Medicine, University of Milan Bicocca, Milan, Italy

Keywords: Chorea, Genetics, Huntington Disease, Next-Generation Sequencing, Medium Spiny Neurons.

Curr Opin Neurol. 2016 Aug;29(4):486-95.
doi:10.1097/WCO.0000000000000352.

Abstract

Purpose of review: Chorea presenting in childhood and adulthood encompasses several neurological disorders, both degenerative and non-progressive, often with a genetic basis. In this review, we discuss how modern genomic technologies are expanding our knowledge of monogenic choreic syndromes and advancing our insight into the molecular mechanisms responsible for chorea.

Recent findings: A genome-wide association study in Huntington Disease identified genetic disease-modifiers involved in controlling DNA repair mechanisms and stability of the CAG repeat expansion. Chorea is the cardinal feature of newly recognized genetic entities, *ADCY5* and *PDE10A*-related choreas, with onset in infancy and childhood. A phenotypic overlap between chorea, ataxia, epilepsy, and neurodevelopmental disorders is becoming increasingly evident.

Summary: The differential diagnosis of genetic conditions presenting with chorea has considerably widened, permitting a molecular diagnosis and an improved prognostic definition in an expanding number of cases. The identification of Huntington Disease genetic-modifiers and new chorea-causing gene mutations has allowed the initial recognition of converging molecular pathways underlying medium spiny neurons degeneration and dysregulation of normal development and activity of basal ganglia circuits. Signalling downstream of dopamine receptors and control of cAMP levels represent a very promising target for the development of new aetiology-based treatments for chorea and other hyperkinetic disorders.

Introduction

Chorea is a hyperkinetic movement disorder characterized by an excess of brief, continuous, unpatterned involuntary movements [1]. Focal lesions of the striatum and degeneration and/or functional dysregulation of medium spiny neurons (MSNs), which constitute ~95% of the striatal cells and form the striatal output projections, are considered to underlie the pathophysiology of choreic movements [2].

A variety of acquired causes may underlie chorea (recently reviewed in [3]). However, genetic aetiologies play a central role in the differential diagnosis of choreic syndromes. Huntington's disease (HD), with a prevalence of up to 1 in 10,000 subjects in Western countries, is not only the most relevant single cause of chorea, but also the most common monogenic neurodegenerative disorder [4]. In recent years, thanks to the advances in DNA sequencing technologies, the list of genetic entities presenting with chorea, both neurodegenerative and non-progressive forms, is rapidly and largely expanding (Table 1).

In this review we will summarise the most relevant recent progresses in the field of genetics of chorea. Furthermore, we will discuss the advances in the understanding of the molecular mechanisms of basal ganglia disorders, gained thanks to the identification of novel monogenic choreic syndromes. Chorea due to inherited metabolic disorders (e.g. mitochondrial diseases or inborn errors of metabolism) will not be reviewed here.

Advances in the genetics of Huntington's disease

Most of the current research efforts in HD genetics are aimed at

identifying disease modifiers, which may influence the disease progression and determine the age at onset (AAO) of motor symptoms [5]. The length of the CAG expansion is well known to be the most relevant determinant of the age at onset (AAO), with longer repeats associated with an earlier onset [6]. However, the CAG repeat size accounts for only ~50% of the variation in AAO [7] and a substantial portion of the remaining variance in AAO is highly heritable, strongly indicating the existence of other critical genetic determining factors [5]. Neither the size of the non-expanded *HTT* allele, nor the presence of a second smaller CAG pathological expansion, is able to significantly influence AAO [8]. A recent study showed that a variant (rs13102260; G>A) in the *HTT* promoter, located in the site that regulates binding of the transcription factor NF-κB, exerts a bidirectional effect on HD AAO [9]. The authors showed *in vitro* and *in vivo* that the presence of the A allele determined a lower NF-κB-mediated *HTT* transcriptional activity, resulting in delayed AAO when inherited on the same allele of the pathological expansion (reduced expression of the pathological allele). On the contrary, the A allele was associated with an earlier AAO when located on the non-expanded allele (reduced expression of the normal *HTT*). An important corollary of these results is that therapeutic strategies aimed at lowering the expression of the pathological CAG expansion should take into account that non allele-specific silencing of *HTT* could bear undesired effects by decreasing the expression of the normal allele. The most relevant advance toward the discovery of HD genetic modifiers is the recent publication of the genome-wide association study (GWAS) performed by the Genetic Modifiers of Huntington Disease (GeM—HD) Consortium [10]. The authors

identified two GWAS-significant loci, one on chromosome 15 and one on chromosome 8 that significantly modified the AAO of motor symptoms as predicted solely by the CAG expansion length. Other suggestive associations, though not passing the stringent GWAS-significance threshold, were observed on chromosomes 3, 5 and 21. Genes located on chromosome 15 locus are *MTMR10* and *FAN1* and on the chromosome 8 locus are *RRM2B* and *UBR5*. Pathway analysis of the GWAS results indicates that HD modifiers may be involved in control of DNA handling and repair mechanisms. Supporting this view, the chromosome 3 locus centred on *MLH1*, a gene previously identified in a HD mouse model as a modifier of somatic instability of the CAG repeats [11].

Huntington's disease-like syndromes

Around 1% of cases with a HD-like presentation do not carry a pathogenic expansion in *HTT* (HD-lookalikes, HDLs). HDLs are a genetically heterogeneous group of progressive heredo-degenerative conditions. Mutations in both dominant and recessive genes can result into HD mimics (recently reviewed in [12]). Amongst the autosomal dominant causes, it is important to consider pathological expansions in the genes encoding the prion protein (*PRNP*), junctophilin 3 (*JPH3*), TATA box-binding protein (*TBP*; also responsible for the dominant spinocerebellar ataxia type 17), atrophin-1 (*ATN1*), mutations in the ferritin light chain gene (the cause of neuroferritinopathy, an adult-onset dominant form of neurodegeneration with brain iron accumulation), and mutations in the genes responsible for idiopathic basal ganglia calcification (*SLC20A2, PDGFB, PDGFRB, XPR1*) [13-18]. Other

important neurodegenerative conditions mimicking HD are neuroacanthocytosis, caused by recessive *VPS13A* mutations [19], and Macleod syndrome, an X-linked recessive disease caused by mutations in *XK* [20]. Most of the published cases series indicate that a genetic diagnosis can be reached only in a small minority of HDL cases (~1-3%) [15, 21-24]. Exceptions to this are the high prevalence of the *JPH3* expansion in patients of sub-Saharan African descent [15, 25] and the *ATN1* expansions in Japanese patients [26]. Importantly, pathological *C9orf72* exanucleotide repeat expansions, the most common genetic cause of familial frontotemporal lobar degeneration and amyotrophic lateral sclerosis [27, 28], were recently recognised as the single most prevalent cause of HDL in Caucasians [29]. Hensman-Moss et al. assessed a UK cohort of 514 HDL patients and identified ten subjects (1.95%) who carried the expansion. The spectrum of movement disorders observed in these cases included variable combinations of chorea, dystonia, myoclonus, and parkinsonian signs. Behavioural, psychiatric and cognitive difficulties were observed in most expansion carriers. Prominent signs of upper motorneuron involvement (but not lower motorneuron) were evident in four subjects. The *C9orf72* repeat expansion has been subsequently confirmed to be a relevant cause of HDL also in other cohorts [30, 31].

Chorea as the core feature in patients with mutations in cerebellar ataxia-related genes

Chorea is increasingly observed in patients with pathogenic mutations in genes linked to cerebellar ataxia (other than the aforementioned SCA17 expansion). Patients with bi-allelic *ATM* mutations, the cause

of ataxia-telangiectasia (A-T), may present with a broad spectrum of movement disorders, including chorea [32-34], isolated dystonia [35, 36], DOPA-responsive dystonia [37], and myoclonus-dystonia [38-40]. Patients with variant A-T have milder mutations, which allow a degree of residual protein activity [41]. Meneret and colleagues systematically assessed a total of 14 consecutive adult subjects with A-T, and showed that, compared to patients with the classic presentation, all had a movement disorders, had a later age at onset, a milder disease course and longer survival [42]. Of relevance, patients with *ATM*-related chorea and dystonia may completely lack the classic clinical features of A-T [43]. Chorea has been rarely described also in cases with ataxia with oculomotor apraxia type 1, 2 and 4 [44-46], and Friedrich ataxia [21, 47, 48]. Recently, recessive mutations in *RNF216,* a gene previously associated with cerebellar ataxia and hypogonadotropic hypogonadism [49], were identified in two recessive pedigrees with chorea, behavioural problems, and severe dementia [50].

Chorea secondary to *NKX2-1* mutations

Mutations in *NKX2-1*, encoding a transcription factor essential for MSNs development, cause benign hereditary chorea (BHC) [51, 52], an autosomal dominant choreic syndrome with onset in infancy or early childhood, relatively scarce progression of symptoms and absence of other major neurological deficits, in particular progressive cognitive decline [53]. To date ~190 cases and ~100 *NKX2-1* mutations have been reported, allowing a better definition and an expansion of the phenotype associated with mutations in this gene [54-56]. *NKX2-1* mutations lead to a complex multi-systemic disease, featuring not only chorea, but also

thyroid and pulmonary defects (*brain-lung-thyroid* syndrome) in ~80% of cases [54, 56]. It was recently proposed to abandon the term BHC [57] given that (i) 60% of the identified *NKX2-1* mutations are de novo (hence, the disease is not hereditary)[54]; (ii) *NKX2-1*-mutated cases commonly present with a variety of neurological symptoms other than chorea (i.e. hypotonia, neurodevelopmental delay, dystonia, myoclonus, tics and ataxia) [54, 58-61]; (iii) patients with *NKX2-1* mutations may present various degrees of non-progressive intellectual disability, as well as behavioural and psychiatric symptoms (recently reviewed in [62]). Furthermore, while the term BHC is often used to imply the presence of *NKX2-1* mutations, a significant number of families with BHC do not carry mutations in this gene [63, 64]. Thorwarth and colleagues recently published an extensive clinical and genetic study in a large cohort of BHC cases [56]. Pathogenic *NKX2-1* mutations were present in only 26.7% of cases (27/101; 17 point mutations and 10 large deletions), indicating the existence of other undetected pathogenic variants in the *NKX2-1* non-coding regions and/or mutations in other closely functionally related genes. Intriguingly, two of the detected deletions spared the coding region of *NKX2-1,* involving only the neighbouring chromosomal region, which encompasses the *MBIP* gene. The pathogenic mechanism of these deletions is not clear. The deletions may remove regulatory elements essential for *NKX2-1* transcription and affect *NKX2-1* expression. Alternatively, *MBIP* haploinsufficiency may represent a novel cause of a *NKX2-1* deficiency-like presentation [56].

Chorea secondary to *ADCY5* and *PDE10A* mutations

Recently, mutations in *ADCY5* and *PDE10A* have been identified as

important causes of chorea. The first pathogenic *ADCY5* missense mutation (A726T) was identified in a large kindred with an autosomal dominant movement disorder, mainly characterized by early onset of dyskinesias (chorea and dystonia) and facial myokymias [65]. Subsequently, *ADCY5* mutations have been recognized as the cause of a broad range of hyperkinetic movement disorders, mainly including chorea, but also dystonia and myoclonus [66-69]. So far, eight different mutations (de novo or with autosomal dominant transmission) have been reported in 27 unrelated subjects. Mutations affecting the amino acid residues R418 and A726 are recurrent, highlighting a particular relevance of these residues for disease mechanisms. Looking at patients published so far, subjects with the common p.R418W mutation seem to have a more severe presentation, with axial hypotonia and delayed motor milestones. Furthermore, somatic mosaicism may be at least in part responsible for intra-familial clinical variability in these subjects [66, 67]. Red flags for the diagnosis of *ADCY5*-related dyskinesias are (i) an onset of symptoms in the first years of life, (ii) the absence of significant cognitive involvement, (iii) prominent facial twitches, (iv) a marked fluctuations of symptoms (some patients presenting frank paroxysmal attacks, though without specific triggers [70]), (v) a marked exacerbation of the dyskinesias at night and upon awakening. Although *ADCY5*-related chorea is a non-degenerative condition, others and we have observed that the clinical picture of *ADCY5*-mutated cases can evolve, with chorea being more evident during childhood and dystonic and myoclonic elements becoming more prominent over the years [66, 68].

Both de novo dominant and recessive *PDE10A* mutations have been recently described in patients with childhood-onset chorea. Two different de novo mutations (p.F300L and p.F334L) were identified in three unrelated cases with a very similar clinical presentation of childhood-onset chorea (AAO between 5-10 years) and characteristic brain MRI showing symmetrical T2-hyperintense bilateral striatal lesions [71]. Recessive homozygous mutations (p.Y107C and p.A116P) were detected in two consanguineous pedigrees [72]. The phenotype in these cases was more severe, with a much earlier AAO (< 1 year), severe dysarthria, axial hypotonia, cognitive and language development delay. Of interest, despite a more severe neurological involvement, the MRI of the cases with recessive mutations did not show the same abnormal signal observed in the cases with dominant mutations.

ADCY5 and *PDE10A* encode the main enzymes regulating the synthesis (adenyl cyclase 5; AC5) and degradation (phosphodiesterase 10A; PDE10A) of cyclic adenosine monophosphate (cAMP) in MSNs. AC5 activity, and consequently cAMP synthesis in MSNs, is promoted by the stimulation of the G protein-coupled dopamine receptors type 1 and adenosine receptors 2A. Hence dopamine and adenosine-mediated modulation of MSNs activity largely relies on cAMP signalling [73]. *In vitro* and *in vivo* assessment of the effect of the identified PDE10A substitutions showed that both dominant and recessive variants lead to a loss-of-function [71] or reduced protein levels [72]. These data, together with the fact that *ADCY5* pathogenic mutations may increase the AC5 enzymatic activity and the synthesis of cAMP [74], suggest that increased intracellular cAMP levels in MSNs is critical for chorea pathogenesis. Pharmacological modulation of PDE10A is a primary

target in pharmacological research of basal ganglia disorders, including HD and Parkinson disease [75] and a phase II clinical study (the Amaryllis study) of a PDE10A inhibitor is currently ongoing in HD. Importantly, the identification of loss-of-function *PDE10A* mutations as a cause of chorea suggests that pharmacological inhibition of PDE10A may not be the best option for the treatment of hyperkinetic movement disorders. Mutations in *GNAL* [76] and *GPR88* [77], coding for G proteins almost exclusively expressed in MSNs and coupled with dopamine receptors, have been recently linked to dystonia and chorea, respectively, further implicating intracellular signalling downstream of dopamine receptors in MSNs in the pathogenesis of chorea and other hyperkinetic movement disorders.

Chorea in carriers of epileptic encephalopathy genes

An overlap between hyperkinetic movement disorders and epileptic/neurodevelopmental syndromes is emerging. A rapidly expanding number of mutations in genes originally reported in severe early-onset epileptic encephalopathies are now recognised in a spectrum of conditions ranging from isolated movement disorders (most frequently chorea, but also dystonia and stereotypies) to more catastrophic presentations.

GNAO1 mutations, first described in a type of severe epileptic encephalopathy with developmental delay (Ohtahara syndrome; [78]), are described also in cases presenting with a progressive choreic movement disorder, often in absence of epilepsy [79-82]. Mutations in *FOXG1*, a gene which plays a crucial role in the development of the foetal telencephalon, lead to a distinct phenotype manifesting in infancy

and early childhood with microcephaly, epilepsy, delayed milestones and severe intellectual disability without language development (congenital Rett-like syndrome) [83]. Movement disorders have now been recognized as a core feature of this disorder, being present in 100% of cases in a series of 28 patients recently published [84]. Chorea is the most frequent movement disorder in *FOXG1* mutation carriers (88%), followed by orolingual/facial dyskinesias, dystonia, myoclonus and stereotypies, present in various combinations. Importantly, patients with missense mutations (instead of severe truncating mutations) may display a milder phenotype, with independent ambulation, spoken language, and normocephaly [84]. A single missense mutation (p.E1483K) in *SCN8A*, encoding a voltage gated Na-channel subunit widely expressed in the CNS, has recently been linked to paroxysmal kinesigenic dyskinesia and benign familial infantile seizures [85]. This observation expands the phenotypic spectrum associated with mutations in this gene, which also includes severe epileptic encephalopathy and a neurodevelopmental disorder [86]. A *de novo* missense variant in *SYT1*, encoding Synaptogamin-1, a protein essential for synaptic vescicle fusion, has been recently associated with severe developmental delay and an early onset, paroxysmal dyskinetic movement disorder worsening at night (as seen in *ADCY5*-mutated patients), but only a single patient has been described to date [87].

Conclusions

Chorea is observed in an expanding number of genetic diseases. Mutations in *ADCY5* and *PDE10A* represent novel important causes of chorea, frequently featuring also myoclonus and dystonia. Furthermore,

mutations in genes classically associated with other neurological disorders, such as ataxias, developmental delay, and epileptic encephalopathies, are increasingly detected in patients with chorea. Vice versa, mutations in *NKX2-1*, the cause of BHC, are now recognised in patients with a range of movement disorders (i.e. myoclonus, dystonia and ataxia) other than chorea. Importantly, this substantial genetic and clinical overlap suggests that disruption of similar circuits and/or molecular pathways may underlie these neurological conditions.

While individually rare, clinical recognition and molecular diagnosis of monogenic causes of chorea is crucial to define precisely the prognosis and offer a correct genetic counselling to patients with chorea. Furthermore, the identification of genetic HD-modifiers and of a growing number of mutations in novel genes linked to chorea is allowing the definition of converging biological pathways likely to be essential for the survival and physiological activity of MSNs. Different types of disease mechanisms can affect MSNs and clinically lead to chorea, including degenerative processes (e.g. HD and HDL), developmental abnormalities (e.g. *NKX2-1* and *FOXG1*-related choreas) and disrupted post-receptorial intracellular signalling (*ADCY5* and *PDE10A*-related choreas). A better understanding of the molecular mechanisms responsible for these conditions will be the key step to develop specific disease-modifying treatments.

Key points

- The results of the first GWAS in Huntington's disease identified novel genetic modifiers of age at onset located on chromosome 8 and 15 and suggest that DNA handling and repair mechanisms are crucial in controlling the somatic stability of the CAG expansion.
- Thanks to the discovery of mutations in *ADCY5* and *PDE10A* as novel causes of chorea, abnormal cAMP metabolism in medium spiny neurons is emerging as a central molecular mechanism underlying the pathogenesis of basal ganglia disorders
- The *C9orf72* exanucleotide expansion has been recognised as the most common cause of Huntington disease-like syndrome in Caucasian populations
- While mutations in *NKX2-1* have been identified in patients with a range of movement disorders other than chorea, more than to 70% of benign hereditary chorea (BHC) cases do not have mutations in *NKX2-1,* prompting to abandon the use of the term BHC to label patients with *NKX2-1* mutations.
- An expanding genetic and phenotypic overlap between chorea (and other hyperkinetic movement disorders) and other neurological syndromes, including developmental delay, epilepsy and ataxia, is emerging.

Gene	Main associated phenotype	Gene product	Inheritance	Age of onset	Diagnostic clues
HTT	Huntington disease	Huntingtin	AD (CAG expansion)	Childhood to late adulthood	Cognitive decline, psychiatric disturbances Progressive course MRI: caudate nucleus head atrophy
PRNP	HDL1	Prion protein	AD (octapeptide coding repeat expansion)	Adulthood	Dementia and psychiatric features Possible parkinsonism at onset and longer survival than HD
JPH3	HDL2	Junctophilin 3	AD (CAG/CTG expansion)	Adulthood	Parkinsonism may be first manifestation High frequency in people with black African ancestry
TBP	HDL4/ Spinocerebellar ataxia type 17	TATA box-binding protein	AD (CAG expansion)	Childhood to adulthood	Ataxia and cognitive decline Frequent parkinsonism MRI: cerebellar atrophy
ATN1	Dentatorubral-pallidoluysian atrophy	Atrophin-1	AD (CAG expansion)	Childhood to adulthood	Seizures, myoclonus and cognitive decline MRI: Cerebellar and brainstem atrophy (especially pons) High frequency in Japan
C9orf72	FTD/MND	Chromosome 9 Open Reading Frame 72	AD (GGGGCC expansion)	Childhood to adulthood	Prominent cognitive and psychiatric features Pyramidal signs MRI: diffuse cerebral atrophy
FTL	Neuroferritinopathy	Ferritin light chain	AD	Teenage to late adulthood	Action-specific facial dystonia Reduced ferritin plasma levels

					MRI: iron deposition in basal ganglia and cortical pencil lining
SLC20A2 *PDGFB* *PDGFRB* *XPR1*	Idiopathic Basal Ganglia Calcification	Na-dependent phosphate transporter type 2 Platelet-derived growth factor β-polypeptide Platelet-derived growth factor receptor, β Xenotropic and polytropic retroviruses receptor	AD AD AD AD	Symptoms: early to late adulthood Calcium deposition: childhood to adolescence	CT scan: basal ganglia, cerebellar dentate nuclei and subcortical white matter calcification
VPS13A	Chorea-acanthocytosis	Chorein	AR	Early adulthood	Severe oromandibular dystonia with lip and tongue biting Head drops Peripheral axonal neuropathy Elevated serum CK MRI: caudate nucleus head atrophy
XK	Macleod syndrome	Kell blood group protein	X-linked recessive	Adulthood	Peripheral sensorimotor neuropathy Cardiomyopathy Elevated serum CK
ATM	Ataxia-telangiectasia	Ataxia-telangiectasia mutated gene	AR	Childhood to adulthood	Oculocutaneous telangiectases Sensorimotor neuropathy Elevated serum alpha-fetoprotein Predisposition to malignancy MRI: cerebellar atrophy

APTX *SETX* *PNKP*	Ataxia with oculomotor apraxia (AOA) type 1, 2, and 4	Aprataxin Senataxin Polynucleotide kinase 3'-phosphatase	AR	Childhood to adulthood	Sensorimotor neuropathy Hypoalbuminemia in AOA1 Hypercholesterolemia in AOA1 and AOA4 Elevated alpha-fetoprotein in AOA2 and AOA4 MRI: cerebellar atrophy
RNF216	Gordon-Holmes syndrome	Ring finger protein 216	AR	Adulthood	Hypogonadism MRI: cerebellar atrophy
NKX2-1	*NKX2-1*-related chorea (benign hereditary chorea)	Thyroid transcription factor 1	AD/De novo	Infancy	Non-progressive course Hypotonia and early falls Learning difficulties Frequent pulmonary and thyroid involvement
ADCY5	*ADCY5*-related chorea	Adenylate cyclase 5	AD/De novo	Infancy to childhood	Dystonia and myoclonus may become prominent with age Severe diurnal and nocturnal exacerbations Axial hypotonia and delayed milestones in most severe cases
PDE10A	*PDE10A*-related chorea	Phosphodiesterase 10A	De novo/AR	Infancy to childhood	Delayed milestones and language development and dysarthria in cases with recessive mutations MRI: symmetrical T2-hyperintense bilateral striatal lesions in cases with dominant de novo mutations
GPR88	*GPR88*-related chorea	G protein-coupled receptor 88	AR	Childhood	Language delay and learning disabilities

GNAO1	Early infantile epileptic encephalopathy type 17 (Ohtahara syndrome)	Gαo	De novo	Infancy to childhood	Progressive and severe movement disorder associated with developmental delay, with or without seizures
FOXG1	Rett Syndrome, congenital variant	Forkhead Box G1	De novo	Infancy to early childhood	Severe intellectual disability, absent language, acquired microcephaly MRI: corpus callosum abnormalities, frontal or frontotemporal underdevelopment mild cerebellar hypoplasia, and delayed myelination.
SYT1	Severe motor delay and intellectual disability	Synaptotagmin-1	De novo	Infancy	Severe delayed motor development without seizures
SCN8A	- Early infantile epileptic encephalopathy type 13 - BFIS	NaV1.6α-subunit of voltage-gated Na channels	AD/De novo	Infancy to childhood	Paroxysmal dystonia/chorea triggered by sudden movements or emotional stress Focal EEG abnormalities during attacks

Table 1. List of monogenic causes of chorea. AD: autosomal dominant; **AR**: autosomal recessive; **BFIS**: Benign familial infantile seizures; **HDL**: Huntington's disease-like

Acknowledgements

1. Acknowledgements: None.

2. Financial support and sponsorship: This work was supported by a Medical Research Council/Wellcome Trust Strategic Award (WT089698/Z/09/Z). N.E.M receives support from the Department of Health's National Institute for Health Research (NIHR) Biomedical Research Centres. M.C. is funded by the Pierfranco and Luisa Mariani foundation.

