Update on *KMT2B*-related dystonia

Michael Zech, MD[1,2] Daniel D. Lam, PhD[1] Juliane Winkelmann, MD[1,2,3,4*]

Author Affiliations:
[1]Institut für Neurogenomik, Helmholtz Zentrum München, Munich, Germany
[2]Institut für Humangenetik, Klinikum rechts der Isar, Technische Universität München, Munich, Germany
[3]Lehrstuhl für Neurogenetik, Technische Universität München, Munich, Germany
[4]Munich Cluster for Systems Neurology, SyNergy, Munich, Germany

[*]address correspondence to:
Prof. Dr. Juliane Winkelmann, Institut für Neurogenomik, Helmholtz Zentrum München, Ingolstädter Landstraße 1, 85764 Munich-Neuherberg, Germany
Phone: +49 89 3187 1884, Email: juliane.winkelmann@tum.de

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Abstract

Purpose of review: To summarize the molecular and clinical findings of *KMT2B*-related dystonia (DYT-*KMT2B*), a newly-identified genetic dystonia syndrome.

Recent findings: Since first described in 2016, 66 different *KMT2B*-affecting variants, encompassing a set of frameshift, nonsense, splice-site, missense and deletion mutations, have been reported in 76 patients. Most mutations are de novo and expected to mediate epigenetic dysregulation by inducing *KMT2B* haploinsufficiency. DYT-*KMT2B* is characterized phenotypically by limb-onset childhood dystonia that tends to spread progressively, resulting in generalized dystonia with cranio-cervical involvement. Co-occuring signs such as intellectual disability are frequently observed. Sustained response to deep brain stimulation (DBS), including restoration of independent ambulation, is seen in 93% (27/29) of patients.

Summary: DYT-*KMT2B* is emerging as a prevalent monogenic dystonia. Childhood-onset dystonia presentations should prompt a search for *KMT2B* mutations, preferentially via next-generation-sequencing and genomic-array technologies, to enable specific counselling and treatment. Prospective multicenter studies are desirable to establish *KMT2B* mutational status as a DBS outcome predictor.

Introduction

Despite the fact that the concept of de novo mutation has been established as a key pathological mechanism for various early-onset neuropsychiatric disorders [1-3], it took until 2016 before our group published the results of a parent-affected child trio whole-exome sequencing analysis in the field of dystonia [4]. In an individual with severe childhood-onset generalized dystonia, our trio approach uncovered a de novo heterozygous protein-truncating variant (PTV) in *KMT2B* (OMIM606834). A follow-up

genetic screen in 30 generalized dystonia patients identified another three heterozygous *KMT2B*-disrupting PTVs (two de novo events, one co-segregating variant), confirming the causal relationship between *KMT2B* mutations and dystonia [4]. In parallel, Meyer et al. collected a cohort of 28 dystonia-affected individuals who harbored deleterious de novo or dominantly inherited PTVs and missense mutations in *KMT2B* as well as clinically relevant whole-gene deletions of *KMT2B* [5]. *KMT2B*, encoding a histone H3 lysine 4 (H3K4)-specific *N*-methyltransferase responsible for posttranslational modification of histones, plays an essential role in the regulation of human gene expression [6, 7]. Implicating the processes of chromatin remodeling and cell type-specific transcription activation in the control of movements, the identification of *KMT2B* mutations in dystonia has begun to offer fresh insights into the pathogenesis of the disease. Consistent with its ubiquitous expression [8, 9] and developmental importance [10, 11], *KMT2B* is linked to a more complex form of dystonia, in which dystonic symptoms are often accompanied by other neurological and/or non-neurological signs [4, 5]. Although the exact prevalence of *KMT2B*-related dystonia (DYT-*KMT2B*, also known as dystonia 28 or DYT28, OMIM617284) remains to be determined, the disorder is emerging as one of the most frequent genetically mediated dystonias. Dystonia patients who were found to carry pathogenic or likely pathogenic variants in *KMT2B* are being reported almost monthly and individuals with DYT-*KMT2B* have now been identified around the globe including Europe (Austria [4], Czech Republic [12, 13], Germany [4, 5, 14, 15], Italy [16], Netherlands [5], Portugal [17], Spain [18], Switzerland [19], UK [5]), North America (USA [5]), Central America (Mexico [20]), South America (Chile, unpublished results), Asia (Afghanistan [21], China [22-24], Japan [25]), and Australia [5]. Current frequency estimates range from 10% in generalized dystonia cohorts [4] to 22% [16] and 38% [5] in subgroups of

individuals with childhood-onset (progressive) dystonia. In this review, we give an overview of the genetic and clinical characteristics of all individuals with DYT-*KMT2B* who have been published to date (2016 December 1 – 2019 June 30). We provide an up-to-date summary of the mutational spectrum, stress the importance of accurate *KMT2B* variant interpretation, and describe the range of dystonic and non-dystonic disease manifestations. Our intent is that this exploration of the literature prompts clinicians to consider early genetic testing in individuals showing a phenotype consistent with DYT-*KMT2B* and inspires future research and prospective clinical trials in this molecularly distinct condition that appears to be amenable to effective therapeutic intervention.

