Title: Review of the phenotype of early-onset generalised progressive dystonia due to mutations in *KMT2B*

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KTM2B; dystonia; microdeletions; genetic and inherited disorders

Abbreviations

CNV	Copy number variants
GPI-DBS	Globus pallidus interna-deep brain stimulation
ID	Intellectual disability
MLPA	Multiplex ligation-dependent probe amplification
MRI	Magnetic resonance imaging
NGS	Next generation sequencing
PEG	Percutaneous endoscopic gastrostomy
PPTV	Predicted protein-truncating variants
WES	Whole exome sequencing
WGS	Whole genome sequencing

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Highlights

- Microdeletions and intragenic variants in *KMT2B* are associated with early onset progressive dystonia, with predominant cervical and oromandibular involvement.
- GPI-DBS should be considered early in the disease course, as medical therapy is of limited benefit.
- Microarray should be included in first tier genetic testing in children and adults with dystonia.

Abstract

In 2016, two research groups independently identified microdeletions and pathogenic variants in the lysine-specific histone methyltransferase 2B gene, *KMT2B* in patients with early-onset progressive dystonia. *KMT2B*-dystonia (DYT28) is emerging as an important and frequent cause of childhood-onset progressive generalised dystonia and is estimated to potentially account for up to 10% of early-onset generalised dystonia. Herein, we review variants in *KMT2B* associated with dystonia, as well as the clinical phenotype, treatment and underlying disease mechanisms. Furthermore, in context of this newly identified condition, we summarise our approach to the genetic investigation of paediatric dystonia.

Introduction

Dystonia is a hyperkinetic movement disorder characterised by sustained or intermittent muscle contractions causing abnormal, often repetitive movements and postures affecting the limbs, trunk, neck and face. Dystonic movements are typically patterned, twisting, and may be tremulous, and they are often initiated or worsened by voluntary action and associated with overflow muscle activation.[1] Childhood-onset dystonia may be acquired or genetic in origin, and can occur in isolation or in association with other movement disorders, neurological or systemic manifestations.[1]

With the advent of next generation sequencing (NGS), new genetic causes of childhood-onset movement disorders have been identified, as well as phenotypic expansion of known dystonia genes.[2-4] Despite these advances, a significant number of children and adults remain without a genetic diagnosis. It is likely that gene discovery in dystonia is complicated by reduced penetrance and intrafamilial variability, which make the interpretation of new variants more challenging. The identification of a genetic diagnosis is key to optimising clinical care, as it enables informed genetic counselling, disease prognostication and targeted disease-specific treatments.

In 2016, two groups independently identified microdeletions and pathogenic variants in the lysine-specific histone methyltransferase 2B gene, *KMT2B* in patients with early onset progressive dystonia.[5,6] *KMT2B* (Chr. 19:35,717,817-35,738,879 , hg38, OMIM 606834*)* has a key role in gene expression and transcription activation. Though only recently reported, *KMT2B*-dystonia (DYT28) is emerging as an important and frequent cause of childhood-onset progressive generalised dystonia and may account for up to 10% of early-onset generalised dystonia.[2] Herein, we review variants in *KMT2B* associated with dystonia, as well as the clinical phenotype, treatment and underlying disease mechanisms. Furthermore, in context of this newly identified condition, we summarise our approach to the genetic investigation of paediatric dystonia.

Clinical characteristics

To date, 43 patients with *KMT2B* variants are published including cases with microdeletions encompassing the gene (n=14), as well as intragenic predicted protein-truncating variants (PPTV) (n=17) and nonsynonymous missense variants (n= 12).[5-9] The clinical phenotype is of an early onset progressive dystonia, which typically begins in the lower limbs. The dystonia becomes generalised over time (range 1-9 years, mean 4.4 years) with cervical (retrocollis

and torticollis), oromandibular (facial dystonia, and bulbar-oromandibular) and laryngeal (dysphonia and spasmodic laryngeal spasm) involvement. Bulbar features are often predominant and present in the majority; some patients develop disabling dysarthria progressing to anarthria as well as swallowing difficulties necessitating percutaneous endoscopic gastrostomy (PEG) tube for nutrition. Bulbar symptoms may be present at the onset of dystonia or develop over time. The clinical phenotype of previously described cases is summarised in **Table 1**.