3. Conflicts of interest: none.

References

1. Donaldson I, Marsden CD, Schneider SA, Bhatia KB. Clinical approach to movement disorders. In: Donaldson I, Marsden CD, Schneider SA, Bhatia KB, editors. Marsden's Book of Movement Disorders. Oxford, United Kingdom: Oxford University Press; 2012. p. 140-141.

2. Gittis AH, Kreitzer AC. Striatal microcircuitry and movement disorders. Trends Neurosci 2012;35:557-64.

3. Hermann A, Walker RH. Diagnosis and treatment of chorea syndromes. Curr Neurol Neurosci Rep 2015;15:514.

4. Ross CA, Tabrizi SJ. Huntington's disease: from molecular pathogenesis to clinical treatment. Lancet Neurol 2011;10:83-98.

5. Gusella JF, MacDonald ME, Lee JM. Genetic modifiers of Huntington's disease. Mov Disord 2014;29:1359-65.

6. Andrew SE, Goldberg YP, Kremer B, et al. The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. Nat Genet 1993;4:398-403.

7. Langbehn DR, Brinkman RR, Falush D, et al. A new model for prediction of the age of onset and penetrance for Huntington's disease based on CAG length. Clin Genet 2004;65:267-77.

8. Lee JM, Ramos EM, Lee JH, et al. CAG repeat expansion in Huntington disease determines age at onset in a fully dominant fashion. Neurology 2012;78:690-5.

**9. Becanovic K, Norremolle A, Neal SJ, et al. A SNP in the HTT promoter alters NF-kappaB binding and is a bidirectional genetic modifier of Huntington disease. Nat Neurosci 2015;18:807-16.

In this paper the authors elegantly demonstrate that reduced

expression of the normal and pathological *HTT* alleles exert an opposite effect on Huntington disease age at onset. Therapeutic strategies aimed at reducing the expression of the pathological allelle, should take into account that suppression of the normal allele expression may have indesirable effect on disease progression.

**10. Lee J-M, Wheeler Vanessa C, Chao Michael J, et al. Identification of Genetic Factors that Modify Clinical Onset of Huntington's Disease. Cell 2015;162:516-526.

This paper details the results of the first GWAS in Huntington disease, describing the identification of a locus on chromosome 15 and one on chromosome 8, significantly associated with modification of the age at onset of motor symptoms. Pathway analysis of the genes underlying the GWAS hits suggests a role for DNA repair mechanisms in altering the course of Huntington disease.

11. Pinto RM, Dragileva E, Kirby A, et al. Mismatch repair genes Mlh1 and Mlh3 modify CAG instability in Huntington's disease mice: genome-wide and candidate approaches. PLoS Genet 2013;9:e1003930.

12. Martino D, Stamelou M, Bhatia KP. The differential diagnosis of Huntington's disease-like syndromes: 'red flags' for the clinician. J Neurol Neurosurg Psychiatry 2013;84:650-6.

13. Moore RC, Xiang F, Monaghan J, et al. Huntington disease phenocopy is a familial prion disease. Am J Hum Genet 2001;69:1385-8.

14. Holmes SE, O'Hearn E, Rosenblatt A, et al. A repeat expansion

in the gene encoding junctophilin-3 is associated with Huntington disease-like 2. Nat Genet 2001;29:377-8.

15. Stevanin G, Fujigasaki H, Lebre AS, et al. Huntington's disease-like phenotype due to trinucleotide repeat expansions in the TBP and JPH3 genes. Brain 2003;126:1599-603.

16. Nagafuchi S, Yanagisawa H, Sato K, et al. Dentatorubral and pallidoluysian atrophy expansion of an unstable CAG trinucleotide on chromosome 12p. Nat Genet 1994;6:14-8.

17. Curtis AR, Fey C, Morris CM, et al. Mutation in the gene encoding ferritin light polypeptide causes dominant adult-onset basal ganglia disease. Nat Genet 2001;28:350-4.

18. Tadic V, Westenberger A, Domingo A, et al. Primary familial brain calcification with known gene mutations: a systematic review and challenges of phenotypic characterization. JAMA Neurol 2015;72:460-7.

19. Rampoldi L, Dobson-Stone C, Rubio JP, et al. A conserved sorting-associated protein is mutant in chorea-acanthocytosis. Nat Genet 2001;28:119-20.

20. Walker RH, Jung HH, Dobson-Stone C, et al. Neurologic phenotypes associated with acanthocytosis. Neurology 2007;68:92-8.

21. Wild EJ, Mudanohwo EE, Sweeney MG, et al. Huntington's disease phenocopies are clinically and genetically heterogeneous. Mov Disord 2008;23:716-20.

22. Costa Mdo C, Teixeira-Castro A, Constante M, et al. Exclusion of mutations in the PRNP, JPH3, TBP, ATN1, CREBBP, POU3F2 and FTL genes as a cause of disease in Portuguese patients with a Huntington-like phenotype. J Hum Genet 2006;51:645-51.

23. Koutsis G, Karadima G, Pandraud A, et al. Genetic screening of Greek patients with Huntington's disease phenocopies identifies an SCA8 expansion. J Neurol 2012;259:1874-8.
24. Keckarevic M, Savic D, Svetel M, et al. Yugoslav HD phenocopies analyzed on the presence of mutations in PrP, ferritin, and Jp-3 genes. Int J Neurosci 2005;115:299-301.
25. Krause A, Mitchell C, Essop F, et al. Junctophilin 3 (JPH3) expansion mutations causing Huntington disease like 2 (HDL2) are common in South African patients with African ancestry and a Huntington disease phenotype. Am J Med Genet B Neuropsychiatr Genet 2015.
26. Becher MW, Rubinsztein DC, Leggo J, et al. Dentatorubral and pallidoluysian atrophy (DRPLA). Clinical and neuropathological findings in genetically confirmed North American and European pedigrees. Mov Disord 1997;12:519-30.
27. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 2011;72:245-56.
28. Renton AE, Majounie E, Waite A, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 2011;72:257-68.
*29. Hensman Moss DJ, Poulter M, Beck J, et al. C9orf72 expansions are the most common genetic cause of Huntington disease phenocopies. Neurology 2014;82:292-9.
This paper describes the expansion of the clinical phenotype associated with the *C9orf72* pathological expansion to comprise also

a hyperkinetic movement disorder mimicking Huntington disease. The authors show that the *C9orf72* pathological expansion represents the most common cause of a Huntington disease-like phenotype in Caucasians.

30. Kostic VS, Dobricic V, Stankovic I, et al. C9orf72 expansion as a possible genetic cause of Huntington disease phenocopy syndrome. J Neurol 2014;261:1917-21.

31. Koutsis G, Karadima G, Kartanou C, et al. C9ORF72 hexanucleotide repeat expansions are a frequent cause of Huntington disease phenocopies in the Greek population. Neurobiol Aging 2015;36:547 e13-6.

32. Klein C, Wenning GK, Quinn NP, Marsden CD. Ataxia without telangiectasia masquerading as benign hereditary chorea. Mov Disord 1996;11:217-20.

33. Thompson S, Iyer A, Byrd P, et al. Dopa-Responsive Dystonia and Chorea as a Presenting Feature in Ataxia-Telangiectasia. Movement Disorders Clinical Practice 2014;1:249-251.

34. Worth PF, Srinivasan V, Smith A, et al. Very mild presentation in adult with classical cellular phenotype of ataxia telangiectasia. Mov Disord 2013;28:524-8.

35. Saunders-Pullman R, Raymond D, Stoessl AJ, et al. Variant ataxia-telangiectasia presenting as primary-appearing dystonia in Canadian Mennonites. Neurology 2012;78:649-57.

36. Claes K, Depuydt J, Taylor AM, et al. Variant ataxia telangiectasia: clinical and molecular findings and evaluation of radiosensitive phenotypes in a patient and relatives. Neuromolecular Med 2013;15:447-57.

37. Charlesworth G, Mohire MD, Schneider SA, et al. Ataxia telangiectasia presenting as dopa-responsive cervical dystonia. Neurology 2013;81:1148-51.

38. Cummins G, Jawad T, Taylor M, Lynch T. Myoclonic head jerks and extensor axial dystonia in the variant form of ataxia telangiectasia. Parkinsonism Relat Disord 2013;19:1173-4.

39. Termsarasab P, Yang AC, Frucht SJ. Myoclonus in ataxia-telangiectasia. Tremor Other Hyperkinet Mov (N Y) 2015;5:298.

40. Georgiev D, Mehta D, Zacharia A, et al. Bilateral Deep Brain Stimulation of the Globus Pallidus Pars Interna in a Patient with Variant Ataxia-Telangiectasia. Movement Disorders Clinical Practice 2016.

41. Gilad S, Chessa L, Khosravi R, et al. Genotype-phenotype relationships in ataxia-telangiectasia and variants. Am J Hum Genet 1998;62:551-61.

*41. Meneret A, Ahmar-Beaugendre Y, Rieunier G, et al. The pleiotropic movement disorders phenotype of adult ataxia-telangiectasia. Neurology 2014;83:1087-95.

This paper explors sistematically the genotype and the phenotype of ataxia-telangiectasia in adults. Compared to patients with the classic A-T presentatiom, variant A-T cases present more often with a hyperkinetic movement disorder, have a later age at onset, later loss of walking indipendance, and longer survival.

43. Kuhm C, Gallenmuller C, Dork T, et al. Novel ATM mutation in a German patient presenting as generalized dystonia without classical signs of ataxia-telangiectasia. J Neurol 2015;262:768-70.

44. Salvatore E, Varrone A, Criscuolo C, et al. Nigrostriatal involvement in ataxia with oculomotor apraxia type 1. J Neurol

2008;255:45-8.

45. Anheim M, Monga B, Fleury M, et al. Ataxia with oculomotor apraxia type 2: clinical, biological and genotype/phenotype correlation study of a cohort of 90 patients. Brain 2009;132:2688-98.

46. Paucar M, Malmgren H, Taylor M, et al. Expanding the ataxia with oculomotor apraxia type 4 phenotype. Neurology: Genetics 2016;2:e49.

47. Zhu D, Burke C, Leslie A, Nicholson GA. Friedreich's ataxia with chorea and myoclonus caused by a compound heterozygosity for a novel deletion and the trinucleotide GAA expansion. Mov Disord 2002;17:585-9.

48. Hanna MG, Davis MB, Sweeney MG, et al. Generalized chorea in two patients harboring the Friedreich's ataxia gene trinucleotide repeat expansion. Mov Disord 1998;13:339-40.

49. Margolin DH, Kousi M, Chan YM, et al. Ataxia, dementia, and hypogonadotropism caused by disordered ubiquitination. N Engl J Med 2013;368:1992-2003.

50. Santens P, Van Damme T, Steyaert W, et al. RNF216 mutations as a novel cause of autosomal recessive Huntington-like disorder. Neurology 2015;84:1760-6.

51. Breedveld GJ, van Dongen JW, Danesino C, et al. Mutations in TITF-1 are associated with benign hereditary chorea. Hum Mol Genet 2002;11:971-9.

52. Krude H, Schutz B, Biebermann H, et al. Choreoathetosis, hypothyroidism, and pulmonary alterations due to human NKX2-1 haploinsufficiency. J Clin Invest 2002;109:475-80.

53. Kleiner-Fisman G, Lang AE. Benign hereditary chorea

revisited: a journey to understanding. Mov Disord 2007;22:2297-305; quiz 2452.

54. Gras D, Jonard L, Roze E, et al. Benign hereditary chorea: phenotype, prognosis, therapeutic outcome and long term follow-up in a large series with new mutations in the TITF1/NKX2-1 gene. J Neurol Neurosurg Psychiatry 2012;83:956-62.

55. Inzelberg R, Weinberger M, Gak E. Benign hereditary chorea: an update. Parkinsonism Relat Disord 2011;17:301-7.

**56. Thorwarth A, Schnittert-Hubener S, Schrumpf P, et al. Comprehensive genotyping and clinical characterisation reveal 27 novel NKX2-1 mutations and expand the phenotypic spectrum. J Med Genet 2014;51:375-87.

This is the largest study reported to date assessing sistematically the frequency of *NKX2-1* mutations in benign hereditary chorea. The authors screened the gene in a cohort of 101 patients and detected pathogenic mutations in only 27% of cases, strongly indicating genetic heterogeneity. Two large segregating deletions spared the coding region of *NKX2-1*.

57. Morgan JC, Kurek JA, Davis J, et al. ADCY5 mutations are another cause of benign hereditary chorea. Neurology 2016;86:978-9.

58. Asmus F, Devlin A, Munz M, et al. Clinical differentiation of genetically proven benign hereditary chorea and myoclonus-dystonia. Mov Disord 2007;22:2104-9.

59. Armstrong MJ, Shah BB, Chen R, et al. Expanding the phenomenology of benign hereditary chorea: evolution from chorea to myoclonus and dystonia. Mov Disord 2011;26:2296-7.

60. Veneziano L, Parkinson MH, Mantuano E, et al. A novel de novo

mutation of the TITF1/NKX2-1 gene causing ataxia, benign hereditary chorea, hypothyroidism and a pituitary mass in a UK family and review of the literature. Cerebellum 2014;13:588-95.

61. de Gusmao CM, Kok F, Casella EB, Waugh JL. Benign hereditary chorea related to NKX2-1 with ataxia and dystonia. Neurology Genetics 2016;2.

62. Peall KJ, Kurian MA. Benign Hereditary Chorea: An Update. Tremor Other Hyperkinet Mov (N Y) 2015;5:314.

63. Bauer P, Kreuz FR, Burk K, et al. Mutations in TITF1 are not relevant to sporadic and familial chorea of unknown cause. Mov Disord 2006;21:1734-7.

64. Breedveld GJ, Percy AK, MacDonald ME, et al. Clinical and genetic heterogeneity in benign hereditary chorea. Neurology 2002;59:579-84.

65. 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;69:630-5.

66. Carapito R, Paul N, Untrau M, et al. A de novo ADCY5 mutation causes early-onset autosomal dominant chorea and dystonia. Mov Disord 2015;30:423-7.

*67. Mencacci NE, Erro R, Wiethoff S, et al. ADCY5 mutations are another cause of benign hereditary chorea. Neurology 2015;85:80-8.

By studying 18 unrelated cases with benign hereditary chorea without *NKX2-1* mutations, the authors identify the *ADCY5* p.R418W mutation in two cases. The authors observe significant progression of symptoms in *ADCY5* mutation carriers, in contrast

to BHC secondary to *NKX2-1* mutations. This difference in the clinical course is mirrored by brain expression data, showing increasing *ADCY5* expression in the striatum during brain development, whereas *NKX2-1* shows an opposite trend.

**68. Chen DH, Meneret A, Friedman JR, et al. ADCY5-related dyskinesia: Broader spectrum and genotype-phenotype correlations. Neurology 2015;85:2026-35.

This paper report the identification of 3 new families and 12 new sporadic cases with *ADCY5* mutations and show that these mutations cause a mixed hyperkinetic disorder that includes dystonia, chorea, and myoclonus.

69. Chang FC, Westenberger A, Dale RC, et al. Phenotypic insights into ADCY5-associated disease. Mov Disord 2016.

70. Friedman JR, Meneret A, Chen DH, et al. ADCY5 mutation carriers display pleiotropic paroxysmal day and nighttime dyskinesias. Mov Disord 2016;31:147-8.

**71. Mencacci NE, Kamsteeg E-J, Nakashima K, et al. De Novo Mutations in *PDE10A* Cause Childhood-Onset Chorea with Bilateral Striatal Lesions. The American Journal of Human Genetics;98:763-771.

***PDE10A* de novo mutations are reported for the first time in patients with childhood-onset chorea and characteristic bilateral striatal lesions on brain MRI, confirming the crucial role of striatal cAMP signaling in the regulation of basal ganglia circuitry.**

** 72. Diggle CP, Sukoff Rizzo SJ, Popiolek M, et al. Biallelic Mutations in *PDE10A* Lead to Loss of Striatal PDE10A and a Hyperkinetic Movement Disorder with Onset in Infancy. The American

Journal of Human Genetics;98:735-743.

Back-to-back publication with ref. 71, this paper describes the identification of recessive *PDE10A* mutations in patients with a more complex phenotype including childhood-onset chorea, axial hypotonia and developmental delay. Patients' brain MRI did not show striatal lesions as in dominant mutations carriers individuated by Mencacci et al., suggesting different *in vivo* mechanisms of the mutations.

73. Herve D. Identification of a specific assembly of the g protein golf as a critical and regulated module of dopamine and adenosine-activated cAMP pathways in the striatum. Front Neuroanat 2011;5:48.

*74. Chen YZ, Friedman JR, Chen DH, et al. Gain-of-function ADCY5 mutations in familial dyskinesia with facial myokymia. Ann Neurol 2014;75:542-9.

The authors provide *in vitro* evidence that a gain-of-function, resulting in increased enzymatic activity and cAMP synthesis, could be the disease mechanism of pathogenic *ADCY5* mutations.

75. Chappie TA, Helal CJ, Hou X. Current landscape of phosphodiesterase 10A (PDE10A) inhibition. J Med Chem 2012;55:7299-331.

76. Fuchs T, Saunders-Pullman R, Masuho I, et al. Mutations in GNAL cause primary torsion dystonia. Nat Genet 2013;45:88-92.

*77. Alkufri F, Shaag A, Abu-Libdeh B, Elpeleg O. Deleterious mutation in GPR88 is associated with chorea, speech delay, and learning disabilities. Neurology Genetics 2016;2.

A combination of developmental delay, marked speech retardation, learning disability and chorea is described in association with a

homozygous mutation in *GPR88*, an orphan G protein–coupled receptor that is selectively expressed in striatal medium spiny neurons.

78. Nakamura K, Kodera H, Akita T, et al. De Novo mutations in GNAO1, encoding a Galphao subunit of heterotrimeric G proteins, cause epileptic encephalopathy. Am J Hum Genet 2013;93:496-505.

*79. Saitsu H, Fukai R, Ben-Zeev B, et al. Phenotypic spectrum of GNAO1 variants: epileptic encephalopathy to involuntary movements with severe developmental delay. Eur J Hum Genet 2016;24:129-34.

A paper characterizing the heterogeneous clinical expression of *GNAO1* mutations, blurring the boundaries between epilepsy and hyperkinetic movement disorders.

80. Kulkarni N, Tang S, Bhardwaj R, et al. Progressive Movement Disorder in Brothers Carrying a GNAO1 Mutation Responsive to Deep Brain Stimulation. J Child Neurol 2016;31:211-4.

81. Dhamija R, Mink JW, Shah BB, Goodkin HP. GNAO1-Associated Movement Disorder. Movement Disorders Clinical Practice 2016.

82. Ananth AL, Robichaux-Viehoever A, Kim YM, et al. Clinical Course of Six Children With GNAO1 Mutations Causing a Severe and Distinctive Movement Disorder. Pediatr Neurol 2016.

83. Ariani F, Hayek G, Rondinella D, et al. FOXG1 is responsible for the congenital variant of Rett syndrome. Am J Hum Genet 2008;83:89-93.

*84. Papandreou A, Schneider RB, Augustine EF, et al. Delineation of the movement disorders associated with FOXG1 mutations. Neurology 2016.

The authors hihglight that movement disorders are a cardinal feature of *FOXG1*-related disease. All 28 enrolled patients reported in this series displayed chorea, dystonia and myoclonus in various combination.

*85. Gardella E, Becker F, Moller RS, et al. Benign infantile seizures and paroxysmal dyskinesia caused by an SCN8A mutation. Ann Neurol 2016;79:428-36.

This paper identifies mutations in *SCN8A* as a cause of benign infantile seizures and paroxysmal dyskinesias, providing evidence for the existence of a second gene, after *PRRT2,* responsible for this condition. The presence of EEG abnormalities during a paroxysmal dystonic attack suggests a unifying disease mechanism for the seizures and the movement disorder.

86. Larsen J, Carvill GL, Gardella E, et al. The phenotypic spectrum of SCN8A encephalopathy. Neurology 2015;84:480-9.

87. Baker K, Gordon SL, Grozeva D, et al. Identification of a human synaptotagmin-1 mutation that perturbs synaptic vesicle cycling. J Clin Invest 2015;125:1670-8.

Chapter 7

DYT2 screening in early-onset isolated dystonia

Miryam Carecchio[a,b,c,*], Chiara Reale[a,*], Federica Invernizzi[a], Valentina Monti[a], Simona Petrucci[d], Monia Ginevrino[e], Francesca Morgante[f], Giovanna Zorzi[b], Federica Zibordi[b], Anna Rita Bentivoglio[g], Enza Maria Valente[e], Nardo Nardocci[b], Barbara Garavaglia[a,**]

[a] *Molecular Neurogenetics Unit, IRCCS Foundation C. Besta Neurological Institute, Milan, Italy*

[b] *Department of Child Neurology, IRCCS Foundation C. Besta Neurological Institute, Milan, Italy*

[c] *Department of Translational Medicine, University of Milan Bicocca, Milan, Italy*

[d] *Department of Neurological Sciences, Sapienza University, Rome, Italy*

[e] *Department of Medicine and Surgery, University of Salerno, Salerno, Italy*

[f] *Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy*

[g] *Institute of Neurology, Università Cattolica del Sacro Cuore, Rome, Italy*

[*]Shared first authors; [**] Corresponding author

Keywords: dystonia, pediatric, isolated, recessive, DYT2, HPCA.

Eur J Paediatr Neurol. 2017 Mar;21(2):269-271. doi: 10.1016/j.ejpn.2016.10.001

Abstract

Background: mutations in *HPCA*, a gene implicated in calcium signaling in the striatum, have been recently described in recessive dystonia cases previously grouped under the term "DYT2 dystonia". Positive patients reported so far show focal onset during childhood with subsequent generalization and a slowly progressive course to adulthood.

Methods: 73 patients with isolated dystonia of various distribution, manifesting within 21 years of age, were enrolled in this Italian study and underwent a mutational screening of *HPCA* gene by means of Sanger sequencing.

Results/Conclusions: mean age at onset was 10.2 (± 5.1) years and mean age at the time of genetic testing was 33 (±14.2) years. Mean disease duration at the time of enrollment was 22.7 (±12.8) years. None of the patients enrolled was found to carry *HPCA* mutations, rising suspicion that these probably represent a very rare cause of dystonia in childhood-adolescence. Larger studies will help determining the real mutational frequency of this gene also in different ethnic groups.

1. Introduction

Dystonia is a hyperkinetic movement disorder characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements, postures, or both. Dystonia is often initiated or worsened by voluntary action and associated with overflow muscle activation. Dystonia is defined "isolated" if no additional neurological abnormalities with the exception of tremor are detectable on examination. In the current classification by Albanese *et al.*[1], the age to discriminate between childhood- and adult-onset dystonia has been set at 21 years and a detailed categorization has been adopted,subdividing the age at onset as follows: infancy (birth to 2 years); childhood (3-12 years); adolescence (13-20 years); early adulthood (21-40 years); late adulthood (>40 years).

Isolated dystonia in children has a wide differential diagnosis, and early-onset cases generally differ from late-onset ones in terms of anatomical sites affected and rate of generalization; in fact, children and adolescents often show an initial lower limb involvement with a high tendency to spread to other body sites, while adult-onset dystonia commonly remains focal (e.g. blepharospasm) or segmental.

Most genetically inherited dystonias show an autosomal dominant mode of inheritance with reduced penetrance and variable expressivity[2]. However, a few families of different ethnical background have been described in which dystonia is recessively inherited. Some of them were collectively grouped under the umbrella term "DYT2" or "DYT2-like" dystonia[3,4], whereas "DYT17" refers to a locus mapped on chromosome 20p11.2-q13.12 in a single Lebanese family[5].