Molecular genetic characteristics

Mutational spectrum

To date, 66 different rare heterozygous variants affecting the *KMT2B* locus in the chromosomal region 19q13.12 have been described (Fig. 1A and B). Of these, 79% are single-nucleotide variations (SNVs) and small insertions and deletions (indels, < 25 base pairs). By creating premature stop codons, a substantial fraction (52%) of the SNVs and small indels are expected to result in protein truncation (PTVs). The most frequent PTVs are frameshift alterations, followed by nonsense and splice-site mutations. Since first described by Meyer et al. [5], an association between *KMT2B* missense substitutions and dystonia is increasingly being recognized [12, 23, 16]. Whereas PTVs seem to show no specific clustering, the dystonia-related missense variants are overrepresented to a certain extent in conserved regions and functional domains of the protein (Fig. 1A and C). After review, we noted the existence of two apparent mutational hot spots for 11 missense variants between codons 1597 and

1662 (mapping to the PHD-like domain) and codons 1753 and 1781 (mapping to the FYR-N domain) (Fig. 1A). To further evaluate the residue-specific distribution and potential deleteriousness of the reported missense changes, we used the Missense Tolerance Ratio (MTR) Gene Viewer, an algorithm designed to estimate the extent of purifying selection that has been acting against missense variants in the general population [26]. As shown in Figure 1C, the MTR analysis revealed that 19 out of 23 missense variants occur in regions depleted of missense variation in population controls from the Exome Aggregation Consortium (ExAC) Browser [27], consistent with a high physiological importance of the sites involved [26]. In two patients, in-frame deletions (one single-amino acid [14] and one eight-amino acid [16] deletion) were identified, both of which also affect the variant-intolerant sequence of *KMT2B* (Fig. 1C). A total of 14 different dystonia-related gross deletions encompassing *KMT2B* have been cataloged in research articles [5, 28-31, 16] and the DECIPHER database [32] (Fig. 1A). The deletions range in size from 0.2 to 4.9 Mb and the smallest deletion contains only one gene in addition to *KMT2B* [5].

As yet, only a few *KMT2B* variations have been observed recurrently: a p.Lys553Glnfs*46 frameshift mutation [22, 25, 16] and a p.Arg1705Gln missense mutation [5, 16] were detected in three and two independent individuals respectively (Fig. 1A). Moreover, a 2.2 Mb-deletion (chr.19: 35,414,997-37,579,142) was seen once in DECIPHER [32] as well as in two unrelated patients from Meyer et al. [5], and a 1.96 Mb-deletion (chr.19: 35,967,904-37,928,373) in a further two apparently independent subjects [5, 28] (Fig. 1A). It is likely that the number of variant recurrences will increase when more DYT-*KMT2B* patients are identified over time. The majority of *KMT2B* variants were shown to have arisen de novo (70%), whereas the inheritance pattern could not be determined for 21% of the variants due to unavailability of parental

DNA. For 9% of the variants (all missense changes), transmission from a seemingly unaffected parent was described. There are only three examples of variants showing complete co-segregation with disease in autosomal-dominant pedigrees [4, 5, 22]. The scarcity of variant inheritance from affected individuals is likely to be explained by the clinical severity of DYT-*KMT2B*, resulting in compromised reproductive fitness.

Collectively, we found that most *KMT2B* variants discovered so far are de novo mutations and predicted to lead to the loss of one *KMT2B* copy or perturbation of certain invariant sequences of the protein.

Molecular mechanisms of pathogenicity

The predominance of PTVs and *KMT2B*-involving gross deletions seen among individuals with DYT-*KMT2B* (Fig. 1A and B) suggests *KMT2B* haploinsufficiency as a primary disease mechanism [4, 5]. In support of this hypothesis, three independent studies demonstrated that mRNA concentrations of *KMT2B* are reduced up to ~50% in PTV-bearing patient cells [4, 5, 21]. This reduction is likely to be the result of mRNA clearing via nonsense-mediated RNA decay, as shown recently by Kawarai et al. [25]. Dosage sensitivity has also been described for other genes in the lysine *N*-methyltransferase 2 (KMT2) family, and deletions or PTVs in five of these, *KMT2A* and *KMT2C-F*, also produce dominant neuropsychiatric phenotypes [33-37] (Fig. 1D). A causative role for haploinsufficiency in the *KMT2A-F*-related disorders is reflected by the fact that these genes are severely constrained for PTVs in the general population [27]. Constraint calculations in ExAC data reveal that each of the KMT2 group genes has a probability of being loss-of-function intolerant score of 1.0 (Fig. 1D), whereby scores of 1.0 define the most haploinsufficient genes in the human genome [27].