Early phenotype-genotype correlation studies indicate that, chromosomal microdeletions and PPTV present at a statistically significant younger age, when compared to intragenic missense variants (mean age of 4.82 years compared to 11.75 years). In addition, patients with nonsynonymous variants have fewer co-existing systemic and neurological findings or pre-existing development delay (**Table 1**) when compared to those with microdeletions or PPTVs. Dysmorphic features of an elongated face, broad nasal base, bulbous nasal tip, fifth-finger clinodactyly or second and third syndactyly has been identified in some patients, and more frequently in those with microdeletions and PPTV. Other reported systemic features include preceding developmental delay (38%), intellectual disability (ID) (57%, mild to severe), microcephaly (21% of cohort, only reported in PPTV and microdeletions) and short stature (21%). Dermatological (cutis aplasia, abnormal scarring) systemic (renal and respiratory), ophthalmological (oculomotor apraxia, strabismus) and psychiatric symptoms are also reported in some individuals (**Table 1**). As with other dystonia genes, atypical phenotypes are reported including, dystonia presenting later in adulthood (Patient 26b[5], Patient 3[7]), paroxysmal cervical dystonia only (Patient 26a[5]), oromandibular dystonia with no lower limb dystonia (Patient 18[5]), or only dystonia of lower limbs (Patient 10[5]).

Neuroimaging features

Meyer and colleagues noted subtle, symmetrical hypointensity of the globus pallidi (especially the lateral aspect of the globus pallidus externa) on T2, diffusion and susceptibility weighted magnetic resonance imaging (MRI) images in 17 of 22 reviewed scans (**Figure 1**).[5] The significance of this hypointensity is unclear and may be an age-dependent finding. Patient age at the time of scan appears to influence MRI findings, as globus pallidus externa hypointensity was more prevalent in individuals who had neuroimaging performed at a younger age (average age of patients with abnormal imaging, 11.7 years; average age of patients with normal imaging 19 years) and in one patient was seen to diminish with increasing age.[5]

Treatment

Dystonia-specific medications and levodopa trials have had minimal or no clinical benefit in patients with *KMT2B*-dystonia. To date, 13 patients have had globus pallidus interna-deep brain stimulation (GPI-DBS, mean age of insertion 21.7 years, range 6-53 years) with a clinical response evident in all patients. In some patients after GPI-DBS, there was a remarkable improvement in motor function and return of independent ambulation.[5-7] Published data therefore suggests that GPI-DBS should be considered early in the disease course of *KMT2B*-dystonia.

KMT2B Variants reported to date

To date, 40 different variants are reported including heterozygous interstitial microdeletions (n=14), PPTV (frameshift, splice-site and stop-gain variants) (n= 15) and nonsynonymous variants of *KMT2B* (n=11) (**Table 1**).[5-9] There are no recurring variants or mutation hotspots, though described variants are frequently located in key protein domains, including the catalytic SET domain. The majority of the variants in *KMT2B* occurred *de novo,* but rarely autosomal dominant inheritance with reduced penetrance is reported. Symptomatic relatives (patient 26b[5], F4-II-4 and F4-I-3[6]) and asymptomatic carriers [mothers of patient 22 and 27[5], adult daughters (aged 32 and 34 years) of patient 3[7]] are described. Symptomatic parents appear to have a milder phenotype than their children, often with later onset dystonia and fewer systemic features. Incomplete penetrance is not unique to *KMT2B*-dystonia and reported in many other genetic dystonia (*DYT1, DYT25*).[2-4]

Disease Mechanisms in KMT2B dystonia

KMT2B encodes lysine methyltransferase 2B, specifically involved in the methylation of histone H3 at lysine 4 (H3K4). This is an important epigenetic regulator involved in gene expression and transcription activation, considered essential for normal development and to maintain proper neural function. The underlying mechanism of how *KMT2B* causes dystonia is not fully elucidated. KMT2B is ubiquitously expressed during brain development and in the adult brain, with the highest expression in areas of motor control and cerebellum.[5,13] *KMT2B* gene expression on qRT-PCR analysis was significantly reduced for microdeletion and PPTV variants compared to control fibroblasts.[5,6] Preliminary work has also shown that *KMT2B* variants are associated with reduced transcript levels of both *THAP1* and *TOR1A* in fibroblasts. It is proposed that variants in *KMT2B* affect the expression (including transcriptional stability and consistency) of a specific set of genes crucial to normal motor control.[5]

How to approach the investigation of a child with dystonia

The discovery of *KMT2B*-related disease further highlights the clinical need for a systematic approach to the genetic investigation of early-onset dystonia. Dystonia may occur in isolation or associated with other neurological and systemic features**.** Careful clinical history and detailed examination can aid diagnosis and direct further diagnostic testing.