Thanks to Next Generation Sequencing (NGS) techniques, the identification of dystonia-related genes has significantly improved in recent years, making it possible to formulate a definite genetic diagnosis in an increasing proportion of patients. In 2015, using a combination of homozygosity mapping and whole exome sequencing, Charlesworth *et al.*[6] identified mutations in Hippocalcin (*HPCA*) as the cause of DYT2 early-onset recessive dystonia in a previously-published Sephardic Jewish kindred from Iran[3] and in an additional unrelated case from Sri Lanka. *HPCA* encodes a neuronal calcium sensor protein expressed mainly in the striatum which exerts its Ca^{2+}-dependent activity by interacting with downstream proteins still under investigation; perturbation of calcium signaling and neuronal excitability has thus been proposed as an important mechanism in the pathogenesis of this kind of genetic dystonia.

The frequency of *HPCA* mutations in isolated dystonia is unknown and the only available genetic screening investigating it[7], including a heterogeneous population of patients with dystonia has been recently published, failing to identify new positive cases.

However, no studies focusing only on pediatric-onset dystonia are available. In this paper, we screened a cohort of genetically undefined isolated dystonia cases for *HPCA* mutations, focusing on patients with onset from infancy to adolescence, namely within 21 years of age.

2. Methods

Patients previously referred either to the Carlo Besta Neurological Institute, Milan, or the Mendel Institute, Rome for clinical assessment of dystonia were included in this study. Subjects with isolated dystonia

with various distribution and onset before 21 years of age, lacking a definite genetic diagnosis were enrolled. All patients tested negative for the recurrent GAG deletion of the DYT1/*TOR1A* gene. Moreover, in all patients Dopa-Responsive Dystonia (DRD) had been previously ruled out either genetically, on the basis of cerebrospinal fluid neurotransmitter profile or on clinical grounds after an appropriate Levodopa trial. Among patients enrolled, 34 also tested negative for mutations in the *PRKRA* gene (DYT16), a rare cause of early-onset, recessive dystonia-parkinsonism described in few families so far, which can be characterized only by dystonia at onset and for several years over the disease course[8].

After obtaining informed consent, the subjects included in this study were blood sampled and DNA was extracted from peripheral blood lymphocytes according to standard procedures. In some cases genetic analysis was performed after a long disease history, thanks to the availability of patients' DNA in our biobank. Also in these cases, patients' consent was retrieved. All exons and flanking intronic regions of *HPCA* were Sanger sequenced (primer sequences and conditions available upon request - disturbimovimento@istituto-besta.it). Clinical and demographic information were obtained by direct interview and by reviewing patients' clinical records and videos.

3. Results

A total of 73 patients (28 females, 45 males) were enrolled. All but three patients (1 from Albania, 1 from China and 1 from India) were of Italian origins. The mean age of onset of dystonia was 10.2 (± 5.1) years and

mean age at the time of genetic testing was 33 (±14.2) years. Mean disease duration at the time of enrollment was 22.7 (±12.8) years.

Parental consanguinity was documented in 4 (5.5%) patients, possible in 1 (1.4%) and absent in 68 (93.1%) enrolled subjects. Eight patients (11%) had at least one sibling affected by dystonia, indicating a possible recessive pattern of inheritance, but in none of these cases parental consanguinity was documented.

Clinical features of enrolled subjects are shown in **Table 1**.

Onset of dystonia (defined by direct patients' observation or review of records) was in the lower limbs in 26% of cases; upper limbs, the cervical region and a multifocal involvement were present at the beginning in 15% of patients each, whereas in 20.5% of patients a generalized distribution was noted since the first clinical evaluation.

Sanger sequencing did not reveal exonic *HPCA* mutations in any subject enrolled. Exonic or genomic rearrangements involving the *HPCA* gene were not ruled out.

4. Discussion

Since the discovery of *TOR1A* gene in 1997[9], 27 dystonia loci have been mapped, and 17 dystonia-related genes have been identified. DYT1 mutations remain the most common genetic cause of dystonia, especially in Ashkenazi Jews [10]. However, for some recently identified genes, a limited number of mutated patients have been reported, and the pathogenic role of some of them has been questioned[11]. In 2015 *HPCA* was discovered in a consanguineous Sephardic Jewish kindred including three affected siblings with childhood-onset dystonia, with a slowly progressive course and generalization without major functional

limitations in adulthood. The age of onset varied between 1 and 8 years and sites initially affected were lower limbs and the cranial-cervical region; the upper body resulted more markedly affected in adulthood.

Based on the observation that the only cases described so far were affected since childhood, we selected a population of dystonic patients with onset during childhood and adolescence according to the current classification of dystonia[1] to assess the mutational frequency of this gene in a specific age-selected population. We excluded all probands with clear autosomal dominant inheritance of dystonia, but purposely included in the study also sporadic cases, who could also have inherited recessive mutations from unaffected healthy parents, even in the absence of obvious consanguinity or of positive family history. The only *HPCA* mutational screening available in the literature has been recently published by Dobričić and colleagues[7] and included 435 patients with isolated dystonia, of which 107 were ≤ 20 years at the time of disease onset. None of the patients enrolled resulted positive for *HPCA* mutations.

Similarly, we failed to identify *HPCA* pathogenic variants in any of the 73 tested patients, indicating that mutations in this gene are a very uncommon in childhood-onset dystonia, as observed for other recently identified dystonia genes. For example, only 53 *GNAL*-positive patients have been reported since the original description of the gene in 2012[12], and only 10 *ANO-3* positive patients have been fully characterized clinically[13]. Notably, four of them had childhood onset dystonia, ranging from 3 to 6 years, but no dedicated studies in children are available.

We acknowledge that multiplex ligation-dependent probe amplification (MLPA) detecting *HPCA* deletions or duplications was not performed in our patients, thus *HPCA* mutational frequency could have overall been underestimated; however, no *HPCA* exonic or genomic rearrangements have been reported in the literature so far.

At present, it is difficult to foresee whether *HPCA* genetic testing would be advisable in sporadic or familial recessive cases with childhood-onset dystonia, and more extensive studies are warranted to assess *HPCA* mutational frequency and related phenotypes in dystonic patients from different populations.

Acknowledgments

We acknowledge the "Cell lines and DNA Bank of Paediatric Movement Disorders and Mitochondrial diseases" of the Telethon Network of Genetic Biobanks (grant GTB12001J), the Eurobiobank Network and the Pierfranco and Luisa Mariani Foundation.

SITE OF ONSET		
	Cranial	2 (2.7%)
	Oro-mandibular	2 (2.7%)
	Cervical	11 (15.1%)
	Trunk	2 (2.7%)
	Upper limb	11 (15.1%)
	Lower limb	19 (26%)
	Multifocal	11 (15.1%)
	Generalized	15 (20.5%)
FAMILY HISTORY		
	N	57 (78.1%)
	Y	12 (16.4%)
	P	4 (5.5%)
CONSANGUINITY		
	N	68 (93.1%)
	Y	4 (5.5%)
	P	1 (1.4%)

Table 1. Clinical features of subjects enrolled. P: possible

References

1. Albanese A, Bhatia KP, Bressmann S, et al. Phenomenology and classification of dystonia: a consensus update. Mov Disord 2013;(7):863-873

2. Charlesworth G, Bhatia KP, Wood NW. The genetics of dystonia: new twists in an old tale. Brain 2013;136:2017-37

3. Khan NL, Wood NW, Bhatia KP. Autosomal recessive, DYT2-like primary torsion dystonia: a new family. Neurology 2003;61(12):1801-3

4. Moretti P, Hedera P, Wald J, et al. Autosomal recessive primary generalized dystonia in two siblings from a consanguineous family. Mov Disord. 2005;20(2):245-7

5. Chouery E, Kfoury J, Delague V, et al. A novel locus for autosomal recessive primary torsion dystonia (DYT17) maps to 20p11.22-q13.12. Neurogenetics 2008;9(4):287-93

6. Charlesworth G, Angelova PR, Bartolomé-Robledo F, et al. Mutations in HPCA cause autosomal-recessive primary isolated dystonia. Am J Hum Genet. 2015;(4):657-65

7. Dobričić V, Kresojević N, Marjanović A, et al. HPCA-related dystonia: Too rare to be found? Mov Disord. 2016; 31(7):1071

8. Camargos S, Scholz S, Simón-Sánchez J, et al. DYT16, a novel young-onset dystonia-parkinsonism disorder: identification of a segregating mutation in the stress-response protein PRKRA. Lancet Neurol. 2008;7(3):207-15

9. Ozelius LJ, Hewett JW, Page CE, et al. The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein. Nat Genet 1997;17(1):40-8

10. Müller U. The monogenic primary dystonias. Brain. 2009;132(8):2005-25

11. Lohmann K, Schlicht F, Svetel M, et al. The role of mutations in COL6A3 in isolated dystonia. J Neurol. 2016;263(4):730-4

12. T. Fuchs, R. Saunders-Pullman, I. Masuho, et al. Mutations in GNAL cause primary torsion dystonia, Nat Genet 45 (2013) 88-92

13. Stamelou M, Charlesworth G, Cordivari C, et al. The phenotypic spectrum of DYT24 due to ANO3 mutations. Mov Disord. 2014;29(7):928-34

Chapter 8

ADCY5-related movement disorders: frequency, disease course and phenotypic variability in a cohort of paediatric patients.

Miryam Carecchio[a,b,c*], Niccolò E. Mencacci[d,e,*], Alessandro Iodice[f], Roser Pons[g], Celeste Panteghini[a], Giovanna Zorzi[b], Federica Zibordi[b], Anastasios Bonakis[h], Argyris Dinopoulos[i], Joseph Jankovic[l], Leonidas Stefanis[h], Kailash P. Bhatia[m], Valentina Monti[a], Lea R'Bibo[d], Liana Veneziano[n], Barbara Garavaglia[a], Carlo Fusco[f], Nicholas Wood[d], Maria Stamelou[o#], Nardo Nardocci[b#]

[a] *Molecular Neurogenetics Unit, IRCCS Foundation Neurological Institute C. Besta, Via L. Temolo 4, 20126 Milan, Italy*

[b] *Department of Pediatric Neurology, IRCCS Foundation Neurological Institute C. Besta, Via Celoria 11, 20133 Milan, Italy*

[c] *Department of Medicine and Surgery, PhD Programme in Molecular and Translational Medicine, University of Milan Bicocca, Via Cadore 48, 20900 Monza, Italy*

[d] *Department of Molecular Neuroscience, UCL Institute of Neurology, WC1N 3BG London, United Kingdom*

[e] *Department of Neurology, Northwestern University, Feinberg School of Medicine, Chicago, 60611 Illinois, USA*

[f] *Child Neurology and Psychiatry Unit, Department of Pediatrics, IRCCS Santa Maria Nuova Hospital, Viale Risorgimento 80, 42123 Reggio nell'Emilia, Italy*

[g] *First Pediatric Clinic, University of Athens, Agia Sofia Children's Hospital, Thivon and Levadias, 11527 Athens, Greece*

[h] *Second Department of Neurology, Attiko University Hospital, University of Athens, Greece*

[i] *Third Department of Paediatrics, Attiko University Hospital, University of Athens, Athens, Greece*

[l] *Parkinson's Disease Center and Movement Disorders Clinic, Department of Neurology, Baylor College of Medicine, 7200 Cambridge, Houston, Texas 77030-4202, USA*

[m] *Sobell Department of Motor Neuroscience and Movement Disorders, University College London, Institute of Neurology, London WC1N 3BG, United Kingdom*

[n] *Institute of Translational Pharmacology, National Research Council, Via Fosso del Cavaliere, 100, 00133 Rome, Italy*

[o] *Movement Disorders Department, HYGEIA Hospital, Athens, Greece*

[p] *Second Department of Neurology, Attiko University Hospital, University of Athens, Greece*

**These authors contributed equally to this manuscript*

[#]These authors contributed equally to this manuscript

Parkinsonism Relat Disord. 2017 Aug;41:37-43. doi: 10.1016/j.parkreldis.2017.05.004.

Abstract

Introduction. *ADCY5* mutations have recently been identified as an important cause of early-onset hyperkinetic movement disorders. The phenotypic spectrum associated with mutations in this gene is expanding. However, *ADCY5* mutational frequency in patients with childhood-onset hyperkinetic movement disorders is not known.

Methods. We performed a mutational screening of the entire *ADCY5* coding sequence in 44 unrelated subjects with genetically undiagnosed childhood-onset hyperkinetic movement disorders, featuring chorea alone or in combination with myoclonus and dystonia. All patients had normal CSF analysis and brain imaging and were regularly followed-up in tertiary centres for paediatric movement disorders.

Results. We identified five unrelated subjects with *ADCY5* mutations (11% of the cohort). Three carried the p.R418W mutation, one the p.R418Q and one the p.R418G mutation. Mutations arose *de novo* in four cases, while one patient inherited the mutation from his similarly affected father. All patients had motor and/or language delayed milestones with or without axial hypotonia and showed generalized chorea and/or dystonia, with prominent myoclonic jerks in one case. Episodic exacerbations of the baseline movement disorder were observed in most cases, being the first disease manifestation in two patients. Evolution of movement disorder was variable, from stability to spontaneous improvement during adolescence.

Conclusion. Mutations in *ADCY5* are responsible for a hyperkinetic movement disorder that can be preceded by dystonic and other hyperkinetic episodic attacks before the movement disorder becomes

persistent. A residual degree of neck hypotonia and a myopathy-like face are frequently observed in mutation-positive patients, who are not infrequently misdiagnosed as dyskinetic cerebral palsy.

1. Introduction

Adenyl cyclase 5, encoded by *ADCY5*, is a striatal-specific enzyme that converts adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP), an intracellular second messenger crucial for several molecular pathways [1].

The role of pathogenic mutations in *ADCY5* was first recognized in 2012, when a segregating missense change in the gene was discovered in a large dominant kindred with multiple affected members presenting with an early-onset hyperkinetic movement disorder named Familial Dyskinesia with Facial Myokymia (FDFM; OMIM 600293) [1,2]. *A* second *de novo* mutation (p.R418W) in *ADCY5* was subsequently found in two unrelated patients presenting with childhood-onset chorea and dystonia [3] and mutation-positive subjects were also found in a cohort of patients with a clinical diagnosis of benign hereditary chorea (BHC) but no *NKX2-1* mutations [4]. The clinical phenotype associated with *ADCY5* mutations includes in most cases childhood-onset chorea with episodic exacerbations observed more frequently upon awakening, when falling asleep or during intercurrent illnesses [5-8]. Besides chorea, various hyperkinetic movement disorders such as myoclonus and dystonia have been described in *ADCY5* positive subjects, but the prevalence of *ADCY5* mutations in such patients is unknown.

The aim of this study was to establish the contribution of *ADCY5* mutations in a multi-centric cohort of patients with early-onset hyperkinetic movement disorder who lacked a definite genetic diagnosis.

We identified six new European cases with pathogenic *ADCY5* mutations belonging to five different families, showing the clinical course of disease at different ages, phenotypic heterogeneity and variability of movement disorder.

2. Materials and methods

In this study, we included patients displaying paediatric onset hyperkinetic movement disorder featuring chorea alone or in combination with myoclonus and dystonia, including patients diagnosed with dyskinetic cerebral palsy (CP). Patients with secondary movement disorders, such as documented hypoxic injury at birth or with detectable structural brain lesions were not included. Patients enrolled had previously undergone extensive metabolic screening (plasma and urinary aminoacids and organic acids, lactate/pyruvate, cerebrospinal fluid analysis including neurotransmitters and biopterins dosage) and multiple MRI brain scans that were unrevealing. Mutations in the *NKX2-1* gene, a significant though rare cause of childhood-onset chorea, were excluded in all of these patients [9].

44 unrelated patients were included from five different European Centers (IRCCS C. Besta Neurological Institute, Milan; IRCCS Santa Maria Nuova Hospital, Reggio Emilia; Movement Disorders Department, HYGEIA Hospital, Athens; Second Department of Neurology, Attikon Hospital, University of Athens; First Pediatric

Clinic, University of Athens, Agia Sofia Hospital, Athens). Details on clinical history were obtained by direct interviewing the patients and their relatives; in some cases, home-made videos were retrieved and reviewed by the authors to better define the clinical phenotype at earlier disease stages.

After obtaining informed consent (parental consent for minors where applicable), patients were blood sampled and DNA was extracted from peripheral blood lymphocytes according to standard procedures. *ADCY5* exons 2 and 10, in which mutations have been identified in most of the families published to date, were Sanger sequenced. Samples without mutations in these two exons were submitted for Whole Exome Sequencing (WES), which was performed as previously reported [10]. Segregation analysis in available family members was performed in all positive cases.

3. **Results**

Five out of 44 unrelated patients (11%) carried *ADCY5* mutations. Four patients were sporadic and carried *de novo* changes, while one had an autosomal dominant family history and inherited the mutation from his 47-year-old father, who also suffered from childhood-onset generalized chorea and dystonia. All mutations detected were located in *ADCY5* exon 2, at amino acidic residue 418 (p.R418W in 3 patients, p.R418G and p.R418Q in one each). Analysis of WES data did not reveal any additional mutation in *ADCY5* located outside exons 2 and 10 in the remainder 39 patients.

Clinical features of positive patients are summarized in **Table 1**.

Patient 1 (p.R418W; *de novo* mutation) is a 15-year-old girl born pre-term from healthy parents. She presented with axial hypotonia (**Video 1 - Segment 1**) and delayed language. Around 11 months she developed abrupt brief generalized dystonic attacks when falling asleep. Between age 1 and 2, generalized chorea also appeared during attacks, that occurred in clusters on a weekly basis. Around 18 months of age she developed generalized chorea with a slowly progressive course until age 13 (V**ideo 1 - Segment 2**), and subsequent spontaneous improvement; at age 9 she developed left foot dystonia (in-turning). Due to chorea and severe axial hypotonia she could walk independently only at age 5; residual neck hypotonia is still present to date. Routine EEG and sleep studies did not show cortical correlates of movement disorder and brain MRI was unremarkable. She initially received a diagnosis of dyskinetic CP. On examination at age 15 (**Video 1 – Segment 3**), her mouth was slightly open, she showed generalized chorea involving also perioral muscles, dystonic posturing of upper and lower limbs, head drop and severe dysarthria with saliva drooling. Her total IQ (84) was in the borderline range (WISC). During teen age, episodic exacerbations of chorea and dystonia during sleep became shorter and less frequent and are now present about once a month. Episodic exacerbations also occur during the day with two distinct patterns: 1) sudden give-way of legs with falls to the ground with preserved consciousness and 2) generalized dystonic-choreic attacks favored by tiredness and narrow passages. Trihexyphenidyl up to 32 mg/day did not improve significantly motor symptoms.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2017.05.004.

Patient 2 (p.R418Q; *de novo* mutation) is an Italian 18-year-old boy born from healthy parents. He presented with delayed motor milestones and a tendency to tiptoe walking at 18 months. Since 6 months of age, nocturnal attacks of generalized dystonia with inconsolable crying, lasting up to some hours, disrupted his sleep. During infancy he developed generalized chorea and mild myoclonic jerks also involving facial muscles (**Video 2 – Segment 1**) and he showed a mildly scissoring gait with pyramidal signs in the lower limbs, for which he underwent tendon elongation. Episodic worsening of dyskinesias accompanied by hyperventilation, lasting about half an hour, were noticed during childhood, triggered by emotions and stress; sometimes hyperventilation and tachypnoea occurred without exacerbation of dyskinesias. These episodes initially recurred at weekly intervals and then spontaneously decreased over disease course. Currently, diurnal and nocturnal exacerbations are almost abolished (about one episode a year is reported), the most recent one being triggered by a minor orthopedic injury. Chorea slowly improved during teen-age years, and the clinical picture became dominated by dystonia and myoclonus. On examination at age 17, he showed mildly scissoring gait with pyramidal signs in the lower limbs, mild dysarthria with a tendency to keep his mouth open, cervical dystonia, multifocal non-stimulus sensitive myoclonic jerks at rest and on posture more prominent in the upper body, also involving the perioral muscles (**Video 2 - Segment 2**). Standard EEG and sleep studies showed no EEG correlates of hyperkinesias. EMG recordings revealed bursts of 100-120 ms in the upper limbs and neck, alone or superimposed on dystonic co-contraction of antagonistic muscle groups, consistent with the co-

occurrence of myoclonus and dystonia, as observed in DYT11 positive patients [11]. Clonazepam was not beneficial in reducing myoclonus. The patient's IQ (WISC) at age 7 was 86.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2017.05.004.

Patient 3 (p.R418G; inherited mutation) is a 3-year-old Italian boy born full-term after normal pregnancy. Motor and language development were delayed, he managed to sit unsupported at 17 months and to walk unassisted at 2 years of age. Generalized chorea appeared in the first months of life. Neurological examination at age 2.5 showed axial hypotonia, mild generalized chorea and dystonic posturing of the limbs (tiptoe walking). To date, the patient has not presented diurnal or nocturnal paroxysmal exacerbations of chorea. His father (**Patient 4**), 47 years old, had delayed motor and language milestones. Generalized chorea with dystonic posturing of upper limbs appeared around age 3. Since childhood, he has suffered from severe and painful exacerbations of dyskinesias triggered by emotions and tiredness and also present during sleep. Chorea is currently worsened by action, emotions and stress (**Video 3**). Acetazolamide significantly improved its severity, whereas tetrabenazine, baclofen and trihexyphenidyl were not effective. His son was started on acetazolamide (125 mg/day) with no substantial changes in his mild movement disorder.

Both the patient and his father carried the p.R418G change; visual inspection of the chromatograms showed an imbalanced ratio between the wild-type and the mutated allele, with the latter significantly less represented (**Figure 1**). This was not observed in the son, suggesting that the father could be a mosaic for the mutation.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2017.05.004.

Patient 5 (p.R418W; *de novo* mutation) is a 35-year-old woman of Greek origin, born full term after an uneventful pregnancy. At 3 months of age she had failure to thrive, feeding difficulties, and developed choreic movements. At the age of 9 months she could not sit unsupported and showed axial hypotonia. At age two, she had a mild cognitive and motor developmental delay. She initially received a diagnosis of dyskinetic CP, with normal intelligence. Her clinical picture remained stable until age 7, when she developed sudden attacks characterized by hip and trunk flexion that made her collapse to the ground with no alteration of consciousness (**Video 4 – Segment 1**). She also developed sustained dystonic postures of the limbs both throughout the day and night, particularly upon awakening, which at times were extremely painful. Multiple brain MRI scans and EEGs were normal. Over the following years, chorea remained stable and the paroxysmal episodes had a variable course, with spontaneous remission for about two years and reappearance at age 23 after a traumatic event. The episodes lasted up to 30 minutes and could occur many times a day and were diagnosed as functional (psychogenic). On examination at age 35 (**Video 4 – Segment 2**), her mouth was slightly open and she had some drooling, and a dysarthric speech. Chorea was present in the face, involving mainly the mouth, and limbs were mildly affected as well, especially the arms. There was dystonic posturing of the feet and hands when outstretched. She was hypotonic, reflexes were present and symmetric throughout. Sleep studies showed hypoventilation triggering paroxysmal episodes at night, for which she was given a CPAP

treatment with some improvement of the nocturnal episodes. Tetrabenazine, levodopa, trihexyphenidyl, various anticonvulsants were not helpful. Clonazepam significantly improved the frequency and severity of the paroxysmal episodes. The patient was found to carry a *de novo* p.R418W mutation.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2017.05.004.

Patient 6 (p.R418W; *de novo* mutation) is a 5 year-old Greek child born full term from healthy parents. He had delayed milestones and generalized hypotonia and was able to sit unassisted and to stand with support only at 20 months of age. He also had delayed language development, though verbal understanding was good. Generalized chorea appeared around age one, and about one year later he developed brief diurnal paroxysmal events lasting less than one minute, that were characterized by limb posturing, more severe in the arms, associated with possible axial posturing. Brain MRI and CSF analysis were unremarkable. On examination at age 5, he was able to follow simple commands and had moderate generalized chorea involving also the face, along with generalized hypotonia. Paroxysmal dystonic attacks are still present, especially upon awakening. A trial of Levodopa was not effective and no additional medication was started.

4. Discussion

Since the original report in 2001 [2], in which the authors described the movement disorder in the affected family members as “Familial Dyskinesia with Facial Myokymia”, the phenotypic spectrum

associated with *ADCY5* mutations has expanded and a more detailed delineation of movement disorders has been provided in subsequent reports [3-5, 7].