The pathomechanistic consequences of *KMT2B* mutations require further study. However, it is likely that aberrant brain transcriptional signatures arising as a result of epigenetic dysregulation contribute to the evolution of disease [4, 5]. *KMT2B* is strongly expressed during brain development and in the adult brain [10, 11], and especially high *KMT2B* mRNA levels are found in neural structures responsible for movements (e.g., the cerebellum) [5]. Although *Kmt2b*$^{+/-}$ heterozygous mice, the counterparts to *KMT2B* haploinsufficient human subjects, appear to be neurologically normal [38], Kerimoglu et al. demonstrated that dosage reduction of *Kmt2b* in mouse neurons leads to significantly impaired transcription of genes known to be implicated in dystonia [39]. In agreement, using patient-derived fibroblasts, different authors have shown that *KMT2B* mutations are associated with decreased expression levels of the dystonia-related genes *TOR1A* and *THAP1* [5, 21]. In light of these findings, it is intriguing to consider that the elucidation of *KMT2B*-sensitive transcriptomes could aid in the identification of novel dystonia-causing genes and pathways [40].

We conclude that, like in other Mendelian conditions linked to defects in the H3K4 methylation pathway, haploinsufficiency is a key pathological event in DYT-*KMT2B*. While in silico modeling has predicted some harmful effects for reported *KMT2B* missense and in-frame deletion variants [5, 14-16], it remains to be determined whether these mutations behave as “functional” null alleles or act through mechanisms other than haploinsufficiency. The knowledge that epigenetic deregulation of brain-specific transcription is involved in the genesis of dystonic movements paves the way for the development of novel research strategies in dystonia.

Variant detection methods and variant interpretation

More than 90% of the reported SNVs and indels were found by next-generation sequencing (NGS)-based methods such as panel sequencing, whole-exome or whole-genome sequencing. Only a handful of these variant types were detected primarily via conventional Sanger evaluation of the entire *KMT2B* coding sequence [5, 17]. The 19q13.12 deletions that included a whole-gene deletion of *KMT2B* were identified by chromosomal microarray analysis or, as described in two instances [31, 16], by NGS techniques. In the diagnostic workup of patients considered to have DYT-*KMT2B*, NGS strategies may be superior to targeted Sanger testing for the following reasons: (1) *KMT2B* is a large gene (37 exons spanning 8,469 kb), making Sanger sequencing expensive and time consuming; (2) in case a clinical suspicion of DYT-*KMT2B* cannot be confirmed genetically, NGS data allow for simultaneous interrogation of other dystonia-causing genes and may thus aid in reaching a diagnosis [41]; and (3) NGS with deep sequence coverage enables the assessment of mosaic mutations that would be unidentifiable by Sanger analysis [42]. Indeed, in our in-house DYT-*KMT2B* patient cohort, we observed the presence of a p.Thr176Aspfs*8 mutation [19] in 1 of 216 reads of NGS data from an affected subject`s mother (M. Wagner, personal communication). Indicative of low-level mosaicism, this result has important consequences for genetic counselling in that the recurrence risk to siblings in this family might be significantly higher than the empirical 1% estimate.

In the clinical diagnostic setting, it is important to interpret the medical relevance of identified *KMT2B* variants according to standardized protocols [12]. Under a haploinsufficiency paradigm, most PTVs and whole-gene deletions of *KMT2B* can be readily classified as pathogenic. By contrast, caution is needed in clinical interpretation of a missense variant whose precise impact on protein function remains unknown [12]. Criteria for considering a missense variant as pathogenic or likely pathogenic include

high deleteriousness prediction scores, the variant`s absence from controls, and a proven de novo status of the variant [12]. In accord with recommended guidelines [43], we deemed those published *KMT2B* missense variants to be variants of uncertain significance (VUS) that were (i) not tested for a de novo origin, (ii) inherited from a healthy parent, or (iii) present in population controls (Fig. 1A-C, Table 2). Regarding the latter two points, it should be taken into consideration that the appearance of missense variants in phenotypically unaffected individuals could be attributable to reduced disease penetrance [5, 12, 16]. Alternatively, such unaffected subjects might be mosaic carriers of the variants [44], as discussed by Meyer et al. [5]. Future studies are warranted to decisively answer the question of incomplete penetrance in DYT-*KMT2B* and improve our ability to address the disease relevance of *KMT2B* missense changes. We stress that the classification approach for missense variants applied in this review was very conservative [12, 43]. Based on the typical DYT-*KMT2B* phenotypes of the carriers, some of the missense changes that we cautiously classified as VUS were considered to be likely disease-causing in the original articles [5, 16].