Children with dystonia may often be labelled early in their disease course as having *"dyskinetic" or "dystonic cerebral palsy".* However, a number of inherited conditions can mimic cerebral palsy and should be suspected, especially in the presence of "clinical red flags" which are rarely seen in acquired forms of cerebral palsy. Indeed, absence of a perinatal hypoxic ischemic insult, similarly affected family members, normal MRI brain (or neuroimaging features atypical for acquired cerebral palsy) and a progressive disease course would all increase clinical suspicion of an underlying genetic or neurometabolic condition (**Yellow box, Figure 2**). If neuroimaging and first-line neurometabolic investigations are negative, then genetic testing is the next step in the diagnostic algorithm (**Figure 2**).

Chromosomal microarray should be considered as a first-line genetic evaluation in children and adults presenting with dystonia, especially in the presence of additional features such as dysmorphism or ID. In a single centre review of children and adults with movement disorders, 28% had a significant copy number variant (CNV) detected on routine diagnostic microarray.[11] Pathogenic microdeletions encompassing causative genes have been described in individuals with dystonia (*KMT2B*), myoclonus-dystonia (*SGCE*) and benign hereditary chorea (*TITF1*).[5,6,14,15] CNVs and are not usually detected on gene-panels or whole exome sequencing (WES), and pathogenic deletions and duplications could be potentially missed. However, over time, the increased availability of whole genome sequencing (WGS) will facilitate future CNV analysis.[16] Chromosomal microarray should therefore be performed in all individuals presenting with dystonia associated with additional features. CNVs should also be excluded in those with a very distinct phenotype (e.g. myoclonus-dystonia) when direct Sanger sequencing is negative, either via microarray or targeted gene multiplex ligation-dependent probe amplification (MLPA).

In recent years, **single gene testing** has been generally surpassed by **gene panels**, a cost-effective route by which to analyse multiple genes associated with a specific disorder. However, single gene testing may still have some clinical utility for distinct phenotypes (e.g. movement disorder with a low CSF:plasma glucose ratio – *SLC2A1*).[17] A number of diagnostic targeted next-generation gene panels are now available, which sequence genes

associated with movement disorders. Genes included in a gene panel will vary depending on the laboratory, and may not include newly identified dystonia genes. A retrospective review in a single tertiary centre estimated the diagnostic yield of a panel at 14.8% compared to 7.4% if the traditional route of consecutive single gene testing was undertaken.[18] Some diagnostic gene panels will also offer MLPA to identify CNVs.

At present, WES is becoming increasingly available in a clinical setting with a number of diagnostic laboratories undertaking clinical exomes for targeted gene analysis. The ability to interrogate WES data for recently identified genes is an added advantage, many of which are not always available immediately on established gene panels. However differential coverage of individual genes can be an issue.[19] Research WES and WGS has certainly aided the identification of new disease-causing dystonia genes, as well as expanding the genotype and phenotype of known dystonia genes.[2,4,9] Next generation sequencing should, therefore, be considered in patients with negative first tier investigations or atypical phenotypes. The diagnostic rate of WES in a single publication was 37.5%, with the yield likely to be higher in early onset generalised dystonia cohorts, where genetic dystonia is highly suspected.[20]

Conclusion

In conclusion, microdeletions and intragenic variants of *KMT2B* are an important cause of childhood-onset progressive dystonia. Clues to diagnosis include early onset dystonia (usually of the lower limbs) with generalisation, and predominant cervical, oromandibular and laryngeal involvement. Additional clinical features include neurological, dermatological, psychiatric and systemic features. The identification of *KMT2B* should prompt early referral for GPI-DBS. For childhood dystonia suspected to be genetic in origin, microarray should be considered as a first-tier genetic investigation before performing gene panels and diagnostic/research WES and WGS.