So far, 60 genetically confirmed patients (24 sporadic, 36 familial cases) belonging to 36 different families have been reported (**Table 2**). The mutational frequency of *ADCY5* in homogeneous cohorts of patients with early-onset non-progressive hyperkinetic movement disorders has not been assessed in previous publications, and some authors identified positive subjects in extensive screenings of patients affected by heterogeneous movement disorders [12]. In this study, we aimed at defining the contribution of *ADCY5* mutations in a cohort of patients with a childhood-onset hyperkinetic movement disorder. We found that 11% of our cohort carried *ADCY5* pathogenic mutations. These patients displayed most of the previously described features of *ADCY5*-associated disease: (a) onset in infancy-childhood with delayed milestones and axial hypotonia, (b) a mixed hyperkinetic movement disorder mostly characterized by generalized chorea and dystonia and (c) frequent exacerbations of dyskinesias upon awakening and when falling asleep. Importantly, differently from subjects with *ADCY5* mutations, these core features were not observed all together in any of the 39 subjects without mutations, suggesting that their concomitant presence is a strong predictor of the mutational status.

Some patients were previously diagnosed with dyskinetic CP despite normal MRI findings and no clear perinatal injury. While movement disorders in CP can show significant worsening during intercurrent infections, a clear relationship between the exacerbations of movement

disorders and sleep should raise the suspicion of a misdiagnosis and lead to consider underlying *ADCY5* mutations.

Chorea presenting in infancy is often due to acute basal ganglia damage in the context of metabolic encephalopathies (aminoacidopathies, organic acidurias, Lesh-Nyhan syndrome), while in childhood auto-immune causes with normal brain imaging prevail (Sydenham's chorea, autoimmune encephalitides) and rare non-metabolic genetic conditions must also be considered (*NKX2-1, PDE10A, GNAO1* mutations) [13].

Age at onset of paroxysmal dyskinesias and dystonia ranged between 6 months and seven years of age in our study, being the first disease manifestation (along with delayed milestones) in one third of positive patients, and developing before a chronic movement disorder became evident. Onset in the first months of life and relation to sleep is unusual in paroxysmal movement disorders due to *PRRT2*, *PNKD* and *SLC2A1* mutations, thus mutations in *ADCY5* should be considered in the differential of paroxysmal movement disorders with a very early onset even in the absence of a detectable chronic movement disorder. On the other hand, as exemplified by Patient 3, who displays mild chorea and dystonia without paroxysmal exacerbations, the absence of such manifestations, suggested as a "red flag" for *ADCY5*-related dyskinesias [14], does not rule *ADCY5* mutations out, at least in the first years of life.

The natural history of *ADCY5*-related dyskinesias is still poorly defined and no or little progression of movement disorder has been observed in previous reports. Phenotypic variability has been partly attributed to genotype-phenotype correlation, with available evidence suggesting that the A726T mutation is associated with a milder phenotype [5],

whereas the p.R418W is responsible for a more severe clinical picture. A lesser degree of severity of movement disorder has also been explained with somatic mosaicism [4,5]. In our series, chromatograms of Patient 4 (**Figure 1**) were suggestive of somatic mosaicism; however, he displayed a relatively severe phenotype as compared to his son. This might indicate that additional genetic and/or environmental factors may play a role in determining *ADCY5* phenotypic variability.

In our series, patients were regularly assessed for several years, thus disease course and phenomenology could be documented at different ages. All patients presented with delayed ability to sit unsupported or walk independently and showed a combination of axial hypotonia and chorea/dystonia affecting also the lower limbs together with spasticity in one case (Patient 2). Axial hypotonia slowly improved over disease course, but a residual degree of cervical hypotonia could still be observed in adolescence in some affected subjects (Patient 1 and 2). Cervical hypotonia in adolescents and young adults can mimic dystonic anterocollis, and it is detectable in other positive patients from previously published videos [7], thus representing a potential additional clue to individuate *ADCY5* mutation carriers, as observed by Meijer *et al*. [15]. Abrupt violent head drops are considered characteristic of chorea-acanthocytosis [16], but are phenomenologically different from what has been observed in *ADCY5* cases.

The course of dyskinesia exacerbations was variable in our series, including spontaneous amelioration in frequency and severity (Patient 1), almost complete remission during teen age (Patient 2), stability since onset (Patient 4 and 6) and stability with relatively long attack-free

periods (Patient 5). Peripheral trauma was reported in two cases to trigger recrudescence of attacks after free periods.

Movement disorder disease course was variable as well, with spontaneous improvement of chorea observed in some cases during adolescence and relative stability since childhood in others.

Patient 2 switched from a choreic/dystonic phenotype in childhood to a clinical and electrophysiological picture consistent with myoclonus-dystonia in his late teens, and was in fact previously tested for DYT11 mutations; however, the presence of pyramidal signs in the lower limbs and delayed milestones in infancy were not consistent with the classic myoclonus-dystonia phenotype due to DYT11 mutations.

In terms of treatment, several agents such as tetrabenazine, trihexyphenidyl, levodopa and anticonvulsant were not beneficial. Of note, acetazolamide, a carbonic anhydrase inhibitor, significantly improved chorea in Patient 4; response of dyskinesia to this drug, though considered nonspecific, was reported in two patients of the original family published by Fernandez *et al*. [2]. Clonazepam reduced the dystonic episodes in Patient 5 but was ineffective in ameliorating myoclonus in Patient 2. Given the unsatisfactory response of *ADCY5*-related movement disorders to several drugs, bilateral GPi Deep Brain Stimulation (DBS) has been recently performed in four patients, with moderate reduction of hyperkinetic movements [8,15].

In our series, all positive patients carried mutations involving the arginine 418, and half of them carried the p.R418W mutation, which is by far the most frequently encountered missense variant, being present in 19/36 (53%) unrelated probands reported to date. With our series, the total number of *ADCY5* positive patients reaches 66 cases and the

number of unrelated patients carrying the p.R418W mutation increases up to 22/41 (54%) (**Table 3**). We therefore confirm that arginine 418 is a mutational hot spot in *ADCY5* with a relevant pathogenic role.

5. Conclusions

Mutations in *ADCY5* represent a significant genetic cause of early-onset non-progressive hyperkinetic movement disorders, with a frequency of 11% in our series. The increasing number of cases reported is contributing to define the phenotypic spectrum of this disorder. Delayed milestones and axial hypotonia seem to be almost universal features in infancy, while onset of movement disorder and its episodic or chronic nature are variable in the first disease phases and tend sometimes to spontaneously improve with age. We suggest testing for *ADCY5* mutations patients previously diagnosed with dyskinetic cerebral palsy when exacerbations of dyskinesia are clearly sleep-related. The knowledge about long-term motor outcome in affected children is still limited, given the relatively small number of cases reported so far. We observed some common features in most patients, including the presence of "head drop" probably due to residual cervical hypotonia as well as a myopathy-like appearance of face with mouth kept slightly open. These characteristics may be relevant in patients without frequent episodic movement disorder exacerbations and suggest underlying *ADCY5* mutations, although their pathophysiology still needs to be elucidated.

Author roles

Miryam Carecchio: concept and design, data collection, data analysis, drafting of manuscript, manuscript revision; Niccolò E. Mencacci: concept and design, data collection, data analysis, manuscript revision; Alessandro Iodice: data collection, data analysis; Celeste Panteghini: data collection, data analysis, manuscript revision; Roser Pons: data collection, manuscript revision; Giovanna Zorzi: data collection; Federica Zibordi: data collection; Anastasios Bonakis: data collection; Argyris Dinopoulos: data collection; Joseph Jankovic: data collection, manuscript revision; Leonidas Stefanis: data collection; Kailash P. Bhatia: data collection, manuscript revision; Valentina Monti: data collection; Lea R'Bibo: data collection, data analysis; Barbara Garavaglia: manuscript revision; Nicholas Wood: data collection; Carlo Fusco: data collection, manuscript revision; Maria Stamelou: concept and design, data collection, manuscript revision; Nardo Nardocci: manuscript revision.

Fundings

This work received financial support from the Fondazione Pierfranco e Luisa Mariani. This work was supported financially by a Medical Research Council/Wellcome Trust Strategic Award (WT089698/Z/09/Z) and grants from the Bachman-Strauss Dystonia Parkinsonism Foundation, and NIHR Bioresource Rare Diseases. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The work was undertaken at University College London (UCL), who receive support

from the Department of Health's National Institute for Health Research (NIHR) Biomedical Research Centers funding streams. N.E.M. is funded by a NIHR funding scheme.

Financial disclosures/conflict of interest

None to declare

Acknowledgements

We acknowledge the "Cell lines and DNA Bank of Paediatric Movement Disorders and Mitochondrial diseases" of the Telethon Network of Genetic Biobanks (grant GTB12001J) and the Eurobiobank Network.

References

[1] Y.Z. Chen, M.M. Matsushita, P. Robertson, M. Rieder, S. Girirajan, F. Antonacci, H. Lipe, E.E. Eichler, D.A. Nickerson, T.D. Bird, W. H. Raskind. Autosomal dominant familial dyskinesia and facial myokymia: single exome sequencing identifies a mutation in adenylyl cyclase 5, Arch Neurol. 69 (2012) 630-635.

[2] M. Fernandez, W. Raskind, J. Wolff, M. Matsushita, E. Yuen, W. Graf, H. Lipe, T. Bird. Familial dyskinesia and facial myokymia (FDFM): a novel movement disorder, Ann Neurol. 49 (2001) 486-492.

[3] Y.Z. Chen, J.R. Friedman, D.H. Chen, G.C. Chan, C.S. Bloss, F.M. Hisama, S.E. Topol, A.R. Carson, P.H. Pham, E.S. Bonkowski, E.R. Scott, J.K. Lee, G. Zhang, G. Oliveira, J. Xu, A.A. Scott-Van Zeeland, Q. Chen, S. Levy, E.J. Topol, D. Storm, P.D. Swanson, T.D. Bird, N.J. Schork, W.H. Raskind, A. Torkamani, Gain-of-function ADCY5 mutations in familial dyskinesia with facial myokymia, Ann Neurol. 75 (2014) 542-549.

[4] N.E. Mencacci, R. Erro, S. Wiethoff, J. Hersheson, M. Ryten, B. Balint, C. Ganos, M. Stamelou, N. Quinn, H. Houlden, N.W. Wood, K.P. Bhatia, ADCY5 mutations are another cause of benign hereditary chorea, Neurology 85 (2015) 80-88.

[5] D.H. Chen, A. Méneret, J. R. Friedman, O. Korvatska, A. Gad, E.S. Bonkowski, H.A. Stessman, D. Doummar, C. Mignot, M. Anheim, S. Bernes, M.Y. Davis, N. Damon-Perrière, B. Degos, D. Grabli, D. Gras, F.M. Hisama, K.M. Mackenzie, P.D. Swanson, C. Tranchant, M. Vidailhet, S. Winesett, O. Trouillard, L.M. Amendola, M.O. Dorschner, M. Weiss, E.E. Eichler, A. Torkamani A, E. Roze, T.D. Bird, W.H.

Raskind, ADCY5-related dyskinesia: Broader spectrum and genotype-phenotype correlations, Neurology 85 (2015) 2026-2035.

[6] R. Carapito, N. Paul, M. Untrau, M. Le Gentil, L. Ott, G. Alsaleh, P. Jochem, M. Radosavljevic, C. Le Caignec, A. David, P. Damier, B. Isidor, S. Bahram. A de novo ADCY5 mutation causes early-onset autosomal dominant chorea and dystonia, Mov. Disord. 30 (2015) 423-427.

[7] F.C. Chang, A. Westenberger A, R.C. Dale, M. Smith, H.S. Pall, B. Perez-Dueñas, P. Grattan-Smith, R.A. Ouvrier, N. Mahant, B.C Hanna, M. Hunter, J.A. Lawson, C. Max, R. Sachdev, E. Meyer, D. Crimmins, D. Pryor, J.G. Morris, A. Münchau, D. Grozeva, K.J. Carss, L. Raymond, M.A. Kurian, C. Klein, V.S. Fung, Phenotypic insights into ADCY5-associated disease, Mov. Disord. 31 (2016) 1033-1040.

[8] M.E. Dy, F.C. Chang, S.D. Jesus, I. Anselm, N. Mahant, P. Zeilman, L.H. Rodan, K.D. Foote,
W.H. Tan, E. Eskandar, N. Sharma, M.S. Okun, V.S. Fung, J.L. Waugh. Treatment of ADCY5-Associated Dystonia, Chorea, and Hyperkinetic Disorders With Deep Brain Stimulation: A Multicenter Case Series, J Child Neurol. 31 (2016) 1027-1035.

[9] N.J. Patel, J. Jankovic. NKX2-1-Related Disorders. Updated 2016. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2016.

[10] N.E. Mencacci, E.J. Kamsteeg, K. Nakashima, L. R'Bibo, D.S. Lynch, B. Balint, M.A. Willemsen, M.E. Adams, S. Wiethoff, K. Suzuki, C.H. Davies, J. Ng, E. Meyer, L. Veneziano, P. Giunti, D. Hughes, F.L. Raymond, M. Carecchio, G. Zorzi, N. Nardocci, C.

Barzaghi, B. Garavaglia, V. Salpietro, J. Hardy, A.M. Pittman, H. Houlden, M.A. Kurian, H. Kimura, L.E. Vissers, N.W. Wood, K.P. Bhatia. De Novo Mutations in *PDE10A* Cause Childhood-Onset Chorea with Bilateral Striatal Lesions, Am. J. Hum. Gen. 98 (2016) 763-771.

[11] C. Marelli, L. Canafoglia, F. Zibordi, C. Ciano, E. Visani, G. Zorzi, B. Garavaglia, C. Barzaghi, A. Albanese, P. Soliveri, M. Leone, F. Panzica, V. Scaioli, A. Pincherle, N. Nardocci, S. Franceschetti. A neurophysiological study of myoclonus in patients with DYT11 myoclonus-dystonia syndrome, Mov. Disord. 23 (2008) 2041-2048.

[12] M. Zech, S. Boesch, A. Jochim, S. Weber, T. Meindl, B. Schormair, T. Wieland, C. Lunetta, V. Sansone, M. Messner, J. Mueller, A. Ceballos-Baumann, T.M. Strom, R. Colombo, W. Poewe, B. Haslinger, J. Winkelmann. Clinical exome sequencing in early-onset generalized dystonia and large-scale resequencing follow-up, Mov. Disord. 2016 Sep 26. doi: 10.1002/mds.26808.

[13] N.E. Mencacci, M. Carecchio. Recent advances in genetics of chorea, Curr Opin Neurol. 29(2016) 486-495.

[14] J.R. Friedman, A. Méneret, D.H. Chen, O. Trouillard, M. Vidailhet, W.H. Raskind, E. Roze. ADCY5 mutation carriers display pleiotropic paroxysmal day and nighttime dyskinesias, Mov. Disord. 31 (2016) 147-148.

[15] I.A. Meijer, J. Miravite, B.H. Kopell, N. Lubarr. Deep Brain Stimulation in an Additional Patient With ADCY5-Related Movement Disorder, J Child Neurol. 32 (2017) 438-439.

[16] S.A. Schneider, A.E. Lang, E. Moro, B. Bader, A. Danek, K.P. Bhatia. Characteristic head drops and axial extension in advanced

chorea-acanthocytosis, Mov. Disord. 25 (2010) 1487-1491.
[17] A. Westenberger, C. Max, N. Brüggemann, A. Domingo, K. Grütz, H. Pawlack, A. Weissbach, A.A. Kühn, J. Spiegler, A.E. Lang, J. Sperner, V.S. Fung, J. Schallner, G. Gillessen-Kaesbach, A. Münchau, C. Klein. Alternating Hemiplegia of Childhood as a New Presentation of Adenylate Cyclase 5-Mutation-Associated Disease: A Report of Two Cases, J Pediatr. 181 (2017) 306-308.
[18] A.G. Douglas, G. Andreoletti, K. Talbot, S.R. Hammans, J. Singh, A. Whitney, S. Ennis,
N.C. Foulds. ADCY5-related dyskinesia presenting as familial myoclonus-dystonia. Neurogenetics 18 (2017) 111-117.

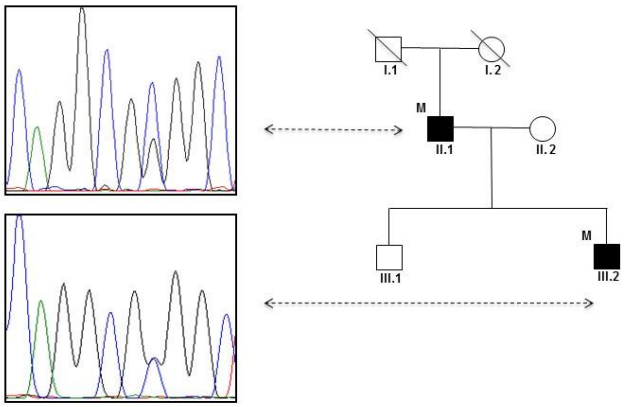


Figure 1. Pedigree of patients 3 and 4. The electropherogram of Patient 4 (II.1) shows an unbalanced ratio between the wild-type and the mutant allele (top panel), with the wild-type significantly more represented, suggesting somatic mosaicism. This was not observed in Patient 3 (III.2), where the chromatograms of the normal and mutated allele are equivalently represented (bottom panel).

Pat #	*ADCY5* mutation	Sex	Fam. Hist	AAO (MD)	Current age	Additional signs at onset	MD at onset	Current MD	Nocturnal paroxysms	Diurnal paroxysms	Paroxysmal episodes amelioration	Motor delay	Language delay
Pt 1	c.1252C>; p.R418W	F	N	1.5	15	Axial hypotonia	Paroxysmal dystonic episodes	Chorea, dystonia	Y	Y	Y	Y	Y
Pt 2	c.1253G>A; p.R418Q	M	N	1	18	Spastic gait	Paroxysmal dystonic episodes	Myoclonus, dystonia	Y	Y	Y	Y	N
Pt 3	c.1252C>G; p.R418G	M	Y	1	3	Axial hypotonia	Chorea, dystonia	Chorea, dystonia	N	N	NA	Y	Y
Pt 4	c.1252C>G; p.R418G	M	Y	3	47	UK	Chorea	Chorea, dystonia	Y	Y	N	Y	Y
Pt 5	c.1252C>T; p.R418W	F	N	3 mo	35	Axial hypotonia	Chorea	Chorea, dystonia	Y	Y	Y	Y	Y
Pt 6	c.1252C>T; p.R418W	M	N	2	5	Axial hypotonia	Chorea	Chorea, dystonia	N	Y	N	Y	Y

Table 1. Clinical features of *ADCY5*-positive patients. Fam. Hist family history; MD: movement disorder; AAO: age at onset; UK: unknown; NA: not applicable; mo: months.

Publication	*ADCY5* positive patients	Affected subjects reported*	No of kindred	*ADCY5* mutation (no of kindred, no of positive patients)
Chen *et al*., 2012[1]	10	18	1	p.A726T
Chen *et al*., 2014[3]	2	2	2	p.R418W
Chen *et al*., 2015[5]	24	30	15	p.R418W (8 K, 9) p.R418Q (3 K, 3) p.A726T (1 K, 6) p.L720P (1 K, 1) p.R438P (1 K, 1) p.M1029K (1K, 4)
Mencacci *et al*., 2015[4]	3	3	2	p.R418W
Carapito *et al.*, 2015[6]	2	2	1	c.2088+1G>A
Chang *et al.*, 2016[7]	6	10	6	p.R418W (4 K, 4) p.R418G (1 K, 1) p.R418Q (1 K, 1)
Dy *et al.*, 2016[8]	3	4	3	p.R418W (2 K, 2) p.K694_M696 (1 K, 1)
Zech *et al.*, 2016[12]	3	3	2	p.I460F (1K, 1) p.R727K (1K, 2)
Meijer *et al.*, 2016[15]	1	1	1	p.R418W
Westenberger *et al.*, 2016[17]	2	2	2	p.D1015E (1K, 1) p.E1025V (1K, 1)
Douglas *et al*., 2017[18]	4	5	1	p. M1029R
TOTAL	**60**	**80**	**36**	

Table 2. *ADCY5* positive patients and kindred reported in the literature to date. K: kindred.
* including clinically affected subjects lacking genetic confirmation

ADCY5 mutation	Affected cases reported (%)*	Number of kindred reported (%)*
c.1252C>T; p.R418W	24 (36,4%)	22 (54%)
c.2176G>A; p.A726T	16 (24,2%)	2 (4,9%)
c.1253G>A; p.R418Q	5 (7,6%)	5 (12.2%)
c.3086T>A; p.M1029K	4 (6%)	1 (2,4%)
c.3086T>G; p. M1029R	4 (6%)	1 (2,4%)
c.1252C>G; p.R418G	3 (4,5%)	2 (4,9%)
c.2088+1G>A	2 (3%)	1 (2,4%)
c.2180G>A; p.R727K	2 (3%)	1 (2,4%)
c.2159T>C; p.L720P	1 (1,5%)	1 (2,4%)
c.1313G>C; p.R438P	1 (1,5%)	1 (2,4%)
c.2080_2088del; p.K694_M696	1 (1,5%)	1 (2,4%)
c.3045C>A; p.D1015E	1 (1,5%)	1 (2,4%)
c.3074A>T; p.E1025V	1 (1,5%)	1 (2,4%)
c.1378A>T; p.I460F	1 (1,5%)	1 (2,4%)
Total	**66 (100%)**	**41 (100%)**

Table 3. Frequency of *ADCY5* mutations reported. *Including the present paper.

Legends to Videos

Video 1 (Patient 1), segment 1 (age 3): severe developmental delay with axial and cervical hypotonia, tiptoe walking and generalized chorea; **(age 4)**: improvement of gait, persistence of cervical hypotonia and chorea; **segment 2 (age 9)**: generalized chorea, left foot dystonia (inturning), dystonic posturing of upper limbs when outstretched; **segment 3 (age 15)**: generalized chorea involving also facial muscles, myopathy-like face with mouth kept open, dysarthria, residual cervical hypotonia (neck flexion).

Video 2 (Patient 2), segment 1: (age 7 and 8): generalized chorea with facial involvement and superimposed myoclonic jerks, more severe in the upper limbs; cervical hypotonia, myopathy-like face; **segment 2 (age 17)**: mildly scissoring gait, multifocal myoclonic jerks, cervical dystonia (left torticollis) and right upper limb posturing; chorea involving perioral muscles, myopathy-like face with mouth kept open.

Video 3 (Patient 4, age 47): generalized chorea also involving facial muscles; myopathy-like face with mouth kept open.

Video 4 (Patient 5), segment 1 (age 10): severe generalized chorea and axial hypotonia; **age 17**: episodic falls to ground; **segment 2 (age 35)**: facial grimacing, distal chorea of upper and lower limbs.

Chapter 9

Rare causes of early-onset dystonia-parkinsonism with cognitive impairment: a *de novo PSEN-1* mutation.

Miryam Carecchio[1,2,3§], MD, Marina Picillo[4§], MD, Lorella Valletta[1], BSc, Antonio E. Elia[5], MD, Tobias B. Haack[6,7,8], MD, PhD, Autilia Cozzolino[4], MD, Annalisa Vitale[4], MD, Barbara Garavaglia[1], PhD, Arcangela Iuso[6,7], PhD, Caterina F. Bagella[5], MD, PhD, Sabina Pappatà[9], MD, Paolo Barone[4], MD, Holger Prokisch[6,7], PhD, Luigi Romito[5], MD, Valeria Tiranti[1*], PhD

[1] *Molecular Neurogenetics Unit, IRCCS Foundation C. Besta Neurological Institute, Via L. Temolo 4, 20126 Milan, Italy;* [2] *Department of Child Neurology, IRCCS Foundation C. Besta Neurological Institute, Via Celoria 11, 20133 Milan, Italy*
[3]*Department of Medicine and Surgery, PhD Programme in Molecular and Translational Medicine, University of Milan Bicocca, Via Cadore 48, 20900 Monza, Italy;* [4] *Neurodegenerative Diseases Centre (CEMAND), Department of Medicine and Surgery, Neuroscience section, University of Salerno, Via Allende 84131, Baronissi (Salerno), Italy;* [5] *Department of Movement Disorders, IRCCS Foundation C. Besta Neurological Institute, Via Celoria 11, 20133 Milan, Italy;* [6] *Institute of Human Genetics, Technische Universität München, Trogerstraße 32, 81675 München, Germany;* [7] *Institute of Human Genetics, Helmholtz Zentrum München, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany;* [8]*Institute of Medical Genetics and Applied Genomics, University of Tübingen, Calwerstraße 7, 72076 Tübingen, Germany;* [9]*Institute of Biostructure and Bioimaging, National Research Council, Via De Amicis 95, 80145 Naples, Italy*

§ These authors contributed equally to this manuscript

*Corresponding Author

Neurogenetics. 2017 Jul;18(3):175-178. doi: 10.1007/s10048-017-0518-4.