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Table 1: Summary of microdeletions and pathogenic variants reported to date

Pat +	*KMT2B* variant Inheritance	Age (y) Sex	Presenting dystonia and other features Age (y)	Current pattern of dystonia	ID	Dysmorphic features	Other features	Treatment-Benefit	DBS (age)-response	MRI pattern * Age
1[5] (1)	Deletion: chr.19:35,608,666 -36,233,508 *De novo*	14 M	RLL R foot posturing Gait disturbance 4	BiLL, BiUL Oromandibular (dysarthria, swallowing difficulties) Laryngeal (dysphonia)	Mild	Elongated face	NR	L-dopa trial - no benefit	No	Yes 9y5m
2[5] (2)	Deletion: chr.19:35,197,252 –38,140,100 *De novo*	14 F	BiLL Limping Gait disturbance 7	BiLL, BiUL Oromandibular (dysarthria, drooling) Laryngeal (dysphonia)	No	Elongated face Bulbous nasal tip	NR	L-dopa trial - no benefit BLF – no benefit	No	Yes 13y1m
3[5] (3)	Deletion: chr.19:34,697,740 –37,084,510 *De novo*	9 M	RLL R foot posturing Gait disturbance 2.5	BiLL, BiUL Oromandibular (dysarthria, drooling, swallowing difficulties) Laryngeal (dysphonia)	Mod	Elongated face	Dermatological (cutis aplasia) Systemic (retinal dystrophy) Mild global DD	GBP - improvement in tone	No	Yes 10y
4[5] (4)	Deletion: chr.19:36,191,100 –36,376,860 *De novo*	11 F	LLL L toe walking Gait disturbance 4	BiLL, BiUL Oromandibular (dysarthria, drooling, swallowing difficulties) Laryngeal (dysphonia)	Very mild	Elongated face Bulbous nasal tip Broad nasal bridge	Psychiatric (prone to anxiety)	L-dopa trial - minimal benefit THP – minimal benefit	No	Yes 10y9m
5[5] (5)	Deletion: chr.19:31,725,360 –36,229,548 *De novo*	20 M	DD Gait disturbance Childhood-onset	BiLL, BiUL Oromandibular (nasal voice)	Mod	Sparse hair Blepharophimosis Absent eye lashes of lower eyelids Low-set rotated ear Epicanthic folds Large bifid tongue Micrognathia Teeth overcrowding Finger contractures 5th finger clinodactlyly	Dermatological (cutis aplasia) Neurological (microcephaly) Systemic (small echogenic kidneys, renal transplant at 17y) Global DD	None	No	NR
6[5] (6)	Deletion: chr.19:35,017,972 –36,307,788 *De novo*	10 F	RLL R foot inversion 2.5	BiLL, BiUL Cervical (torticollis, retrocollis) Oromandibular (dysarthria, then anarthria, jaw-opening dystonia, swallowing difficulties requiring PEG)	No	NR	Neurological (microcephaly)	L-dopa trial – no benefit THP- no benefit	Yes (7y)-clinical response	Yes 6y10m
7[5] (7)	Deletion: chr.19:35,414,997 –37,579,142 *De novo*	21 M	RLL R foot dragging Gait disturbance 7	BiLL, BiUL Oromandibular (dysarthria, swallowing difficulties) Laryngeal (dysphonia)	Mild	Elongated face	Neurological (absence seizure) Systemic (absent R testis) Mild global DD	L-dopa trial – no benefit BLF- no benefit	No	Yes 13y3m
8[5] (8)	Deletion: chr.19:35,414,997 – 37,579,142 *De novo*	17 F	RLL R foot posturing 4	BiLL, BiUL Cervical (torticollis) Oromandibular (drooling, dysarthria) Laryngeal (dysphonia)	Mild	Fifth finger clinodactlyly	Dermatological (ectodermal dysplasia)	L-dopa trial -no benefit	Yes (6y)-clinical response	Yes 10y7m Yes 12y
9[5] (9)	Deletion: chr.19:35,967,904 – 37,928,373 *De novo*	14 M	BiLL Gait disturbance 4	BiLL, BiUL Oromandibular (dysarthria) Laryngeal (dysphonia)	Mild	Elongated face	Neurological (strabismus) Systemic (cleft palate) Mild global DD	L-dopa trial-initial benefit, not sustained	Yes (14y)-clinical response	Yes 15y1m

Pat +	*KMT2B* variant Inheritance	Age (y) Sex	Presenting dystonia and other features Age (y)	Current pattern of dystonia	ID	Dysmorphic features	Other features	Treatment-Benefit	DBS (age)-response	MRI pattern * Age
10[5] (10)	Deletion: chr.19:35,794,775 – 38,765,822 *De novo*	7 F	BiLL Intermittent toe walking Gait disturbance 4	BiLL	Mod	NR	Neurological (strabismus) Systemic (short stature, bronchiectasis) Mild global DD	No	No	NR
11[6,10]	Deletion: chr.19:33,203,635 - 38,108,990 *De novo*	23 F	NR NR	Not specified	Yes	Long face High forehead Sparse eye lashes and eye brows Retrognathia, thin lips Clinodactlyly, long fingers, dysplastic nails Overlapping toes	Neurological (microcephaly) Dermatological (cutis aplasia) Systemic (IUGR, CHD, short stature) DD	NR	NR	NR
12[6,11]	Deletion: chr.19:40,300,506 – 40,925,348 *De novo*	NR NR	NR 4	Progressive dystonia Gait disturbance	Mild	NR	NR	NR	NR	No^
13[6,12]	Deletion: chr.19:34,916,872 - 36,661,836 *De novo*	14 M	BILL 4	Generalised Oromandibular Laryngeal (spasmodic dystonia with life threatening breathing episodes) Tremor Myoclonus	Sev	Narrow face Thick medially sparse eyebrows Hypertelorism Retrognathia. thin lips Low set ears with large lobules Clinodactyly of 5th fingers 2nd and 3rd finger syndactyly R hand Bi-syndactyly of toes	Neurological (microcephaly) Systemic (short stature, partial GH deficiency, low weight) DD	Intrathecal BLF-some response Botox– some response Pimozide-some response	NR	No^
14[6]	Deletion: chr. 19:35,414,997 - 37,579,142 *De novo*	NR F	NR NR	NR	NR	NR	Dermatological (fine hair)	NR	NR	NR
15[5] (11)	c.402dup p.Ser135Glnfs*23 *De novo*	25 F	RUL R hand cramps and posturing 6	BiLL, BiUL Oromandibular (tongue thrusting, anarthria, swallowing difficulties needing PEG) Laryngeal	No	Bulbous nasal tip	NR	L-dopa trial-no benefit	No	Yes 21y
16[5] (12)	c.1690C>T p.Arg564* *De novo*	6 F	BiLL Toe walking 4	BiLL, BiUL Oromandibular (dysarthria, swallowing difficulties)	Mod	Elongated face Bulbous nasal tip Short nasal root Hypertelorism Large mouth, full lower lip	Neurological (epilepsy) Speech delay	L-dopa trial-no benefit	No	NA 3y3m
17[5] (13)	c.3023_3027del p.Glu1009Glyfs*9 *De novo*	11 M	BiUL Tremor Difficulty with hand-writing 8	BiLL, BiUL Oromandibular (dysarthria) Laryngeal (dysphonia)	Mild difficulties with attention	Elongated face	Fine motor delay	L-dopa trial-no benefit	No	Yes 11y3m