Abstract

Mutations in PSEN1 are responsible for familial Alzheimer's disease (FAD) inherited as autosomal dominant trait, but also de novo mutations have been rarely reported in sporadic early-onset dementia cases. Parkinsonism in FAD has been mainly described in advanced disease stages. We characterized a patient presenting with early-onset dystonia-parkinsonism later complicated by dementia and myoclonus. Brain MRI showed signs of iron accumulation in the basal ganglia mimicking Neurodegeneration with Brain Iron Accumulation (NBIA) as well as fronto-temporal atrophy. Whole exome sequencing revealed a novel PSEN1 mutation and segregation within the family demonstrated the mutation arose de novo.

We suggest considering PSEN1 mutations in cases of dystonia-parkinsonism with positive DAT-Scan, later complicated by progressive cognitive decline and cortical myoclonus even without a dominant family history.

Introduction

Early-onset dystonia-parkinsonism is a heterogeneous clinical entity including several rare genetic conditions. Dominant mutations in Presenilin-1 (*PSEN1)* are responsible for familial Alzheimer's disease (FAD), with parkinsonism mainly appearing in advanced stages.[1]

Here, we report on a *de novo PSEN1* mutation in a patient with early-onset dystonia-parkinsonism later complicated by dementia and myoclonus, with brain MRI mimicking basal ganglia iron accumulation. We suggest considering PSEN1 mutations among rare genetic causes of early-onset dystonia-parkinsonism with cognitive impairment.

Case description

The patient was born full-term from healthy non-consanguineous Italian parents and had no family history of neurological or psychiatric disorders. Developmental milestones were normal but he performed poorly at school. His total IQ at age 12 was in the low-average range (92; Wechsler Intelligence Scale for Children, WAIS). At age 25, he presented with progressive slowness of movements, walking difficulties, slurred speech and apathy. First examination was performed at age 26 and disclosed dystonia-parkinsonism with pyramidal signs in lower limbs (**Video 1 – Segment 1**). The patient's IQ score (WAIS) was significantly lower than normal (total IQ <45). **Figure 1A and 2B-E** show neuroimaging data at this stage.

No mutations or exonic rearrangements in *Parkin*, *PINK-1*, *LRRK2*, *GBA*, *ATP1A3*, *SNCA*, *DJ1*, *FBX07*, *SYNJ1*, *MAPT* as well as in known

NBIA genes were found. Wilson's and Niemann-Pick type C disease were both ruled out with appropriate testing.

Levodopa initially improved bradykinesia and rigidity, but dyskinesias, paranoid ideation and auditory hallucinations appeared and were treated with quetiapine up to 300 mg/day. At age 29, he needed assistance to walk due (**Video 1 – segment 2**). Over the following years, parkinsonism progressed with unsatisfactory dopaminergic response; cognitive decline, generalized myoclonic jerks, progressive aphasia and dysphagia appeared. At age 31 (**Video 1 – segment 3**), the patient was bed-ridden with advanced dementia. Brain MRI at this stage is shown in **Figure 1B-D**. EEG showed diffuse slowing in the theta-delta band; EMG recordings showed time-locked cortical potentials consistent with cortical myoclonus.

Eventually, exome sequencing was performed and four genes were prioritized: *PSEN1, GBA, BSCL2, IDH1* (details in supplemental material). Among them, the *PSEN1* S170P was considered the best candidate, given the patient's phenotype with prominent dementia and the previous report of a different change at the same aminoacid residue (S170F) in a patient with early-onset dementia, parkinsonism and myoclonus[2]. The heterozygous variant (c.508T>C) was neither found in ExAC nor in 1000Genomes, and was predicted to be damaging by *in silico* analysis (PolyPhen-2, Mutation Taster). Moreover, the minor allele C was observed only in this case among the samples collected in our own WES dataset (n=11545 exomes) available at the Helmholtz Zentrum. Therefore, the calculated MAF for this variant is 0,00004331 in our internal database. No additional variants in PSEN1 were identified in the index patient and extensive bioinformatics analysis

excluded the presence of Copy Number Variation in *PSEN1*, *APP* and in other genes possibly related to dementia. Sanger sequencing confirmed the mutation, and segregation analysis demonstrated it was absent in the patient's parents and in his unaffected sister, thus arising *de novo*. False paternity was excluded using PCR on a highly polymorphic repetitive-sequence marker at locus D11S533. The same marker was utilized to confirm biological relationship with the mother (supplementary figure 1).

Discussion

Early-onset dystonia-parkinsonism with cognitive impairment can be due to a variety of genetic disorders. Here, we report on a case of a *de novo* yet unreported *PSEN1* mutation presenting with a predominant motor phenotype. *PSEN1* mutations (http://www.molgen.ua.ac.de/ADMutations) are responsible for FAD, however sporadic cases due to *de novo* mutations have been described[3,4,5]. While frequently observed in late phases[1], parkinsonism at onset has been associated with specific *PSEN1* mutations (G217D and V272A)[6,7]. Patients described so far carrying the S170F mutation display a different phenotype from our patient, including myoclonus, seizures and cerebellar ataxia[8,9]. Unlike our patient, in all these cases progressive cognitive decline dominated the clinical picture and started earlier than movement disorders[6-9]. However, in our case minor deficits of intelligence were demonstrated since childhood and could represent a long prodromal phase preceding overt motor and cognitive deterioration[10].

The S170P substitution affects a highly conserved and functional residue of PSEN1 and, similarly to what has been suggested for the S170F mutation, it could affect interactions with other proteins resulting in an altered function of the gamma-secretase complex[2]. Interestingly, neuropathology suggests the involvement of the substantia nigra in PSEN1 carriers[8], possibly explaining the presence of parkinsonism in such patients as well as SPECT findings in our case.

Our report further expands the link between movement disorders and dementia, yet not completely explored. As such, our case presenting with predominant motor symptoms while harboring a PSEN1 mutation (i.e. a gene associated with dementia), parallels a recently described case presenting predominant cognitive symptoms but harboring a synuclein mutation (i.e. a gene associated with movement disorders[11]). In addition, this experience further confirms NGS as a powerful tool to assist clinicians in diagnosing rare genetic disorders with unusual phenotypes.

We recognize the lack of objective evaluation of B-amyloid status, with either CFS or PET radiotracer, represent a major drawback of our report and prevent from drawing firm conclusions. Notwithstanding, we believe our case may prompt the clinician to consider PSEN1 in the differential diagnosis of unusual phenotypes characterized by dystonia-parkinsonism with cognitive impairment.

Acknowledgements
We acknowledge the "Cell lines and DNA Bank of Paediatric Movement Disorders and Mitochondrial diseases" of the Telethon Network of Genetic Biobanks (project no. GTB09003) and the Eurobiobank Network. This work received financial support from the Fondazione Pierfranco e Luisa Mariani, Milan and TIRCON project (FP7/2007-2013, HEALTH-F2-2011, #277984 to VT and HP). CFB was partially supported by the Italian Ministry of Health grant GR-2009-1594645. TBH work was supported by the German Federal Ministry of Education and Research (BMBF) within the framework of the e:Med research and funding concept (grant #FKZ 01ZX1405C).

Author contributions
All Authors have been involved in critical revision and authorization of the draft manuscript.
MC, MP, VT: conceived the study, analysed the results, and drafted the manuscript. LV, BG, TBH, AI, HP: performed exome sequencing and genetic investigation. AEE, AC, AV, CFB, SP, PB, LR: provided clinical details and followed the patient.

Compliance with ethical standards

Conflict of interest: none to declare

Ethical Approval
Subject' consent was obtained according to the Declaration of Helsinki: BMJ 1991; 302, 1194. In addition, we obtained institutional review board–approved informed consent from patient for videotape and its publication. Health professionals and patient's mother also gave their consent for videotape and publication.

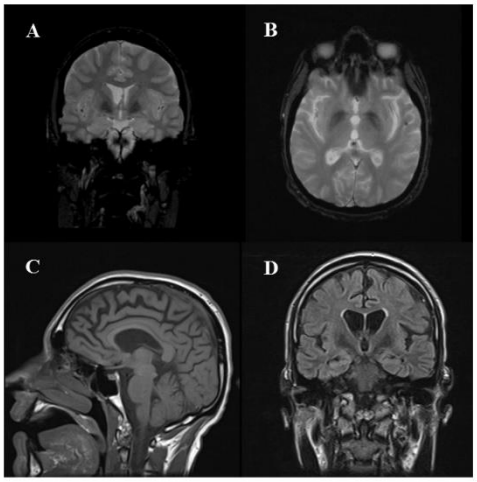


Figure 1: **A)** coronal brain MRI T2* sequences showing bilateral putaminal and globus pallidus hypointensity at age 26, possibly consistent with iron deposition, without "eye-of-the tiger" sign; **B)** axial brain MRI T2* sequences showing hypointensity of putamina, GPi, and substantia nigra at age 31; **C-D)** fronto-temporal cortical atrophy at age 31.

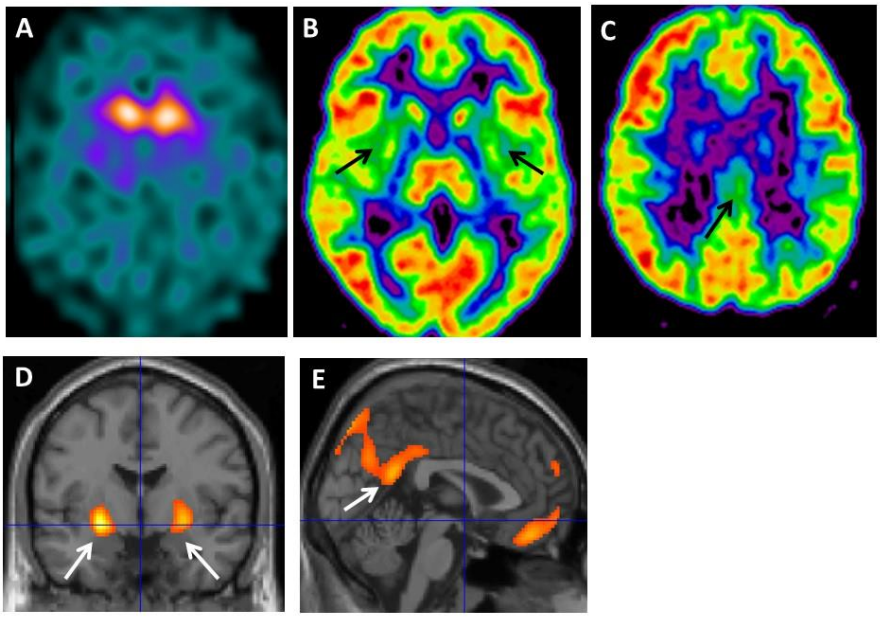


Figure 2: A) SPECT with FP-CIT (DAT-Scan) showing severe nigrostriatal dopaminergic deficit bilaterally, more marked in the putamen; Positron Emission Tomography (PET) with 18F-fluorodeoxyglucose showing severe striatal (**B**) and posterior cingulate (**C**) hypometabolism (black arrows). Results of SPM analysis highlight the location of hypometabolic deficit in the striatum (**D**) and posterior cingulate (**E**) (white arrows) in our patient as compared to controls (p 0.02 uncorrected; patient vs 17 healthy controls, age range: 35-63; age was considered in the statistical model of SPM as nuisance covariate).

References

1. Chen JY, Stern Y, Sano M, Mayeux R. Cumulative risk of developing extrapyramidal signs, psychosis or myoclonus in the course of Alzheimer's disease. Arch Neurol 1991; 48:1141-1143.

2. Golan MP, Styczyńska M, Jóźwiak K, et al. Early-onset Alzheimer's disease with a de novo mutation in the presenilin 1 gene. Exp Neurol 2004;208:264-268.

3. Dumanchin C, Brice A, Campion D, et al. De novo presenilin 1 mutations are rare in clinically sporadic, early onset Alzheimer's disease cases. French Alzheimer's Disease Study Group, J Med Genet 1998; 35:672-673.

4. Devi G, Fotiou A, Jyrinji D, et al. Novel presenilin 1 mutations associated with early onset of dementia in a family with both early-onset and late-onset Alzheimer disease. Arch Neurol 2000; 57:1454-1457.

5. Lou F, Luo X, Li M, Ren Y, He Z. Very early-onset sporadic Alzheimer's disease with a de novo mutation in the PSEN1 gene. Neurobiol Aging. 2017; 53:193.e1-193.e5.

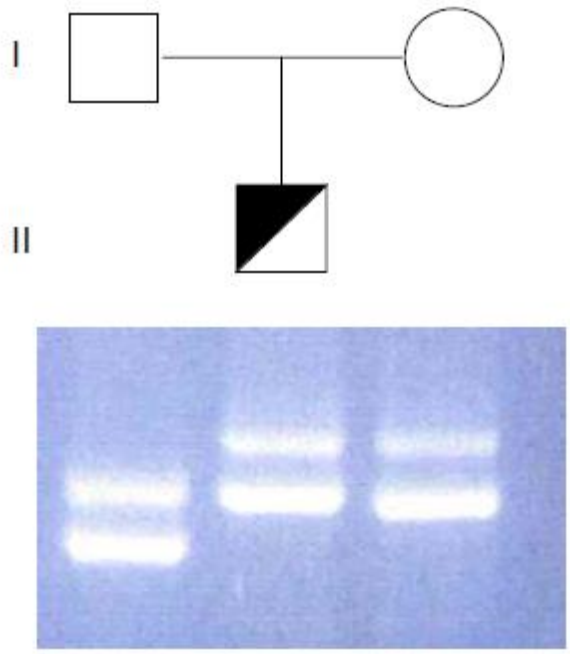
6. Takao M, Ghetti B, Hayakawa I, et al. A novel mutation (G217D) in the Presenilin 1 gene (PSEN1) in a Japanese family: presenile dementia and parkinsonism are associated with cotton wool plaques in the cortex and striatum. Acta Neuropathol (Berl) 2002;104:155-170.

7. Jimenez-Escrig A, Rabano A, Guerrero C, et al. New V272A presenilin 1 mutation with very early onset subcortical dementia and parkinsonism. Eur J Neurol 2004;11:663-669.

8. Snider BJ, Norton J, Coats MA, et al. Novel presenilin 1 mutation (S170F) causing Alzheimer disease with Lewy bodies in the third decade of life. Arch Neurol 2005;62:1821-1830.
9. Piccini A, Zanusso G, Borghi R, et al. Association of a presenilin 1 S170F mutation with a novel Alzheimer disease molecular phenotype. Arch Neurol 2007;64:738-745.
10. Godbolt AK, Cipolotti L, Watt H, Fox NC, Janssen JC, Rossor MN. The natural history of Alzheimer disease: a longitudinal presymptomatic and symptomatic study of a familial cohort. Arch Neurol 2004; 61:1743-1748.
11. Bougea A, Koros C, Stamelou M, et al. Frontotemporal dementia as the presenting phenotype of p.A53T mutation carriers in the alpha-synuclein gene. Parkinsonism Relat Disord 2017;35:82-87.

.

Supplementary material



Supplementary Figure 1. Haplotypes of polymorphic repetitive sequence marker at locus D11S533. Lane 1: proband's father; lane 2: proband; lane 3: proband's mother.

Electronic supplementary material. The online version of this article (doi:10.1007/s10048-017-0518-4) contains supplementary material, which is available to authorized users.

Video Legend

Video 1: **Segment 1 (age 26):** absent arm swings with dystonic posturing of the right arm; mild bradykinesia on tapping; **segment 2 (age 29):** freezing of gait, lower limb dystonia (tip-toe walking), axial dystonia and loss of postural reflexes; severe bilateral bradykinesia; hypomimia; severe dysarthria and hypophonic speech; mild Levodopa-induced dyskinesias are present at rest; **segment 3 (age 33)**: severe akinetic-rigid parkinsonism, anarthria; stimulus-sensitive myoclonic jerks in the upper limbs on posture; pyramidal signs in the lower limbs; frontal release signs (grasping, Myerson's sign).

Chapter 10

A *PDE10A de novo* mutation causes childhood-onset chorea with diurnal fluctuations

S. Esposito, MD, PhD[1], **M. Carecchio**, MD[1,2,3], D. Tonduti, MD, PhD[1], V. Saletti, MD[1], C. Panteghini, MSc[2], L. Chiapparini, MD[4], G. Zorzi, MD[1], C. Pantaleoni, MD[1], B. Garavaglia, PhD[2], D. Krainc, MD, PhD[5], S. Lubbe, PhD[5], N. Nardocci, MD[1], N. E. Mencacci, MD, PhD[5]

[1] *Department of Paediatric Neuroscience, IRCCS Foundation Carlo Besta Neurological Institute, Via Celoria 11, 20131 Milan, Italy*

[2] *Molecular Neurogenetics Unit, IRCCS Foundation Carlo Besta Neurological Institute, Via L. Temolo 4, 20126 Milan, Italy*

[3]*Department of Medicine and Surgery, PhD Programme in Molecular and Translational Medicine, Milan Bicocca University, Via Cadore 48, 20900 Monza, Italy*

[4] *Neuroradiology Department, IRCCS Foundation Carlo Besta Neurological Institute, Via Celoria 11, 20131 Milan, Italy*

[5] *Department of Neurology, Northwestern University, Feinberg School of Medicine, Chicago, 60611 Illinois, USA*

Key Words: *PDE10A*, chorea, childhood, dystonia, fluctuations

Mov Disord. 2017 Nov;32(11):1646-1647. doi: 10.1002/mds.27175

Recently, both *de novo* and bi-allelic mutations in *PDE10A*, encoding a cyclic nucleotide phosphodiesterase selectively expressed in striatal medium spiny neurons, have been recognized as a rare cause of childhood-onset chorea[1,2]. Brain MRI consistently showed striking bilateral striatal lesions in the three patients with *de novo* dominant mutations identified to date[1]. Interestingly, these radiological features were not observed in any of the patients with recessive mutations, despite a more severe clinical presentation[2].

Herein, we describe a patient carrying a *de novo PDE10A* mutation presenting with bilateral striatal MRI abnormalities and a yet unreported circadian pattern of non-progressive chorea.

The patient is an Italian 5-year-old boy born full term after an uneventful pregnancy and delivery. Motor and language milestones were normally achieved. At age 2.5 he presented with sub-acute onset of chorea involving the lower limbs causing frequent falls. No febrile illness preceded the onset of movement disorder. Over the following three months, chorea slowly progressed and became generalized with sparing of the oro-mandibular and facial muscles and he developed mild dystonic posturing of upper limbs. At this stage, diurnal fluctuations of chorea became evident, with increased severity lasting about two hours after waking up in the morning (**Video 1**). Chorea slowly improved during the day, showed no worsening before falling asleep and was absent at night. Cognitive assessment was normal (total IQ=114).

Brain MRI performed 2 months after onset of symptoms revealed bilateral symmetrical hyperintense lesions on T2-weighted, FLAIR and diffusion weighted images (DWI) involving the putamen and caudate nuclei (**Figure 1**). An extensive diagnostic workup, including CSF

analysis (neurotransmitters, folates), plasma lactate/pyruvate, activity of the respiratory chain enzymes in muscle, basic metabolic panel, a screening for autoimmune and infectious conditions was unremarkable. Targeted sequencing of bilateral striatal necrosis-related genes[3] yielded negative results.

Whole-exome sequencing was then performed as previously described[1] and revealed a heterozygous known pathogenic *PDE10A* missense variant (c.1000T>C, p.Phe334Leu; transcript ENST00000539869) located in the regulatory GAF-B domain of the protein. Sanger sequencing confirmed the presence of the variant and segregation analysis in the parents demonstrated it arose *de novo* in the proband. Only one additional dominant pathogenic variant located in the same domain has been identified to date (p.Phe300Leu)[1] suggesting that these residues are mutational hot spots.

After two years of follow-up, chorea showed no progression, but diurnal fluctuations consistently persisted (**Video 1)** and brain MRI was unchanged.

This report confirms the homogeneous phenotype related to dominant *PDE10A* mutations. The unique clinical presentation of childhood-onset chorea with a scarcely progressive course associated with bilateral striatal lesions is highly suggestive of dominant *PDE10A* mutations.

Unlike previously reported *PDE10A* patients, our case showed marked diurnal fluctuations with chorea being more severe upon awakening in the morning. If observed in other patients, this might be an additional clue for the differential diagnosis of pediatric movement disorders characterized by fluctuations of symptoms during the day. Unlike our

case, in fact, *ADCY5*-related chorea shows characteristics exacerbations lasting up to hours both upon awakening and when falling asleep[4], while Dopa-responsive Dystonia due to *GCH* mutations presents with marked worsening of dystonia and parkinsonism over the day[5].

Author Roles

1) Research project: A. Conception, B. Organization, C. Execution; 2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique; 3) Manuscript: A. Writing of the first draft, B. Review and Critique

E.S.: 1A, 1B, 3A, 3B

C.M.: 1A, 1B, 1C, 3A, 3B

T.D.: 1B, 3B

S.V.: 3B

P.C.: 1C, 3B

C.L.: 1C, 3B

Z.G.: 3B

P.C.: 3B

G.B.: 1C, 3B

K.D.: 3B

L.S.: 1C, 3B

N.N.: 1A, 1B, 3A, 3B

M.N.E.: 1B, 1C, 3B

References

1. Mencacci NE, Kamsteeg E-J, Nakashima K, et al. De Novo Mutations in PDE10A Cause Childhood-Onset Chorea with Bilateral Striatal Lesions. The American Journal of Human Genetics 2015; 98: 763-771
2. Diggle CP, Sukoff Rizzo SJ, Popiolek M, et al. Biallelic Mutations in PDE10A Lead to Loss of Striatal PDE10A and a Hyperkinetic Movement Disorder with Onset in Infancy. The American Journal of Human Genetics 2015; 98: 735-743
3. Tonduti D, Chiapparini L, Moroni I, et al. Neurological Disorders Associated with Striatal Lesions: Classification and Diagnostic Approach. Curr Neurol Neurosci Rep. 2016; 16:54
4. Carecchio M, Mencacci NE, Iodice A, et al. ADCY5-related movement disorders: Frequency, disease course and phenotypic variability in a cohort of paediatric patients. Parkinsonism Relat Disord. 2017 (*in press*)
5. Lee WW, Jeon BS. Clinical spectrum of dopa-responsive dystonia and related disorders. Curr Neurol Neurosci Rep. 2014; 14:461

Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Video legend

Video 1: **Segment 1** (age 3), early in the morning. Generalized chorea also involving the lower limbs with imbalance and wobbling gait; dystonic posturing of upper and lower limbs distally. **Segment 2** (age 3 years and 9 months), afternoon: mild generalized chorea with less marked severity and impairment of gait.

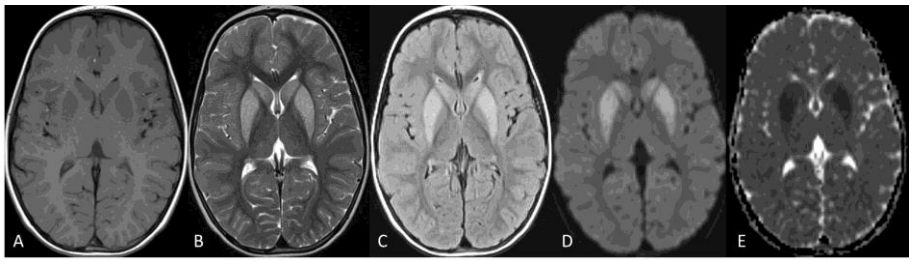


Figure 1. Brain MRI at age 3. Axial T1-weighted (**A**), T2-weighted (**B**) and FLAIR (**C**) images showing selective, bilateral and symmetrical mild hypointensity (**A**) and marked hyperintensity (**B-C**), of both caudate nuclei and putamina. The nuclei are slightly swollen. Axial diffusion-weighted imaging (DWI b1000; **D**), and apparent diffusion coefficient maps (ADC; **E**) show restricted diffusion of the nuclei, representing cytotoxic edema. Brain MRI at age 5 was unchanged (data not shown).