Pat +	*KMT2B* variant Inheritance	Age (y) Sex	Presenting dystonia and other features Age (y)	Current pattern of dystonia	ID	Dysmorphic features	Other features	Treatment-Benefit	DBS (age)-response	MRI pattern * Age
18[5] (14)	c.3143_3149del p.Gly1048Glufs*132 *De novo*	18 M	BiUL Posturing of hands Myoclonic jerks 8	BILL, BiUL Oromandibular (dysarthria, swallowing difficulties requiring) Laryngeal (dysphonia)	No	Elongated face Bulbous nasal tip	NR	L-dopa trial-no benefit	No	Yes 10y7m
19[5] (15)	c.4545C>A p.Tyr1515* *De novo*	20 F	BiLL Toe walking Clumsy 2	BILL, BiUL Oromandibular (dystonia, dysarthria, swallowing difficulties requiring PEG) Laryngeal (dysphonia)	No	Elongated face Bulbous nasal tip	NR	L-dopa, THP, CLZ, BLF-moderate response	No	Yes 18y
20[5] (16)	c.4688del p.Ala1563Aspfs*83 *De novo*	6 F	BiLL Gait disturbance Increase falls 3	BiLL, BiUL Oromandibular (dysarthria) Laryngeal (dysphonia)	No	Elongated face	NR	L-dopa trial-no benefit THP-initial benefit, not sustained	No	Yes 4y9m Yes 5y9m
21[5] (17)	c.6515_6518delinCCCAA p.Val2172Alafs*11 *De novo*	17 M	BiLL Toe walking Gait disturbance 1	BiLL, BiUL Oromandibular (dysarthria, swallowing difficulties) Laryngeal (dysphonia)	No	Elongated face	Dermatological (phimosis)	L-dopa trial-no benefit TBZ-no benefit BLF and THP-minimal benefit	Yes (16y) Clinical response, independent walking	Yes 14y8m
22[5] (18)	c.8061del p.Tyr2688Thrfs*50 *De novo*	20 F	Clumsy movements Difficulties with speech articulation 1	Oromandibular (swallowing difficulties, dysarthria) Laryngeal (dysphonia)	Mild	Micrognathia Atrophic tongue Bulbous nasal tip 5th-finger clinodactyly	Delayed speech	None	No	No 20y
23[5] (19)	c.8079del p.Ile2694Serfs*44 *De novo*	28 M	Bi LL Toe walking, Severe speech delay 2	BiLL, BiUL Cervical (torticollis, laterocollis) Oromandibular (jaw-opening dystonia, tongue protrusion, anarthria, swallowing difficulties, PEG feeding)	No	No	Neurological (delay in saccade initiation, hypometric vertical saccades) Systemic (short stature) Psychiatric (ADHD)	L-dopa trial – no benefit TBZ and THP-reduced tongue protrusion	Yes (27y)-Clinical response	No 23y
24[8]	c.4966_4968TCCdel p.Ser1656del *De novo*	10 F	Microcephaly R hand dystonic tremor 4	BILL, BiUL, trunk Cervical Oromandibular (dysarthrophonia) Action induced myoclonus	Mild	No	Neurological (microcephaly) Other (scoliosis)	Anticholinergics-improved motor symptoms	No	No^
25[5] (20)	c.3528+2T>A Unknown	40 M	L LL Dragging L foot Gait disturbance Clumsiness 4	BiLL, BiUL Cervical (torticollis) Oromandibular (dysarthria) Laryngeal (dysphonia)	Mod	NR	NR	L-dopa trial-no benefit TBZ, THP, SUL-no benefit	Yes (32y)-Clinical response	No 34y
26[6] (F1-II-5)	c.6406delC p.Leu2136Serfs*17 *De novo*	31 F	Inversion of L foot 7	BILL, BiUL Cervical Oromandibular (tongue) Laryngeal	No	NR	NR	L-dopa– no benefit	Yes (23y)-Clinical response	No^