Chapter 11

Emerging monogenic complex hyperkinetic disorders.

Miryam Carecchio[1,2,3], Niccolò E. Mencacci[4,5]

[1] *Molecular Neurogenetics Unit, IRCCS Foundation Carlo Besta Neurological Institute, Via L. Temolo 4, 20126 Milan, Italy*

[2] *Department of Pediatric Neurology, IRCCS Foundation Carlo Besta Neurological Institute, Via Celoria 11, 20131 Milan, Italy*

[3] *Department of Medicine and Surgery, PhD Programme in Molecular and Translational Medicine, Milan Bicocca University, Via Cadore 48, 20900 Monza, Italy*

[4] *Department of Neurology, Northwestern University, Feinberg School of Medicine, Chicago, 60611 Illinois, USA*

[5] *Department of Molecular Neuroscience, UCL Institute of Neurology, WC1N 3BG London, United Kingdom*

Keywords: hyperkinetic; movement disorders; genetics; complex; epilepsy.

Curr Neurol Neurosci Rep. 2017 Oct 30;17(12):97. doi: 10.1007/s11910-017-0806-2.

ABSTRACT

Purpose of review: Hyperkinetic movement disorders can manifest alone or as part of complex phenotypes. In the era of Next-Generation Sequencing (NGS), the list of monogenic complex movement disorders is rapidly growing. This review will explore the main features of these newly identified conditions.

Recent findings: Mutations in *ADCY5* and *PDE10A* have been identified as important causes of childhood-onset dyskinesias and *KMT2B* mutations as one of the most frequent causes of complex dystonia in children. The phenotypic spectrum of *ATP1A3*-related disorders has expanded. Moreover, an expanding overlap is emerging between epileptic encephalopathies, developmental delay/intellectual disability and hyperkinetic movement disorders, with several genes involved (*FOXG1*, *GNAO1*, *GRIN1*, *FRRS1L*, *TBC1D24*).

Summary: Thanks to NGS the etiology of several complex hyperkinetic movement disorders has been elucidated. Importantly, NGS is changing the way clinicians diagnose these complex conditions. The dissection of the genetics of complex hyperkinetic disorders is helping to delineate how shared molecular pathways, involved in early stages of brain development and normal synaptic transmission, may underlie basal ganglia dysfunction, epilepsy and other neurodevelopmental disorders.

Introduction

Hyperkinetic movement disorders are a heterogeneous group of neurological disorders defined by an excess of involuntary movement production.

Based on the clinical phenomenology, hyperkinetic movement disorders are classified in different clinical entities, including among others dystonia, chorea, and myoclonus. Dystonia is characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive and patterned movements and/or postures. Chorea features continuous and brief involuntary movements, typically flowing from one body part to another in an unpredictable fashion in terms of timing, speed and direction. Myoclonus defines shock-like involuntary jerks caused by rapid muscle contractions [1].

The aetiology of these disorders is often genetically determined, especially in paediatric cases, and mutations in a rapidly growing number of genes have been causally linked to hyperkinetic movement disorders.

Traditionally, a detailed characterisation of the predominant movement disorder observed on examination would lay the bases for subsequent investigations, including targeted genetic analysis of mutations in genes that are known to be associated with a specific movement disorder.

However, there are several pitfalls that can make establishing a precise diagnosis on clinical grounds a true challenge. First, multiple hyperkinetic movement disorders are frequently observed together in the same patient with a considerable degree of overlap, which makes defining the predominant type of movement disorder very difficult. Second, the clinical presentation of hyperkinetic movement disorders is

often complex and highly variable, which may lead different neurologists to label differently the same movement disorder. Finally, additional neurological features (including intellectual disability, epilepsy, spasticity, ataxia and structural abnormalities of the brain) are often observed, in variable combinations, especially in cases with paediatric onset.

Hence, it is not surprising how the diagnostic work-up for complex hyperkinetic movement disorders may easily turn into long and painful diagnostic odysseys for patients and their families.

Importantly, the advent of next-generation sequencing (NGS) is rapidly changing the way clinicians diagnose and identify these conditions. Diagnostic approaches based on NGS technologies (i.e. targeted gene sequencing panels or whole-exome sequencing) are progressively becoming a first-line asset in the diagnostic pipeline for these complex disorders, partially bypassing the difficulties of the clinical assessment.

To increase awareness of these individually rare conditions, in this review we will summarise the main clinical and genetic features of the genetically determined complex hyperkinetic movement disorders identified in the last 5 years (summarised in **Table 1**).

Complex hyperkinetic movement disorders without epilepsy as core feature

ADCY5-related disorders

The first pathogenic dominant mutation in *ADCY5* was identified in a large kindred of German descent initially described in 2001 by

Fernandez *et al*. [2,3] Affected subjects showed an early-onset hyperkinetic movement disorder initially named Familial Dyskinesia with Facial Myokimia (FDFM). Subsequently, mutations in this gene were found in patients with childhood-onset chorea and dystonia, as well as in patients with a non-progressive condition resembling Benign Hereditary Chorea (BHC) who tested negative for *NKX2-1* mutations [4,5]. So far, 70 genetically confirmed cases belonging to 45 different families have been reported [2-16]. Since the first description, the phenotype associated to *ADCY5* mutations has largely broadened and consequently the original term FDFM has been replaced by a more comprehensive definition (*ADCY5*-related dyskinesias). Patients present virtually in all cases with axial hypotonia and delayed motor and/or language milestones during infancy, associated with early-onset chorea with a generalized distribution, classically involving also the facial muscles and the perioral region [5,15]. Dystonic posturing of the limbs and myoclonic jerks can be prominent, mimicking a myoclonus-dystonia-like phenotype but without the classical upper-body distribution observed in *SGCE* mutation carriers [15,17]. Episodic exacerbations of movement disorder lasting up to hours have been described in most *ADCY5*-positive cases, being frequently related to sleep but also triggered by febrile illnesses and other various stressors [18]. Such episodes can precede the onset of the chronic movement disorder that eventually dominate patients' clinical picture [15]. Pyramidal signs in the lower limbs and dysarthria are common clinical findings; in a single kindred, dilated cardiomyopathy was reported to co-segregate with *ADCY5* mutations in affected individuals, but this finding has never been observed in other families [3]. Both dominant

families and sporadic cases due to *de novo* mutations have been published, with a recurrent missense mutation (p.R418W) reported in the majority the affected cases. Two additional mutations at aminoacidic residue 418 (p.R418Q and p.R418G) have been subsequently reported, thus indicating that arginine 418 is a mutational hot-spot with a relevant pathogenetic role [6,8]. The severity of the movement disorder and the consequent degree of functional disability are variable in affected subjects. The available evidence suggests that the p.R418W mutation is responsible for a more severe clinical picture, whereas the p.A726T mutation is associated with a milder phenotype [6,15]. Besides genotype-phenotype correlation, somatic mosaicism detected in some mildly affected patients further explains the clinical heterogeneity of *ADCY5* mutated subjects [5,6].

In terms of therapy, anticholinergics, benzodiazepines (clonazepam), tetrabenazine, baclofen, neuroleptics, anticonvulsants alone or in combination, have been administered to reduce dyskinesias, with variable response; acetazolamide, a carbonic anhydrase inhibitor, has been successfully used in two patients [2]. Deep brain stimulation (DBS) of bilateral globus pallidus interna (GPi) has been performed in four patients, with moderate improvement of chorea [9,11].

From a disease mechanism point of view, *ADCY5* encodes adenyl cyclase 5 (AC5), an enzyme most abundantly expressed in striatal neurons that converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). Importantly, dopamine and adenosine modulation of striatal medium spiny neurons (MSNs) is largely mediated through cAMP signalling, as AC5 activity is promoted by the

stimulation of the G protein-coupled dopamine receptors type 1 and adenosine receptors 2A [19].

PDE10A-related disorders

Both dominant and recessive mutations in this gene have been recently reported in patients with childhood-onset chorea with a generalized distribution and also involving the facial muscles. Two recurrent dominant mutations (p.F300L and p.F334L) have been found so far in five unrelated patients worldwide, arising *de novo* in all but one subject, whose family showed a dominant pattern of inheritance with complete penetrance [20-22]. Recessive homozygous mutations (p.Y107C and p.A116P) have been detected in eight patients from two consanguineous pedigrees [23]. Clinically, carriers of dominant mutations display a homogeneous phenotype characterized by early onset (5-15 years) chorea, normal development and cognition and characteristic symmetrical T2-hyperintense bilateral striatal lesions on brain MRI. Disease course seems to be non-progressive although diurnal fluctuations in childhood and a progressive spreading of chorea during life with increased severity in the elderly have been reported [20,21]. Also, levodopa-responsive parkinsonism with abnormal DAT-Scan has been described in an adult positive patient [20]. However, more evidence is needed to establish whether this is truly part of the *PDE10A*-realted phenotype in the elderly or a chance association. Patients carrying biallelic *PDE10A* mutations show a more severe phenotype, with markedly delayed motor and language milestones, axial hypotonia, an earlier age at onset of chorea (within 6 months of

age), severe dysarthria and mild intellectual disability in some cases. In a single affected subject, childhood-onset epilepsy was also reported. Despite a more severe neurological involvement, the MRI of these cases is normal, without striatal abnormalities observed in cases with dominant mutations [23].

PDE10A encodes phosphodiesterase 10A, which regulates the degradation of cAMP in MSNs of the corpus striatum, where it is highly and selectively expressed. Both recessive and dominant mutations in *PDE10A* have been shown to lead to a loss of enzymatic function or reduced striatal protein levels [20, 23].

Interestingly, preliminary evidence suggests that pathogenic mutations in *ADCY5* may act through a gain of function mechanism [4], overall supporting the hypothesis that abnormally increased levels of intracellular cAMP in striatal neurons may represent a key mechanism in the pathogenesis of chorea.

KMT2B-related disorders

KMT2B, also known as *MLL4*, encodes a ubiquitously expressed histone lysine methyltransferase involved in methylation of histone H3 at lysine 4 (H3K4). This gene, located in chromosomal region 19q13.12, belongs to the SET/MLL family of proteins, which are essential for activating specific sets of genes during normal development [24]. Consistently, loss-of-function mutations in other MLL-encoding genes have been reported in a number of human developmental disorders, such as Kabuki syndrome [25]. Mutations in *KMT2B*, including interstitial microdeletions detected by microarray at

19q13.11-19q13.12, have been reported in patients with childhood-onset dystonia with a progressive course and a variable number of additional clinical features [26,27]. Patients classically present with lower limb dystonia in early childhood, with subsequent generalization as observed in DYT1 mutation carriers; however, unlike DYT1 patients, affected subjects develop a prominent oro-mandibular and laryngeal involvement that can lead to severe dysarthria or even anarthria. Most cases carry *de novo* dominant mutations, but a limited number of families with an autosomal dominant transmission have been reported as well [27,28]. Intra-familial clinical heterogeneity, with variable severity of dystonia as well as incomplete penetrance, either true o due to possible parental mosaicism have been observed [26-27]. So far, 33 unrelated patients carrying *KMT2B* variants with convincing evidence of pathogenicity have been reported, of which 19 carried genomic microdeletion involving *KMT2B* and different contiguous genes [16;26-28]. *KMT2B*-related dystonia has been defined "complex" in that additional neurological and systemic features have been recognized in some of the mutation carriers, including psychomotor and language delay, minor dysmorphic traits and a characteristic facial appearance (bulbous nasal tips and elongated face), mild-to-moderate intellectual disability, short stature, skin abnormalities and psychiatric disturbances [26]. In some patients, the complexity of the clinical phenotype could be partially related to the extension of microdeletions on chromosome 19 leading to haploinsufficiency of a variable number of genes contiguous to *KMT2B*. Notably, a marked improvement of dystonia with sustained clinical benefit on long-term follow-up has been reported following bilateral GPi DBS, whereas no oral medication

is reported to be particularly effective in alleviating motor manifestations [26-28].

Meyer *et al*. reported a detection rate of *KMT2B* mutations in up to 38% of patients with early-onset progressive dystonia, suggesting that the contribution of this recently discovered gene to the pathogenesis of childhood-onset dystonia is far higher than most other dystonia-related genes [26].

ATP1A3-related disorders

ATP1A3 gene encodes the α3 isoform of the catalytic subunit of the Na^+/K^+ pump, which is an adenosine triphosphatase (ATPase) cation transporter playing a crucial role in maintaining electrochemical gradients for Na^+ and K^+ across the plasma membrane of different cellular types [29]. Mutations affecting the α3 subunit, which is selectively expressed in neurons, were initially linked to three distinct neurological phenotypes, including rapid-onset dystonia parkinsonism (RDP), alternating hemiplegia of childhood (AHC), and cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS) syndrome [30-33]. Other presentations that do not fall within these neurological entities, as well as intermediate and overlapping phenotypes have emerged in recent years, providing evidence that these conditions are different manifestations of a wide phenotypic spectrum rather than allelic disorders [34,35].

Mutations in *ATP1A3* arise *de novo* in most cases of AHC, whereas autosomal dominant transmission has been documented in RDP and CAPOS syndrome cases; moreover, germline mosaicism has been

recently reported in two families with recurrence of AHC in offspring of unaffected parents [35,36].

In AHC symptoms begin before 18 months, and developmental delay is the rule. Clinical manifestations consist of paroxysmal episodes of unilateral hemiplegia or quadriplegia, dystonia, or oculomotor abnormalities (such as monocular nystagmus) which disappear upon sleeping, sometimes only transiently. Episodes last from minutes to several days and can occur with a variable frequency, up to multiple times a day. About half of cases develop epilepsy [37].

In RDP patients present with abrupt onset of asymmetric dystonia with generally minor features of parkinsonism, with a clear rostro-caudal spreading (face>arm>leg) and prominent bulbar involvement. Symptoms evolve over a few minutes to 30 days, with subsequent stabilization within one month; disease course is often biphasic, with a sudden second worsening of symptoms during life. Age at onset ranges from infancy to the fifth decade [38].

In AHC and RDP clinical manifestations are typically triggered by environmental, physical or emotional stressors (excitement, strong emotions, physical exertion, febrile illness, excessive environmental stimuli – sounds, light etc). The recognition of provocative factors triggering paroxysmal neurological symptoms with an initial hemisomatic distribution represents the most pathognomonic feature to diagnose *ATP1A3*-related disorders, and must be carefully investigated in the patients' clinical history [35].

Atypical phenotypes include paroxysms of unresponsiveness, bulbar signs, ataxia, fever-induced encephalopathy, prolonged flaccid tetraplegia with persistent choreo-athetosis between episodes,

catastrophic epilepsy, progressive childhood-onset cerebellar syndrome with step-wise deterioration [34;39-41].

From a genetic point of view, mutations are distributed over almost all ATP1A3 coding sequence, but RPD phenotypes are mainly associated with mutations in exons 8, 14 and 17, whereas the majority of mutations in patients with AHC are located in exons 17 and 18 [34]. Available evidence supports a genotype-phenotype correlation with mutations causing classic AHC affecting trans-membrane and functional protein domains; two recurrent missense mutations (p.Asp801Asn and p.Glu815Lys) have been detected in 50% of AHC cases reported. Moreover, only four missense variants have been detected so far in AHC-RDP intermediate phenotypes, and a single recurrent missense mutation (p.Glu818Lys) has been identified in all CAPOS cases [34,35].

No specific drugs targeting the altered ionic transport across the Na^+/K^+ pump are available, and symptomatic treatment of acute attacks mostly with benzodiazepines and other sleep inducers is the most frequent therapeutic approach in AHC. Flunarizine is widely used as a prophylactic agent; in a series of 30 AHC patients, it was effective to reduce frequency and duration of attacks in 50% of cases, but no controlled trials are available [42]. Topiramate is also used with the same aim based on anecdotal reports. In RDP treatment of dystonia and parkinsonism does not benefit from dopaminergic drugs, and GPi DBS has proven ineffective in a very limited number of cases and also in the authors' experience [43].

GPR88-related disorders

Alkufri *et al*. recently individuated a recessive homozygous truncating mutation (C291X) in *GPR88* in three affected children from a consanguineous Palestinian family [44]. Patients (all females) presented with developmental delay in infancy, markedly delayed speech and learning disability followed, around 9 years of age, by chorea initially affecting the facial muscles and subsequently spreading to involve upper limbs (mainly distally), trunk and tights. Chorea showed a slow but constant progression over a period of months, without further worsening few years after the onset. Severe mental retardation (IQ 40 in one subject) and a scarcely progressive movement disorder therefore seem to be key phenotypic features of this disorder. So far, no additional cases following the original publication have been reported.

The *GPR88* gene encodes a G protein-coupled receptor (GPCR) abundantly expressed both in D1R- and D2R-expressing MSNs, which are, respectively, part of the direct and indirect pathway [45].

MSNs from *GPR88* knock-out mice show increased glutamatergic excitability and reduced GABAergic inhibition, which results in enhanced firing rates *in vivo*, producing a murine phenotype characterized by hyperactivity, impaired motor coordination and motor learning [46].

Hyperkinetic movement disorders in epileptic-dyskinetic encephalopathies

Early onset encephalopathies are a heterogeneous group of diseases characterized by severe dysfunction of cognitive, sensory and motor

development. The etiology of these disorders is variable and includes acquired causes such as prematurity, congenital infections and hypoxic insult at birth, as well as various genetic defects that disrupt brain function, or its normal structure and development. Early-onset, often drug-resistant seizures are a recurrent feature of several encephalopathies. In recent years, the co-occurrence of hyperkinetic movement disorders (chorea, dystonia, ballismus, complex stereotypies) in early-onset epileptic encephalopathies (EOEE) has been increasingly recognized and detailed, to the point that movement disorders are now considered a core feature of several EOEE. These conditions, currently referred to as "epileptic-dyskinetic encephalopathies" (MIM: 308350), are clinically and genetically heterogeneous and encompass various degrees of intellectual disability and severe, often intractable epilepsy in association with hyperkinetic movement disorders. Altered functioning of glutamatergic NMDA and AMPA receptors as well as impaired neurotransmission and synaptic plasticity in early neurodevelopmental stages seem to be relevant pathogenetic mechanisms, that can give rise to a wide spectrum of variably associated neurological symptoms including movement disorders, intellectual disability and epilepsy.

FOXG1-related disorders

FOXG1 (Forkhead Box G1) gene, a transcription repressor, plays a crucial role in fetal telencephalon development and is an important component of the transcription regulatory network that controls proliferation, differentiation, neurogenesis, and neurite outgrowth in the cerebral cortex, hippocampus and basal ganglia [47,48]. Mutations in

FOXG1 cause a distinct developmental encephalopathy manifesting in infancy or early childhood with severe developmental delay, acquired microcephaly, profound intellectual disability, epilepsy and absent language (so called “congenital Rett syndrome”; OMIM 613454) [49]. Corpus callosum hypoplasia or aplasia, delayed myelination, simplified gyration and fronto-temporal abnormalities are frequent radiological findings. Beyond these core features, the phenotypic spectrum of *FOXG1* mutations has recently expanded to include early-onset, complex hyperkinetic movement disorders featuring various combinations of chorea, dystonia, dyskinesia, myoclonus and hand stereotypies that are virtually observed in all positive patients and become evident since the first years of life [50]. In a series of 28 patients, chorea was the most frequent movement disorder (88%), followed by orolingual/facial dyskinesia (80%) and dystonia (76%); movement disorders course was progressive in about half of cases, with remarkable severity and disability [51]. In a recent review of 83 novel and published cases, dyskinesias and hand stereotypies were both reported in 90% of patients whose clinical data were available. A definite genotype-phenotype correlation has not been established, although truncating *FOXG1* mutations in the N-terminal and the forkhead domains (except conserved site 1) are associated with more severe phenotypes, whereas missense variants in the forkhead conserved site 1 seem to be responsible for milder phenotypes, with independent ambulation, spoken language, normal head growth and ability to use hands [51,52].

Several drugs have shown little or no benefit in alleviating *FOXG1* movement disorders, although levodopa, tetrabenazine and pimozide were partially beneficial in single cases [50,51].

Hyperkinetic movements similar to those observed in *FOXG1* have also been reported in carriers of *CDKL5* mutations, transmitted as an X-linked trait. Mutations in this gene are associated with variant Rett syndrome characterized by onset of refractory seizures within the first weeks of life inconstantly associated with movement disorders [53].

The differential diagnosis of *FOXG1*-related phenotypes includes other epileptic-dyskinetic encephalopathies such as *ARX*-related encephalopathy (characterized by infantile spasms, neonatal-onset progressive dystonia with recurrent status dystonicus and severe mental retardation) and three conditions caused by *de novo* mutations in genes essential for neurotransmitter release through synaptic vesicle fusion [54-56]. These include mutations in *STXBP1* (featuring infantile-onset epilepsy with good prognosis, tremor and frequent paroxysmal non-epileptic movement disorders), *SYT1* (associated with severe developmental delay and an early onset, paroxysmal dyskinetic movement disorder worsening at night) and *UNC13A* (linked to developmental and speech delay, intellectual disability, dyskinesias and intention tremor, with febrile seizures as a minor feature) [57-59].

GNAO1-related disorders

GNAO1 encodes a subclass (Gαo) of the Gα subunit of heterotrimeric guanine nucleotide-binding proteins which is highly expressed in the brain, where it is involved in the regulation of neuronal excitability and

neurotransmission. *De novo* mutations in *GNAO1* were initially associated with Ohtahara syndrome, a severe type of early epileptic encephalopathy characterized by neonatal tonic spasms, severe motor developmental delay and intellectual disability with a suppression-burst pattern on EEG [60,61]. *GNAO1*-related encephalopathy has been further characterized following the individuation of additional mutation carriers, and hyperkinetic movements have emerged as an important core feature, being universally present in affected subjects. Patients present in most cases a combination of generalized chorea associated with dystonia, which manifest within the first months or years of life, with a median age at onset around two years [62]. Facial and oro-lingual dyskinesia and complex stereotypies have been reported as well. Movement disorders display a chronic course with characteristic episodic exacerbations triggered by high temperature, infections, emotions and purposeful movements lasting from minutes to days and even months and often being accompanied by dysautonomic manifestations (sweating, tachycardia, hypertemia, diaphoresis) and thus being potentially life-threatening [63]. Exacerbations have a variable frequency and can present in clusters up to several times a day. While developmental delay and severe intellectual disability are features consistently associated with *GNAO1* mutations, epilepsy is variably present, and often follows the onset of movement disorders of months or years [64].

So far, 45 genetically proven patients have been reported, harbouring 25 different mutations (23 missense, 1 splice-site, 1 deletion) [62, 64-69]. Glutamine at position 246 (Glu246) and arginine at position 209 (Arg209), both highly conserved amino acids, are *GNAO1* mutational

hotspots and missense mutations involving these residues have been reported in about half (21/45, 46.7%) of published cases. A genotype-phenotype correlation has recently been suggested in functional *in vitro* studies, with *GNAO1* loss-of-function mutations associated with epileptic encephalopathy and gain-of-function or normally functioning alleles leading to phenotypes dominated by movement disorders [70]. Tetrabenazine and neuroleptics seem to be the most effective drugs to treat movement disorders in *GNAO1* mutation carriers; in severe drug-resistant cases, bilateral GPi DBS has significantly improved the frequency and severity of exacerbations as well as patients' motor performances, although the baseline movement disorder seems to remain rather constant even after DBS implant [66; 71,72].