Pat +	*KMT2B* variant Inheritance	Age (y) Sex	Presenting dystonia and other features Age (y)	Current pattern of dystonia	ID	Dysmorphic features	Other features	Treatment-Benefit	DBS (age)-response	MRI pattern * Age
27[6] (F2-II-1)	c.1633C>T p.Arg545* *De novo*	11 F	BiLL 3	BiLL, BiUL	No	NR	Neurological (microcephaly and strabismus) Systemic (short stature, VUR) Moderate motor delay	L-dopa- no benefit Anticholinergics –NR	No	No^
29[6] (F4-III-2)	c.2482C>T p.Gln810* Paternal	6 F	L foot 4	BiLL Cervical	Mild	NR	Neurological (microcephaly, astigmatism) Systemic (short stature) Global DD	L-dopa- NR	No	No^
30[6] (F4-II-4)	c.2482C>T p.Gln810* Paternal	36 M	Hand clumsiness 9	Hand- writer cramp Forearm Oromandibular (dysarthria)	Mild	NR	Neurological (microcephaly, astigmatism) Systemic (short stature) Speech delay	No	No	NR
31[6] (F4-I-3)	c.2482C>T p.Gln810* Unknown	61 M	Hand clumsiness 11	Limb (writer-cramp) Forearm Oromandibular (dysarthria) R arm action induced tremor	Mild	NR	Speech delay	No	No	NR
32[5] (21)	c.4955G>A p.Gly1652Asp *De novo*	18 M	RLL R leg posturing 6	BiLL, BiUL Oromandibular (dysarthria, swallowing difficulties) Laryngeal (dysphonia)	Mild	Elongated face	Systemic (short stature) Dermatological (hypertrichosis)	L-dopa trial- no benefit THP- not tolerated	Yes (15y)-Clinical response	NA 10y5m 15y3m
33[5] (22)	c.4986C>A p.Phe1662Leu Maternal	20 F	RLL R foot posturing Abnormal gait 5	BiLL, BiUL Cervical (torticollis) Oromandibular (dysarthria, swallowing difficulties) Laryngeal (dysphonia)	No	Elongated face Bulbous nasal tip	NR	L-dopa trial- no benefit THP- mild benefit BTX neck- no functional benefit	Yes (20y)-Clinical response Independent walking	Yes 13y1m Yes 15y3m
34[5] (23)	c.5114G>A p.Arg1705Gln *De novo*	8 M	BiLL Toe walking 3	BiLL, BiUL Cervical (torticollis) Oromandibular (dysarthria)	Mild-mod	Elongated face Bulbous nasal tip Broad philtrum Up-slanted eyes Low-set ears Periorbital fullness Gap between front teeth	Neurological (BILL spasticity) Dermatological (ichthyoid lesion, Systemic (episodic vomiting) Global DD	L-dopa trial- no benefit CLZ, THP, IT BLF- some benefit	Yes (7y)-Clinical response	Yes 6y6m Yes 6y7m
35[5] (24)	c.5284C>T p.Arg1762Cys *De novo*	27 F	LLL Tip-toe walking In-turning L toe 6	BiLL, BiUL Oromandibular (dysarthria, anarthria, reduced tongue movement)	No	No	Neurological (oculomotor apraxia with difficulty initiating saccades) Systemic (short stature)	L-dopa trial- no benefit THP- no benefit	No	No 20y No 27y

Pat +	*KMT2B* variant Inheritance	Age (y) Sex	Presenting dystonia and other features Age (y)	Current pattern of dystonia	ID	Dysmorphic features	Other features	Treatment-Benefit	DBS (age)-response	MRI pattern * Age
36[5] (25)	c.5342T>C p.Leu1781Pro *De novo*	19 F	RLL R foot posturing Gait disturbance 8	BiLL, BiUL Cervical (torticollis) Oromandibular (dysarthria, swallowing difficulties Laryngeal (dysphonia)	No	Elongated face Bulbous nasal tip	NR	L-dopa trial – no benefit LVT – mild benefit	Yes (19y) Clinical response Improved walking	Yes 16y
37[5] (26a)	c.7549C>T p.Arg2517Trp Maternal	8 M	Delayed motor and speech development 8	Cervical (severe paroxysmal retrocollis) Oromandibular (jaw dystonia)	No	Bulbous nasal tip	Psychiatric (ADHD)	NR	No	No 15m No 8y
38[5] (26b)	c.7549C>T p.Arg2517Trp *De novo*	46 F	BiUL UL posturing Torticollis Inability to walk long distances or run 23	BiLL, BiUL Cervical (torticollis) Laryngeal (dysphonia)	No	Bulbous nasal tip	Neurological (Idiopathic intracranial hypertension)	No	No	
39[5] (27)	c.8021T>C p.Ile2674Thr Maternal	19 F	RUL Posturing Tremor Poor handwriting Myoclonic jerks 9	BILL, BiUL Oromandibular Laryngeal (dysphonia)	Mild	Bulbous nasal tip	Psychiatric (anxiety, self-harm, OCD)	L-dopa trial-no benefit THP-no benefit LVT-no benefit CBZ-initial benefit, not sustained CLZ-not tolerated	No	NA 10y10
40[7] (2)	c.3700G>A p.Glu1234Lys *De novo*	21 M	L LL dystonia-exercise induced 17	BiLL, BiUL Cervical Oromandibular (dysarthria) Laryngeal (spasmodic dysphonia)	No	NR	No	L-dopa trial – no benefit Anticholinergics-partial benefit	No	No^
41[7] (3)	c.4622C>T p.Ala1541Val^ Unknown	60 M	Generalised 43	BiUL Cervical Oromandibular (dysarthria) Laryngeal (dysphonia) Tremor	No	NR	No	L-dopa trial	Yes (53y)-clinical response	No^
42[7] (4)	c.5336G>A p.Arg1779Gln^ Unknown	52 F	LL posturing 7	BiLL, BiUL, trunk Cervical (retro-torticollis) Oromandibular (lingual, dysarthria)	No	NR		L-dopa trial	Yes (43y)-clinical response	No^
43[9] (7)	c.4847C>T p.Ala1616Val *De novo*	13 F	Trunk 6	BiLL, BiUL, trunk Cervical	Mod	Bulbous nasal tip	Neurological (strabismus) Other (SNHL)	NR	NR	No^