GRIN1-related disorders

GRIN1 encodes the GluN1 subunit of the glutamatergic N-methyl-D aspartate receptors (NMDAR), which are heteromeric protein complexes acting as ion channels upon ligand activation [73]. GluN1 subunits have a key role in the plasticity of synapses, which underlies memory and learning [74]. *De novo* heterozygous variants of *GRIN1* were first linked to nonsyndromic intellectual disability (ID) with or without epilepsy [75]. So far, 34 positive patients from 30 different families have been reported [76-80]. The vast majority of mutations are heterozygous variants arising *de novo*, but recessive biallelic mutations have also been described in seven patients from three different consanguineous kindred [77,78]. *GRIN1* positive patients present in almost all cases with severe developmental delay, cognitive

dysfunction and profound ID since early infancy. About 70% of cases develop early-onset, polymorphic seizures with non-specific EEG patterns, that are drug-resistant in about one third of cases. Hyperkinetic movement disorders (mainly a combination of chorea and dystonia) have been observed in about 60% of patients, and complex stereotypies as well as oculogyric crises resembling those of monoamine neurotransmitter disorders are frequently reported, being an important diagnostic clue [77]. Additional features include spastic tetraparesis, cortical blindness, non-specific sleep disturbances, subtle dysmorphism and microcephaly. All *de novo GRIN1* mutations cluster within or in close proximity of the trans-membrane domains of GluN1, a highly-conserved region; *in vitro* studies demonstrated that variants in this position lead to a dominant negative effect, whereas one of the reported homozygous variants (c.649C>T; p.Arg217Trp) causes impaired activation of the NMDA receptor [77]. Moreover, a *GRIN1* truncating variant (c.1666C>T; p.Gln556*), resulting in GRIN1 haploinsufficiency, seems to be tolerated in a heterozygous state, not producing a neurological phenotype; however, when in homozygosity, it has proven responsible for a fatal neonatal epileptic encephalopathy [77].

FRRS1L-related disorders

The *FRRS1L* gene encodes a component of the outer core of AMPA receptor accessory proteins. Glutamatergic AMPA receptors represent the most common receptor subtype in the brain, mediating fast glutamatergic excitatory post-synaptic potentials. Using a combination

of homozygosity mapping and WES, Madeo *et al*. recently identified four different homozygous mutations in *FRRS1L* in eight patients from four different pedigrees, two of which were consanguineous [81]. Six additional patients from a large consanguineous Arab kindred have subsequently been reported [82]. Affected subjects present around 20 months of age with psychomotor regression after a phase of normal development, followed by the onset of progressive choreo-atethosis and ballismus and severe encephalopathic epilepsy. Differently from *GNAO1* positive patients, the severity of movement disorders seems to decrease over disease course, giving way to an akinetic-rigid phenotype in late adolescence, and no episodic exacerbations have been reported [81].

TBC1D24-related disorders

TBC1D24 is involved in synaptic vesicles trafficking and is expressed in multiple human tissues, with the highest expression in the brain [83]. Recessive homozygous or compound heterozygous mutations in *TBC1D24* have been linked to several human diseases, ranging from non-syndromic deafness to a wide spectrum of epilepsies, whereas dominant mutations have been linked to a type of non-syndromic hearing loss. The most common epilepsy phenotype consists of early-onset myoclonic epilepsy, myoclonic seizures (often occurring in clusters), and drug-resistance. About 50 epileptic patients carrying *TBC1D24* have been reported worldwide, with heterogeneous presentations and prognosis [84]. In a recent review of new and published cases, 39/48 (81%) presented mild to profound intellectual

disability, which therefore appears to be a frequently encountered clinical feature. Moreover, dystonia (sometimes with a hemisomatic distribution) was reported in 7/48 (14.5%) of patients as well as in a recently published case with a complex phenotype including epilepsy, infantile-onset parkinsonism, cerebellar signs and psychosis [85]. Cortical myoclonus affecting lower limbs with gait impairment has also been reported [86].

Conclusions

In the last 5 years, the list of genes associated with dystonia, chorea, myoclonus and mixed movement disorders, has dramatically expanded and so has the phenotype associated with mutations in individual genes. Our systematic review of the recent literature shows that an unexpected variety of molecular causes underlie complex hyperkinetic disorders. Several genes, though individually very are, can be responsible for the same phenotypes and, on the other hand, mutations in a given gene can be associated with several phenotypes, which are often part of spectrum and not discrete entities (as exemplified by *ATP1A3*-related disorders). With some exceptions (e.g. *ADCY5*-, *KMT2B*-, *ATP1A3*-related movement disorders), the number of reported patients affected by these novel genetic entities is still rather limited. Therefore, our knowledge about the clinical features and natural history of these disorders will only grow once larger case series will become available.

Importantly, NGS is challenging the traditional - and often problematic - approach to patients with hyperkinetic movement disorders, based on the recognition of “core clinical features”. The increasing availability

of NGS in clinical practice will hopefully help to formulate definite diagnoses in a larger number of patients affected by complex movement disorders, allowing clinicians to provide families with appropriate genetic counselling and disease-specific therapies.

References

1. Abdo WF, van de Warrenburg BP, Burn DJ, Quinn NP, Bloem BR. The clinical approach to movement disorders. Nat Rev Neurol 2010:6:29-37.

2. *Chen YZ, Matsushita MM, Robertson P, Rieder M, Girirajan S, Antonacci F, et al. Autosomal dominant familial dyskinesia and facial myokymia: single exome sequencing identifies a mutation in adenylyl cyclase 5. Arch Neurol 2012:69:630-35.

This paper identifies for the first time a pathogenic mutation in *ADCY5* as the cause of familial dyskinesia and facial myokymia, an autosomal dominant movement disorder previously described by Fernandez *et al*. in a large dominant kindred.

3. Fernandez M, Raskind W, Wolff J, Matsushita M, Yuen E, Graf W, et al. Familial dyskinesia and facial myokymia (FDFM): a novel movement disorder. Ann Neurol 2001:49: 486-92.

4. Chen YZ, Friedman JR, Chen DH, Chan GC, Bloss CS, Hisama FM, et al. Gain-of-function ADCY5 mutations in familial dyskinesia with facial myokymia. Ann Neurol 2014:75:542-49.

5. *Mencacci NE, Erro R, Wiethoff S, Hersheson J, Ryten M, Balint B, et al. ADCY5 mutations are another cause of benign hereditary chorea. Neurology 2015:85:80-88.

By studying 18 unrelated cases diagnosed with benign hereditary chorea without *NKX2-1* mutations, the authors identify the *ADCY5* p.R418W mutation in two cases showing chorea with a progressive course, in contrast to BHC secondary to *NKX2-1* mutations. This

difference in the clinical course is mirrored by brain expression data, showing increasing *ADCY5* expression in the striatum during brain development, whereas *NKX2-1* shows an opposite trend.

6. **Chen DH, Méneret A, Friedman JR, Korvatska O, Gad A, Bonkowski ES, et al. ADCY5-related dyskinesia: Broader spectrum and genotype-phenotype correlations. Neurology 2015:85:2026-35.

This paper reports the identification of 3 new families and 12 new sporadic cases carrying *ADCY5* mutations. The authors provide a detailed description of *ADCY5*-related phenotype to include a mixed hyperkinetic disorder characterized by chorea, dystonia and myoclonus preceded by axial hypotonia and developmental delay in infancy/childhood.

7. Carapito R, Paul N, Untrau M, Le Gentil M, Ott L, Alsaleh G, et al. A de novo ADCY5 mutation causes early-onset autosomal dominant chorea and dystonia. Mov Disord 2015:30:423-27.

8. Chang FC, Westenberger A, Dale RC, Smith M, Pall HS, Perez-Dueñas B, et al. Phenotypic insights into ADCY5-associated disease. Mov Disord 2016:31:1033-40.

9. Dy ME, Chang FC, Jesus SD, Anselm I, Mahant N, Zeilman P, et al. Treatment of ADCY5-Associated Dystonia, Chorea, and Hyperkinetic Disorders With Deep Brain Stimulation: A Multicenter Case Series. J Child Neurol 2016:31:1027-35.

10. Zech M, Boesch S, Jochim A, Weber S, Meindl T, Schormair B, et al. Clinical exome sequencing in early-onset generalized dystonia and large-scale resequencing follow-up. Mov Disord 2017:32:549-59.

11. Meijer IA, Miravite J, Kopell BH, Lubarr L. Deep Brain Stimulation in an Additional Patient With ADCY5-Related Movement Disorder. J Child Neurol 2017:32:438-39.
12. Westenberger A, Max C, Brüggemann N, Domingo A, Grütz G, Pawlack H, et al. Alternating Hemiplegia of Childhood as a New Presentation of Adenylate Cyclase 5-Mutation-Associated Disease: A Report of Two Cases. J Pediatr 2017:181:306-08.
13. Douglas AG, Andreoletti G, Talbot K, Hammans SR, Singh J, Whitney A, et al. ADCY5-related dyskinesia presenting as familial myoclonus-dystonia. Neurogenetics 2017:18:111-17.
14. Tunc S, Brüggemann N, Baaske MK, Hartmann C, Grütz K, Westenberger A, et al. Facial twitches in ADCY5-associated disease - Myokymia or myoclonus? An electromyography study. Parkinsonism Relat Disord 2017:40:73-75.
15. *Carecchio M, Mencacci NE, Iodice A, Pons R, Panteghini C, Zorzi G, et al. ADCY5-related
movement disorders: Frequency, disease course and phenotypic variability in a cohort of paediatric patients. Parkinsonism Relat Disord 2017:41:37-43.

In this paper, the authors describe clinical features and disease course of six additional *ADCY5* mutation carriers, highlighting that paroxysms of chorea and/or dystonia can precede the onset of a chronic movement disorder and that the clinical picture can vary over time, sometimes leading to a spontaneous improvement of episodic exacerbations triggered by sleep or other provoking factors.

16. Zech M, Jech R, Wagner M, Mantel T, Boesch S, Nocker M, et al. Molecular diversity of combined and complex dystonia: insights from diagnostic exome sequencing. Neurogenetics 2017, *in press*. doi:10.1007/s10048-017-0521-9.

17. Kinugawa K, Vidailhet M, Clot F, Apartis E, Grabli D, Roze E. Myoclonus-dystonia: an update. Mov Disord 2009:24:479-89.

18. Friedman JR, Méneret A, Chen DH, Trouillard O, Vidailhet M, Raskind WH, et al. ADCY5 mutation carriers display pleiotropic paroxysmal day and nighttime dyskinesias. Mov Disord 2016:31:147-48.

19. Hervé D. Identification of a specific assembly of the g protein golf as a critical and regulated module of dopamine and adenosine-activated cAMP pathways in the striatum. Front Neuroanat 2011:5:48.

20. **Mencacci NE, Kamsteeg E-J, Nakashima K, R'Bibo L, Lynch DS, Balint B, et al. De Novo Mutations in PDE10A Cause Childhood-Onset Chorea with Bilateral Striatal Lesions. American J Hum Gen 2016:98:763-71.

PDE10A de novo **mutations are reported for the first time in patients with childhood-onset chorea and characteristic bilateral striatal lesions on brain MRI, confirming the crucial role of cAMP signalling in the regulation of striatal medium spiny neurons firing and the pathogenesis of chorea.**

21. Esposito S, Carecchio M, Tonduti D, Saletti V, Panteghini C, Chiapparini L, et al. A PDE10A de novo mutation causes childhood-onset chorea with diurnal fluctuations. Mov Disord 2017, *in press*

22. Myatake S, Koshimizu E, Shirai I, Kumada S, Nakata I, Kamemaru A, et al. A familial case of PDE10A-associated childhood-onset chorea with bilateral striatal lesions. Mov Disord 2017, *in press*.

23. Diggle CP, Sukoff Rizzo SJ, Popiolek M, Hinttala R, Schülke JP, Kurian MA, et al. Biallelic Mutations in PDE10A Lead to Loss of Striatal PDE10A and a Hyperkinetic Movement Disorder with Onset in Infancy. Am J Hum Gen 2016:98:735-743.

****Back-to-back publication with ref. 19, this paper describes the identification of recessive *PDE10A* mutations in patients with a more complex phenotype including chorea with onset in infancy, axial hypotonia and developmental delay. Patients' brain MRI was unremarkable, with no striatal lesions as described by Mencacci *et al*. in dominant mutations carriers, suggesting different *in vivo* mechanisms of the mutations.**

24. Shao GB, Chen JC, Zhang LP, Huang P, Lu HY, Jin J, et al. Dynamic patterns of histone H3 lysine 4 methyltransferases and demethylases during mouse preimplantation development. In Vitro Cell Dev Biol Anim 2014:50:603-13.

25. Ng SB, Bigham AW, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, et al.

Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. Nat Genet 2010:42:790-3

26. **Meyer E, Carss KJ, Rankin J, Nichols JM, Grozeva D, Joseph AP, et al. Mutations in the histone methyltransferase gene KMT2B cause complex early-onset dystonia. Nat Genet 2017:49:223-37.

In this multicentric international study, the authors identify 27 unrelated cases carrying dominant, mostly *de novo* mutations in

***KMT2B*, a novel disease-causing gene located on chromosome 19. The patients' phenotype is characterized by childhood-onset generalized dystonia with onset in the lower limbs and prominent oromandibular and laryngeal involvement. Additional neurological and non-neurological features are reported, including developmental delay, minor facial dysmorphisms, and mild mental retardation.**

27. **Zech M, Boesch S, Maier EM, Borggraefe I, Vill K, Laccone F, et al. Haploinsufficiency of KMT2B, Encoding the Lysine-Specific Histone Methyltransferase 2B, Results in Early-Onset Generalized Dystonia. Am J Hum Genet 2016:99:1377-87.

Published in parallel with the manuscript by Meyer *et al*., this study reports different *KMT2B* mutation carriers with strikingly similar clinical features. The authors demonstrate significantly decreased mRNA levels of KMT2B in mutant fibroblasts, thus suggesting haploinsufficiency as the underlying pathogenic mechanism leading to dystonia.

28. Zech M, Jech R, Havránková P, Fečíková A, Berutti R, Urgošík D, et al. KMT2B rare missense variants in generalized dystonia. Mov Disord 2017, *in press*.

29. Shull GE, Greeb J, Lingrel JB. Molecular cloning of three distinct forms of the Na^+, K^+-ATPase α-subunit from rat brain. Biochemistry 1986:25:8125-32.

30. de Carvalho Aguiar P, Sweadner KJ, Penniston JT, Zaremba J, Liu L, Caton M, et al. Mutations in the Na+/K+-ATPase alpha3 gene ATP1A3 are associated with rapid-onset dystonia parkinsonism. Neuron 2004:43:169-75.

31. Heinzen EL, Swoboda KJ, Hitomi Y, Gurrieri F, Nicole S, de Vries B, et al. De novo mutations in ATP1A3 cause alternating hemiplegia of childhood. Nat Genet 2012:44:1030-34.
32. Rosewich H, Thiele H, Ohlenbusch A, Maschke U, Altmüller J, Frommolt P, et al. Heterozygous de-novo mutations in ATP1A3 in patients with alternating hemiplegia of childhood: a whole-exome sequencing gene-identification study. Lancet Neurol 2012:11:764-73.
33. Demos MK, van Karnebeek CD, Ross CJ, Adam S, Shen Y, Zhan SH, et al. A novel recurrent mutation in ATP1A3 causes CAPOS syndrome. Orphanet J Rare Dis 2014:9:15.
34. Rosewich H, Ohlenbusch A, Huppke P, Schlotawa L, Baethmann M, Carrilho I, et al. The expanding clinical and genetic spectrum of ATP1A3-related disorders. Neurology 2014:82:945-55.
*__This paper provides important insights in the clinical spectrum of *ATP1A3*-associated disorders, highlighting the existence of partially overlapping phenotypes and making important observations on genotype-phenotype correlation, localization and clustering of *ATP1A3* mutations in 19 novel and 164 published cases.__
35. Sweney MT, Newcomb TM, Swoboda KJ. The expanding spectrum of neurological phenotypes in children with ATP1A3 mutations, Alternating Hemiplegia of Childhood, Rapid-onset Dystonia-Parkinsonism, CAPOS and beyond. Pediatr Neurol 2015:52:56-64.
36. Hully M, Ropars J, Hubert L, Boddaert N, Rio M, Bernardelli M, et al. Mosaicism in ATP1A3-related disorders: not just a theoretical risk. Neurogenetics 2017:18:23-28.

37. Rosewich H, Sweney MT, DeBrosse S, Ess K, Ozelius L, Andermann E, et al. Research conference summary from the 2014 International Task Force on ATP1A3-Related Disorders. Neurol Genet 2017:3:e139.

38. Brashear A, Dobyns WB, de Carvalho Aguiar P, et al. The phenotypic spectrum of rapid-onset dystonia-parkinsonism (RDP) and mutations in the ATP1A3 gene. Brain 2007:130:828-35.

39. Yano ST, Silver K, Young R, DeBrosse SD, Ebel RS, Swoboda KJ, et al. Fever-Induced Paroxysmal Weakness and Encephalopathy, a New Phenotype of ATP1A3 Mutation. Pediatr Neurol 2017:73:101-05.

40. Kanemasa H, Fukai R, Sakai Y, Torio M, Miyake N, Lee S, et al. De novo p.Arg756Cys mutation of ATP1A3 causes an atypical form of alternating hemiplegia of childhood with prolonged paralysis and choreoathetosis. BMC Neurol 2016:16:174.

41. Jaffer F, Fawcett K, Sims D, Heger A, Houlden H, Hanna MG, et al. Familial childhood-onset progressive cerebellar syndrome associated with the ATP1A3 mutation. Neurol Genet 2017:3:e145.

42. *Pisciotta L, Gherzi M, Stagnaro M, Calevo MG, Giannotta M, Vavassori MR, et al. Alternating Hemiplegia of Childhood: Pharmacological treatment of 30 Italian patients. Brain Dev 2017:39:521-28.

This is the first paper systematically assessing the available evidence about the efficacy and outcome of different treatments used in Alternating Hemiplegia of Childhood due to *ATP1A3* mutations and is relevant for clinicians dealing with this rare disorder.

43. Deutschlander A, Asmus F, Gasser T, Steude U, Botzel K. Sporadic rapid-onset dystonia-parkinsonism syndrome: failure of bilateral pallidal stimulation. Mov Disord 2005:20:254-57.

44. *Alkufri F, Shaag A, Abu-Libdeh B, Elpeleg O. Deleterious mutation in GPR88 is associated with chorea, speech delay, and learning disabilities. Neurol Genet 2016:2:e64.

A combination of marked developmental and speech delay, intellectual disability and chorea is described in association with a homozygous mutation in *GPR88*, an orphan G protein-coupled receptor selectively expressed in striatal medium spiny neurons.

45. Massart R, Guilloux JP, Mignon V, Sokoloff P, Diaz J. Striatal GPR88 expression is confined to the whole projection neuron population and is regulated by dopaminergic and glutamatergic afferents. Eur J Neurosci 2009:30:397-414.

46. Quintana A, Sanz E, Wang W, Storey GP, Güler AD, Wanat MJ, et al. Lack of GPR88 enhances medium spiny neuron activity and alters motor- and cue-dependent behaviors. Nature Neurosci 2012:15:1547-55.

47. Regad T, Roth M, Bredenkamp N, Illing N, Papalopulu N. The neural progenitor-specifying activity of FoxG1 is antagonistically regulated by CKI and FGF. Nat Cell Biol 2007:9:531-40.

48. Brancaccio M, Pivetta C, Granzotto M, Filippis C, Mallamaci A. Emx2 and Foxg1 inhibit gliogenesis and promote neuronogenesis. Stem Cells 2010:28:1206-18.

49. Ariani F, Hayek G, Rondinella D, Artuso R, Mencarelli MA, Spanhol-Rosseto A, et al. FOXG1 is responsible for the congenital variant of Rett syndrome. Am J Hum Genet 2008:83:89-93.

50. Cellini E, Vignoli A, Pisano T, Falchi M, Molinaro A, Accorsi P, et al. The hyperkinetic movement disorder of FOXG1-related epileptic-dyskinetic encephalopathy. Dev Med Child Neurol 2016:58:93-7.

51. **Papandreou A, Schneider RB, Augustine EF, Ng J, Mankad K, Meyer E, et al. Delineation of the movement disorders associated with FOXG1 mutations. Neurology 2016:86:1794-800.

In this series of 28 patients, the authors highlight for the first time that hyperkinetic movement disorders are a cardinal feature of *FOXG1*-related phenotypes and consist mostly of a combination of chorea, dystonia and myoclonus.

52. Mitter D, Pringsheim M, Kaulisch M, Plümacher KS, Schröder S, Warthemann R, et al.

FOXG1 syndrome: genotype-phenotype association in 83 patients with FOXG1 variants. Genet Med 2017, *in press*.

53. Kobayashi Y, Tohyama J, Kato M, Akasaka N, Magara S, Kawashima H, et al. High prevalence of genetic alterations in early-onset epileptic encephalopathies associated with infantile movement disorders. Brain Dev 2016:38:285-92.

54. Guerrini R, Moro F, Kato M, Barkovich AJ, Shiihara T, McShane MA, et al. Expansion of the first polyA tract of ARX causes infantile spasms and status dystonicus. Neurology 2007:69:427-33.

55. Poirier K, Eisermann M, Caubel I, Kaminska A, Peudonnier S, Boddaert N, et al. Combination of infantile spasms, non-epileptic seizures and complex movement disorder: a new case of ARX-related epilepsy. Epilepsy Res 2008:80:224-8.

56. Absoud M, Parr JR, Halliday D, Pretorius P, Zaiwalla Z, Jayawant S. A novel ARX phenotype: rapid neurodegeneration with Ohtahara

syndrome and a dyskinetic movement disorder. Dev Med Child Neurol 2010:52:305-7.

57. Deprez L, Weckhuysen S, Holmgren P, Suls A, Van Dyck T, Goossens D, et al. Clinical spectrum of early-onset epileptic encephalopathies associated with STXBP1 mutations. Neurology 2010:75:1159-65.

58. Baker K, Gordon SL, Grozeva D, van Kogelenberg M, Roberts NY, Pike M, et al. Identification of a human synaptotagmin-1 mutation that perturbs synaptic vesicle cycling. J Clin Invest 2015:125:1670-78.

59. Lipstein N, Verhoeven-Duif NM, Michelassi FE, Calloway N, van Hasselt PM, Pienkowska K, et al. Synaptic UNC13A protein variant causes increased neurotransmission and dyskinetic movement disorder. J Clin Invest 2017:127:1005-18.

60. Nakamura K, Kodera H, Akita T, Shiina M, Kato M, Hoshino H, et al. De Novo mutations in GNAO1, encoding a $G_{\alpha o}$ subunit of heterotrimeric G proteins, cause epileptic encephalopathy. Am J Hum Genet 2013:93:496-505.

61. Ohtahara S, Yamatogi Y. Ohtahara syndrome: with special reference to its developmental aspects for differentiating from early myoclonic encephalopathy. Epilepsy Res 2006:70:S58-S67.

62. **Danti FR, Galosi S, Romani M, Montomoli M, Carss KJ, Raymond FL, et al. GNAO1 encephalopathy: Broadening the phenotype and evaluating treatment and outcome. Neurol Genet 2017:3:e143.

The authors of this paper report the clinical and genetic features of 7 novel and 20 previously reported *GNAO1* mutation carriers underlying that episodic, often long-lasting exacerbations of

movement disorders are an important diagnostic clue, and individuating two mutational hot spots of GNAO1 (Arg209 and Glu246).

63. Ananth AL, Robichaux-Viehoever A, Kim YM, Hanson-Kahn A, Cox R, Enns GM, et al. Clinical course of six children with GNAO1 mutations causing a severe and distinctive movement disorder. Pediatr Neurol 2016:59:81-4.

64. *Saitsu H, Fukai R, Ben-Zeev B, Sakai Y, Mimaki M, Okamoto N, et al. Phenotypic spectrum of GNAO1 variants: epileptic encephalopathy to involuntary movements with severe developmental delay. Eur J Hum Genet 2016:24:129-34.

A paper providing a characterization of the heterogeneous and complex clinical expression of *GNAO1* mutations, blurring the boundaries between epilepsy and hyperkinetic movement disorders.

65. Bruun TUJ, DesRoches CL, Wilson D, Chau V, Nakagawa T, Yamasaki M, et al. Prospective cohort study for identification of underlying genetic causes in neonatal encephalopathy using whole-exome sequencing. Genet Med 2017, *in press*.

66. Waak M, Mohammad SS, Coman D, Sinclair K, Copeland L, Silburn P, et al. GNAO1-related movement disorder with life-threatening exacerbations: movement phenomenology and response to DBS. J Neurol Neurosurg Psychiatry 2017, *in press*.

67. Schorling DC, Dietel T, Evers C, Hinderhofer K, Korinthenberg R, Ezzo D, et al. Expanding Phenotype of De Novo Mutations in GNAO1: Four New Cases and Review of Literature. Neuropediatrics 2017, *in press*.

68. Arya R, Spaeth C, Gilbert DL, Leach JL, Holland KD. GNAO1-associated epileptic encephalopathy and movement disorders: c.607G>A variant represents a probable mutation hotspot with a distinct phenotype. Epileptic Disord 2017:19:67-75.

69. Sakamoto S, Monden Y, Fukai R, Miyake N, Saito H, Miyauchi A, et al. A case of severe movement disorder with GNAO1 mutation responsive to topiramate. Brain Dev 2017:39:439-43.

70. **Feng H, Sjögren B, Karaj B, Shaw V, Gezer A, Neubig RR. Movement disorder in GNAO1 encephalopathy associated with gain-of-function mutations. Neurology 2017:89:762-70.

A paper providing for the first time evidence of the molecular mechanisms underlying the wide spectrum of clinical manifestations of *GNAO1* mutations. By studying in vitro the impact of human mutant alleles on $G_{\alpha 0}$ synthesis, Feng and colleagues suggest that loss-of-function *GNAO1* mutations are associated with epileptic encephalopathy (Ohtahara syndrome), whereas gain-of-function or normally-functioning mutants are responsible for hyperkinetic movement disorder without epilepsy.

71. Kulkarni N, Tang S, Bhardwaj R, Bernes S, Grebe TA. Progressive movement disorder in brothers carrying a GNAO1 mutation responsive to deep brain stimulation. J Child Neurol 2016:31:211-214.

72. Yilmaz S, Turhan T, Ceylaner S, Gökben S, Tekgul H, Serdaroglu G. Excellent response to deep brain stimulation in a young girl with GNAO1-related progressive choreoathetosis. Childs Nerv Syst 2016:32:1567-68.

73. Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, et al. Glutamate receptor ion channels: structure, regulation, and function. Pharmacol Rev 2010:62:405-96.

74. Lau CG, Zukin RS. NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. Nat Rev Neurosci 2007:8:413-26.

75. Hamdan FF, Gauthier J, Araki Y, Lin DT, Yoshizawa Y, Higashi K, et al. Excess of de novo deleterious mutations in genes associated with glutamatergic systems in nonsyndromic intellectual disability. Am J Hum Genet 2011:88:306-16.

76. Ohba C, Shiina M, Tohyama J, Haginoya K, Lerman-Sagie T, Okamoto N, et al. GRIN1 mutations cause encephalopathy with infantile-onset epilepsy, and hyperkinetic and stereotyped movement disorders. Epilepsia 2015:56:841-48.

77. *Lemke JR, Geider K, Helbig KL, Heyne HO, Schütz H, Hentschel J, et al. Delineating the GRIN1 phenotypic spectrum: A distinct genetic NMDA receptor encephalopathy. Neurology 2016:86:2171-78.

A paper delineating the phenotypic spectrum of *de novo* and biallelic mutations in *GRIN1* in 23 patients (both novel and previously reported). The authors indicate profound intellectual disability, a mixed dystonic-dyskinetic movement disorder and oculogyric crises as characteristic phenotypic features. They also characterize functional consequences of *GRIN1* mutations demonstrating that an altered activity of the GluN1 subunit, encoded by *GRIN1*, leads to a loss of normal NMDA receptor function and represents the underlying pathogenic molecular mechanism in affected patients.

78. Rossi M, Chatron N, Labalme A, Ville D, Carneiro M, Edery P, et al. Novel homozygous missense variant of GRIN1 in two sibs with intellectual disability and autistic features without epilepsy. Eur J Hum Genet 2017:25:376-80.
79. Chen W, Shieh C, Swanger SA, Tankovic A, Au M, McGuire M, et al. GRIN1 mutation associated with intellectual disability alters NMDA receptor trafficking and function. J Hum Genet 2017:62:589-97.
80. Zehavi Y, Mandel H, Zehavi A, Rashid MA, Straussberg R, Jabur B, et al. De novo GRIN1 mutations: An emerging cause of severe early infantile encephalopathy. Eur J Med Genet 2017:60:317-20.
81. **Madeo M, Stewart M, Sun Y, Sahir N, Wiethoff S, Chandrasekar I, et al. Loss-of-Function Mutations in FRRS1L Lead to an Epileptic-Dyskinetic Encephalopathy. Am J Hum Genet 2016:98:1249-55.
The authors individuate biallelic mutations in *FRRS1L* as the cause of an epileptic-dyskinetic encephalopathy characterized by initially normal psychomotor development followed by a phase of regression, intractable epilepsy and prominent choreo-athetosis. Chronic alteration of GABAergic neurotransmission in the brain is proposed as the pathogenetic mechanism giving rise to this complex clinical entity.
82. Shaheen R, Al Tala S, Ewida N, Abouelhoda M, Alkuraya FS. Epileptic encephalopathy with continuous spike-and-wave during sleep maps to a homozygous truncating mutation in AMPA receptor component FRRS1L. Clin Genet 2016:90:282-83.
83. Campeau PM, Kasperaviciute D, Lu JT, Burrage LC, Kim C, Hori M, et al. The genetic basis of DOORS syndrome: an exome-sequencing study. Lancet Neurol 2014:13:44-58.

84. *Balestrini S, Milh M, Castiglioni C, Lüthy K, Finelli MJ, Verstreken P, et al. TBC1D24 genotype-phenotype correlation: Epilepsies and other neurologic features. Neurology 2016:87:77-85.

This paper provides a comprehensive review of 48 patients carrying mutations in *TBC1D24*. Detailed EEG findings, neuroimaging, developmental and cognitive features, treatment responsiveness are analysed, delineating the clinical phenotype associated with mutations in this gene.

85. Banuelos E, Ramsey K, Belnap N, Krishnan M, Balak C, Szelinger S, et al. Case Report: Novel mutations in TBC1D24 are associated with autosomal dominant tonic-clonic and myoclonic epilepsy and recessive Parkinsonism, psychosis, and intellectual disability. F1000Res. 2017:6:553.

86. Doummar D, Mignot C, Apartis E, Villard L, Rodriguez D, Chantot-Bastauraud S, et al. A Novel Homozygous TBC1D24 Mutation Causing Multifocal Myoclonus With Cerebellar Involvement. Mov Disord 2015:30:1431-32.

Gene	Main associated phenotype	Gene product	Inheritance	Age of onset	Diagnostic clues
ADCY5	*ADCY5*-related chorea	Adenylate cyclase 5	AD/de novo	Infancy to childhood	Axial hypotonia and delayed milestones Diurnal and sleep-related MD exacerbations Dystonia and myoclonus prominent in some cases
PDE10A	*PDE10A*-related chorea	Phosphodiesterase 10A	De novo/AD/AR	Infancy to childhood	Delayed motor-language milestones and dysarthria in recessive cases MRI: symmetrical T2-hyperintense bilateral striatal lesions in cases with heterozygous *de novo* mutations
FOXG1	Congenital Rett disease	Forkhead Box G1	De novo	Infancy to early childhood	Severe ID, absent language, acquired microcephaly MRI: corpus callosum aplasia/hypoplasia, delayed myelination, simplified gyration
ARX	Early infantile epileptic encephalopathy-type 1; X-linked mental retardation	Aristaless-related homeobox protein	XL	Infancy	Ohtahara/West syndrome, severe mental retardation, generalized dystonia/dyskinesias with recurrent status dystonicus
STXBP1	Early infantile epileptic encephalopathy-type 4	Syntaxin-Binding Protein 1	De novo	Early infancy to childhood	Onset of seizures within one year of age. Developmental delay, ID, autistic-like features, ataxia with or without dyskinesias/dystonia
SYT1	Severe motor delay and intellectual disability	Synaptogamin-1	De novo	Infancy	Severely delayed motor development without seizures
UNC13A	Congenital encephalopathy with dyskinesias	Unc-13 homolog A	De novo	Congenital	Developmental and speech delay; ID, congenital dyskinesias with intention tremor, rare febrile seizures

GNAO1	Early infantile epileptic encephalopathy type 17/Ohtahara syndrome	Gαo subunit of GPCR	De novo	Infancy to childhood	Developmental delay and ID Long-lasting MD exacerbations not related to sleep Epilepsy can be absent or well controlled
GRIN1	Mental retardation, autosomal dominant 8	GluN1 subunit of NMDAR	De novo/AR	Infancy	Severe developmental delay and ID Early-onset epileptic seizures Oculogyric crises Cortical blindness, dysmorphic traits, microcephaly
FRRS1L	Early infantile epileptic encephalopathy-type 37	Ferric Chelate Reductase 1-like	AR	Infancy	Psychomotor regression after normal development Severe encephalopathic epilepsy Choreo-athetosis in infancy/childhood, parkinsonism in adolescence
TBC1D24	Early infantile epileptic encephalopathy type 16	TBC1 domain family, member 24	AR	Infancy	Early-onset myoclonic seizures Variable degrees of ID Dystonia
GPR88	*GPR88*-related chorea	G protein-coupled receptor 88	AR	Infancy to childhood	Developmental and language delay Severe mental retardation Scarcely progressive chorea
KMT2B	DYT28 dystonia	lysine-specific histone methyltransferase 2B	De novo/AD	Childhood-adolescence	Onset in lower limbs and prominent oro-mandibular/laryngeal involvement Mild dysmorphic traits; mild ID Good and sustained response to pallidal DBS
ATP1A3	AHC RDP CAPOS syndrome	Na^+/K^+ ATPase, α3 subunit	De novo/AD	Infancy to fifth decade	Abrupt onset of neurological signs (dystonia, muscular weakness, ataxia) Initial hemisomatic distribution Identifiable triggering factors

Table 1. Synopsis of the most relevant genes associated with complex hyperkinetic movement disorders. **MD**: movement disorders; **GPCR**: Guanine nucleotide-binding protein-coupled receptors; **NMDAR**: Glutamatergic N-methyl-D aspartate receptors; **ID**: intellectual disability;

AHC: alternating hemiplegia of childhood; **RDP**: rapid-onset dystonia parkinsonism; **CAPOS**: cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss; **DBS**: Deep Brain Stimulation.

Chapter 12

SUMMARY

During the DIMET PhD course, my activity as a clinician has been mainly focused on pediatric movement disorders. As a Medical Doctor trained in Neurology, the activities I carried out allowed me to broaden my knowledge in the field of movement disorders, that represents my main topic of interest. In terms of clinical duties, I attended two movement disorders clinics a week in the Department of Pediatric Neurosciences of Carlo Besta Neurological Institute, which is a third-level internationally renewed Institution entirely dedicated to diseases of the central and peripheral nervous system.

In parallel, I worked in the Molecular Neurogenetics Unit of Besta Institute, where I contributed to the interpretation of the results of genetic analyses (NGS) carried out by neurogeneticists for diagnostic purposes, establishing an active link between clinicians and the lab with the aim of formulating definitive diagnosis in patients affected by rare movement disorders.

Movement disorders, especially dystonia, are rare diseases, and childhood-onset cases often have a genetic etiology. The creation of national and international networks bringing together basic scientists and clinicians is of paramount importance in the field of rare diseases, and I personally contributed to establish new international collaborations with the United Kingdom (UCL Institute of Neurology, London) and with the Unites States (Northwestern University, Chicago). This allowed our group to study by means of WES or WGS many patients with childhood-onset movement disorders followed in

our Institute in whom an underlying genetic etiology was suspected, yet not identified despite extensive investigations. Most importantly, we contributed with our cohort of patients to international studies that led to the discovery of new genes causing movement disorders. Moreover, through these collaborations, we were able to screen our patients for mutations in newly-discovered genes, individuating positive subjects and thus increasing the number of cases reported worldwide and widening the associated phenotypic spectrum. The results of the most recent screening (*KMT2B* gene) in our genetically-undefined cohort of dystonic patients have not been submitted yet, hence they have not been included in the present thesis.

The results achieved in our Institute and through international collaboration networks have been published in different papers, a selection of which is included in the previous chapters.

The most important results obtained through NGS analysis of single subjects, families or selected cohorts of patients consist in having individuated carriers of pathogenic mutations in genes associated with movement disorders in the last 5 years (*KCTD17*, *PDE10A*, *HPCA*, *ADCY5* and others), that have rapidly become available as part of the diagnostic offer of the Molecular Genetics Unit of the C. Besta Institute.

This thesis is meant to summarize the most relevant results obtained applying NGS and traditional Sanger sequencing in our lab and as part of international networks that we have actively contributed to create.

CONCLUSIONS AND FUTURE PERSPECTIVES

The opportunities provided by NGS in the past years have given rise to a new exciting era in the field of rare disease, including movement disorders.

However, limitations of NGS techniques must be taken into consideration both in clinical and research settings, and caution is needed when interpreting results. Erroneous interpretations of potentially pathogenic variants can in fact have dramatic consequences for patients and their families, especially in terms of genetic counselling. To minimize the attribution of pathogenicity to erroneously annotated variants, international guidelines and recommendations have been developed[1,2]. Still, variants of unknown significance (VUS) or variants the interpretation of which might change with the availability of novel evidence still represent a major issue in clinical practice. For this reason, patients and families must always been carefully informed about the possible pitfalls of NGS. These include, among others, technical limitations that can make it impossible to detect a pathogenic variant, because of its location within a region with low depth of coverage, copy number variants or long insertions/deletions, or aneuploidy.

When NGS provides negative results despite a strong clinical suspicion of a specific disease, further strategies need to be undertaken, including data reanalysis and use of alternative genetic (e.g. MLPA) or biochemical techniques to detect potentially missed variants. For such cases, a fruitful and tight collaboration between neurologists and diagnostic laboratories is mandatory to confidently rule out a specific

genetic diagnosis. Notably, the quality, accuracy and reproducibility of NGS data provided by different laboratories can be variable, despite substantial technical improvements achieved in the last years.

NGS has provided a major breakthrough in the understanding of the genetic bases of movement disorders. Hopefully, the proportion of patients affected by rare disease with no definite genetic diagnosis will progressively decrease in the near future with the availability of NGS in a larger number of diagnostic laboratories. The growing knowledge in this and other fields of medicine will lay -and is already laying - the bases for the development of experimental models, the understanding of pathophysiological mechanisms and ultimately the development of novel therapeutic strategies.

References

1. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-424.
2. MacArthur DG, Manolio TA, Dimmock DP, et al. Guidelines for investigating causality of sequence variants in human disease. Nature 2014;508:469-476.

Publications

1. Mencacci NE, Rubio-Agusti I, Zdebik A, Asmus F, Ludtmann MH, Ryten M, Plagnol V, Hauser AK, Bandres-Ciga S, Bettencourt C, Forabosco P, Hughes D, Soutar MM, Peall K, Morris HR, Trabzuni D, Tekman M, Stanescu HC, Kleta R, Carecchio M, Zorzi G, Nardocci N, Garavaglia B, Lohmann E, Weissbach A, Klein C, Hardy J, Pittman AM, Foltynie T, Abramov AY, Gasser T, Bhatia KP, Wood NW. **A missense mutation in KCTD17 causes autosomal dominant myoclonus-dystonia**. Am J Hum Genet. 2015;96:938-47.
2. Carecchio M, Schneider SA. **GTP cyclohydrolase 1 mutations and Parkinson's disease: New insights beyond DOPA-responsive dystonia**. Mov Disord. 2015;30:910
3. Mencacci NE, R'bibo L, Bandres-Ciga S, Carecchio M, Zorzi G, Nardocci N, Garavaglia B, Batla A, Bhatia KP, Pittman AM, Hardy J, Weissbach A, Klein C, Gasser T, Lohmann E, Wood NW. **The CACNA1B R1389H variant is not associated with myoclonus-dystonia in a large European multicentric cohort**. Hum Mol Genet. 2015;24:5326-9
4. Zorzi G, Carecchio M, Nardocci N. **Inherited isolated dystonia in children**. J Ped Neurol 2015, 13:174-179
5. Sainaghi PP, Bellan M, Lombino F, Alciato F, Carecchio M, Galimberti D, Fenoglio C, Scarpini E, Cantello R, Pirisi M, Comi C. **Growth Arrest Specific 6 Concentration is Increased in the Cerebrospinal Fluid of Patients with Alzheimer's Disease**. J Alzheimers Dis. 2017;55:59-65.

6. Corrado L, Magri S, Bagarotti A, Carecchio M, Piscosquito G, Pareyson D, Varrasi C, Vecchio D, Zonta A, Cantello R, Taroni F, D'Alfonso S. **A novel synonymous mutation in the MPZ gene causing an aberrant splicing pattern and Charcot-Marie-Tooth disease type 1b**. Neuromuscul Disord. 2016;26:516-20
7. Mencacci NE, Kamsteeg EJ, Nakashima K, R'Bibo L, Lynch DS, Balint B, Willemsen MA, Adams ME, Wiethoff S, Suzuki K, Davies CH, Ng J, Meyer E, Veneziano L, Giunti P, Hughes D, Raymond FL, Carecchio M, Zorzi G, Nardocci N, Barzaghi C, Garavaglia B, Salpietro V, Hardy J, Pittman AM, Houlden H, Kurian MA, Kimura H, Vissers LE, Wood NW, Bhatia KP. ***De Novo* Mutations in PDE10A Cause Childhood-Onset Chorea with Bilateral Striatal Lesions**. Am J Hum Genet. 2016;98:763-71
8. Mencacci NE, Carecchio M. **Recent advances in genetics of chorea**. Curr Opin Neurol. 2016;29:486-95
9. Carecchio M, Panteghini C, Reale C, Barzaghi C, Monti V, Romito L, Sasanelli F, Garavaglia B. **Novel GNAL mutation with intra-familial clinical heterogeneity: Expanding the phenotype**. Parkinsonism Relat Disord. 2016;23:66-71
10. Heywood WE, Galimberti D, Bliss E, Sirka E, Paterson RW, Magdalinou NK, Carecchio M, Reid E, Heslegrave A, Fenoglio C, Scarpini E, Schott JM, Fox NC, Hardy J, Bhatia K, Heales S, Sebire NJ, Zetterberg H, Mills K. **Identification of novel CSF biomarkers for neurodegeneration and their validation by a high-throughput multiplexed targeted proteomic assay**. Mol Neurodegener. 2015;10:64. Erratum in: Mol Neurodegener. 2016;11:20

11. Carecchio M, Reale C., Invernizzi F, Monti V, Petrucci S, Ginevrino M, Morgante F, Zorzi G, Zibordi F, Bentivoglio AR, Valente EM, Nardocci N, Garavaglia B. **DYT2 screening in early-onset isolated dystonia**. Eur J Ped Neurol 2017;21:269-271
12. Carecchio M, Mencacci NE, Iodice A, Pons R, Panteghini C, Zorzi G, Zibordi F, Bonakis A, Dinopoulos A, Jankovic J, Stefanis L, Bhatia KP, Monti V, R'Bibo L, Veneziano L, Garavaglia B, Fusco C, Wood N, Stamelou M, Nardocci N. **ADCY5-related movement disorders: Frequency, disease course and phenotypic variability in a cohort of paediatric patients**. Parkinsonism Relat Disord. 2017;41:37-43
13. Rodriguez-Porcel F, Espay AJ, Carecchio M. **Parkinson disease in Gaucher disease**. J Clin Mov Disord. 2017 May 23;4:7
14. Carecchio M, Picillo M, Valletta L, Elia AE, Haack TB, Cozzolino A, Vitale A, Garavaglia B, Iuso A, Bagella CF, Pappatà S, Barone P, Prokisch H, Romito L, Tiranti V. **Rare causes of early-onset dystonia-parkinsonism with cognitive impairment: a *de novo* PSEN-1 mutation**. Neurogenetics 2017;18:175-178.
15. Esposito S. Carecchio M, Tonduti D, Saletti V, Panteghini C. Chiapaprini L., Zorzi G, Pantaleoni C, Garavaglia B, Krainc D, Lubbe S, Nardocci N, Mencacci NE. **A PDE10A *de novo* mutation causes childhood-onset chorea with diurnal fluctuations**. Mov Disord 2017, 32:1646-1647
16. Carecchio M, Mencacci NE. **Emerging complex monogenic hyperkinetic movement disorders**. Current Neurol Neurosci Reports 2017;17(12):97
17. Mignarri A, Carecchio M, Del Puppo M, Magistrelli L, Di Bella D,

Monti L, Dotti MT. **SPG5 siblings with different phenotypes showing reduction of 27-hydroxycholesterol after simvastatin-ezetimibe treatment**. J Neurol Sci 2017, *in press*

18. Ramos EM*, Carecchio M*, Lemos R, Ferreira J, Legati A, Sears RL, Chan Hsu S, Panteghini C, Magistrelli L, Salsano E, Esposito S, Taroni F, Richard AC, Tranchant C, Anheim M, Ayrignac X, Goizet C, Vidailhet M, Maltete D, Wallon D, Frebourg T, the French PFBC study group, Pimentel L, Geschwind DH, Vanakker O, Galasko D, Fogel BL, A. M. Innes, Ross A, Dobyns WD, Alcantara D, O'Driscoll M, Hannequin D, Campion D, Oliveira JR, Garavaglia B, Coppola G, Nicolas G. **Primary brain calcification: an international study reporting novel mutations and phenotypes**, *submitted* (Eur J Med Gen). *shared first authors

Acknowledgements

When I will be thinking about my PhD in the future, I will probably not recall papers and database, but rather all those whom I had the privilege work with during the last three years of my life.
I wish to list and thank each of them thereafter, well aware that for most of them words will not be enough to fully express my gratitude.

Thanks to Dr. Valeria Tiranti, my PhD tutor, who has constantly supported me during the entire DIMET PhD course always being fully available in any circumstances.

Thanks to Dr. Barbara Garavaglia, Head of the Department of Neurogenetics of the C. Besta Neurological Institute, who has been much more than a simple boss, but a mother, a friend, a trustworthy advisor in some difficult moments of my life.

Thanks to Dr. Nardo Nardocci, Head of the Pediatric Neuroscience Department of the C. Besta Neurological Institute, a world-leading expert in pediatric movement disorders, whose unequal experience in the field literally amazed me. Thanks for being a mentor and teaching me dedication, affection for patients, patience, helpfulness, and for sincerely caring about my well-being.

Thanks to Dr. Giovanna Zorzi and Dr. Federica Zibordi, Consultants in Pediatric Neurology, whom I had the pleasure to closely work with, for transmitting their knowledge in the field to me, and, most importantly,

for being precious friends when I needed help and support, and some warm words of encouragement.

Thanks to the Movement Disorder lab team within the Neurogenetics Unit of the C. Besta Neurological Institute (Dr. Chiara Reale, Dr. Celeste Panteghini, Dr. Federica Invernizzi, Dr. Chiara Barzaghi, Dr. Valentina Monti), for making real all our clinical diagnoses. Clinicians would not formulate many diagnoses without you, girls.

Thanks to Dr. Niccolò E. Mencacci for the exciting opportunity to work together, despite living and working in different countries/continents. Our close collaboration has been a unique experience which I will treasure in my career.

As a Neurologist trained to examine adult and elderly patients, I faced a sweeping change in clinical practice during my PhD, being mainly focused on pediatric patients. The psychological impact that children's disability and sufferance had on me was huge, and difficult to bear on many occasions. However, nothing happens by chance in life. Our pediatric patients, and their families, were of considerable help in a difficult time of my existence, and prompted me to always look on the bright side of life, making me think every day about the privilege of being a healthy, free, highly educated woman, lucky enough to dedicate her life to the most exciting and developing field of Medicine: Neurology.

A special word of thanks goes to some special friends that I consider at all effects a gift of life: Barbara, Nicola, Silvia, Enza Maria, Laura, Claudio, Chiara and many others who were there in time of need.

This thesis is dedicated to you, to myself, and to all those who fight and never (ever!) give up.