^, Not analyzed by Meyer and Colleagues; +, number in brackets refers to patient number in original paper; *, typical pattern of bilateral subtle hypointensity of the globus pallidi (especially the lateral aspect of the globus pallidus externa) on T2, diffusion and susceptibility weighted MRI images; BiLL, Bilateral lower limbs; BiUL, Bilateral upper limbs; BLF, baclofen; BTX, botulinum toxin; CLZ, clonazepam; CHD, congenital heart disease; CLZ, clonazepam; DD, developmental delay; F, female; GD, generalised dystonia; GBP, gabapentin; IT, intrathecal; IUGR, intra-uterine growth retardation; L, left; LL, lower limb; LVT, levetiracetam; M, male; m, months; mod, moderate; NA, not analysed; NR, not recorded; Pat, Patient; PEG, percutaneous endoscopic gastrostomy; Sev, severe; SNHL, sensorineural hearing loss; SUL, sulpiride; R, right; TBZ, tetrabenazine; THP, trihexyphenidyl; UL, upper limbs; y, years

Legend

Figure 1: Radiological features of KMT2B variants.
MR imaging (Patient1, age 9 years, 5 months) T2-weighted(A), echo-planar technique diffusion image with b value of zero(B) and susceptibility-weighted sequences(C). Abnormal findings are indicated by yellow arrows with evidence of bilateral subtle hypointensity of the globus pallidus with hypointense lateral streak of globus pallidus externa.
Figure is modified with permission from Meyer et al., 2017. Mutations in the histone methyltransferase gene KMT2B cause complex early-onset dystonia. *Nat Genet* 2017;**49**(2):223–37.

Figure 2 Diagnostic algorithm for the investigation of a child presenting with dystonia
*Neurometabolic investigations of dystonia
Blood: Amino acids, lactate, creatine kinase, biotinidase, renal and liver profile, glucose, copper, caeruloplasmin, urate, thyroid function tests, ammonia, acylcarnitine profile
Urine: Organic acids, copper, guanidinoacetate
CSF: Neurotransmitters, glucose and lactate (paired with serum samples)
^Sheffield Dystonia and Parkinson Panel (28 genes)
AFG3L2, ANO3, ATP1A3, ATP7B, CYP27A1, FA2H, FTL, GBA, GCH1, GNAL, LRRK2, MAPT, PANK2, PARK2, PARK7, PINK1, PNKD, PRKCG, PRRT2, SGCE, SLC16A2, SLC2A1, SNCA, SPG11, SPR, TH, THAP1, WDR45
CNV, Copy number variants; EBV, Epstein Barr Virus; ID, Intellectual disability; MLPA, Multiplex ligation-dependent probe amplification; MRI, Magnetic resonance imaging; WES, Whole exome sequencing; WGS, Whole genome sequencing

Table 1: Table of KMT2B variants, dystonia phenotype, response to treatment and additional clinical features
^, Not analyzed by Meyer and Colleagues; +, number in brackets refers to number in original paper; *, typical pattern of bilateral subtle hypointensity of the globus pallidi (especially the lateral aspect of the globus pallidus externa) on T2, diffusion and susceptibility weighted MRI images; BiLL, bilateral lower limbs; BiUL, bilateral upper limbs; BLF, baclofen; BTX, botulinum toxin; CLZ, clonazepam; CHD, congenital heart disease; CLZ, clonazepam; F, female; GD, generalised dystonia; GBP, gabapentin; IT, intrathecal; IUGR, intra-uterine growth retardation; L, left; LL, lower limb; LVT, levetiracetam; M, male; m, months; mod; moderate, NA, not analysed; NR, not recorded; Pat, patient; PEG, percutaneous endoscopic gastrostomy; Sev, severe; SNHL, sensorineural hearing loss; SUL, sulpiride; R, right; TBZ, tetrabenazine; THP, trihexyphenidyl; UL, upper limbs; y, years

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