Together, NGS and genomic array approaches enabling molecular testing at a high level of accuracy and cost-effectiveness should be preferentially used in diagnosing DYT-*KMT2B*. We recommend that pathogenicity assessment of *KMT2B* variants follows international sequence interpretation criteria.

Clinical characteristics

Dystonic features

In total, of the 76 *KMT2B*-mutated dystonia patients from 72 families published to date, 63 were described as having a generalized distribution of their symptoms (83%) (Tab.

1). Usually starting in the lower limbs, DYT-*KMT2B* tends to spread to the trunk and adjacent body parts within a few years (approximately 2-10 years). For 67 patients for whom data were available, the mean age at onset was 6.4 ± 5.9 years (range: 6 weeks to 43 years). We note that the only patient who had dystonia onset in late adulthood (43 years) harbored a *KMT2B* missense VUS [12]. More than half of the reported patients display prominent involvement of bulbar muscle groups, causing laryngo-pharyngeal spasms, tongue protrusion, and jaw opening or closing difficulties. Cervical dystonia, sometimes manifesting as massive retrocollis, orofacial dyskinesia, and upper limb dystonic cramps, interfering with dexterity and handwriting, are also common. Although objective dystonia rating scale documentation over the disease course is not available for most reported patients, review of the literature suggests that DYT-*KMT2B* can be generally regarded as a disorder that is (1) progressive in nature and (2) associated with markedly impaired basic life functions. Limb dystonic contractions often lead to disabling gait disturbances, whereas bulbar and orofacial involvement can produce significant swallowing and articulation deficits. In the most severely affected individuals, loss of ambulation with wheelchair-dependence, complete anarthria, and the necessity of feeding via gastrostomy tube are observed [5].

In summary, DYT-*KMT2B* typically manifests as a childhood-onset progressive generalized dystonia syndrome that severely impacts the affected individuals` quality of life. Although most patients tend to follow a similar disease course, there is indication of variable expressivity: we recently phenotypically characterized a three-generation kindred in which the 6-year-old index patient suffered classical lower limb-onset generalized dystonia while the index subject`s father and grandfather solely featured writer`s cramp without clinical evidence of more widespread dystonic

involvement [4]. Further research will likely increase knowledge of how genetic, epigenetic, or other modifying factors influence disease severity in DYT-*KMT2B*.

Additional, non-dystonic features

A diverse array of other neurological and systemic features is part of the emerging clinical picture of DYT-*KMT2B* (Tab. 1). Apart from dystonia, presenting movement disorders include myoclonic-like jerks, mainly apparent in the limbs, as well as choreic and ballistic movements (13%). About 38% of the reported patients reached developmental milestones belatedly, and 51% were characterized as having cognitive impairment. Although not always formally assessed, the severity of intellectual disability ranged from borderline to severe with the majority of patients being mildly affected. Varying degrees of dysmorphia occur in about half of the described patients. The most recurrent features that were noted in this group include minor facial stigmata such as bulbous nasal tip and elongated face. Some patients had mircognathia and clino- or syndactyly [4, 5]. Cerebral morphological abnormalities, consisting of symmetrically distributed T2-hypointense lesions in the external globus pallidus, were evident on MRI scans of 19 patients (25%). Meyer et al. highlighted that these changes are likely to represent an age-dependent phenomenon [5], explaining why their evaluation may be subject to ascertainment bias. Atrophy of the corpus callosum [20], white matter alterations [31], cerebellar vermis hypoplasia [16], and enlarged ventricles [15] were each documented in a single affected individual. Less frequent, albeit possibly underappreciated findings among 76 DYT-*KMT2B* patients encompass short stature, decreased head size, brisk reflexes or spasticity, eye movement disorder, skin lesions, psychiatric illness, seizures, and sensorineural hearing loss (Tab. 1).

It has only recently become evident that, in rare cases, *KMT2B* mutations can give rise to neurodevelopmental disease phenotypes without any signs of dystonia or related movement disorder [45]. A recent case report from China showed that DYT-*KMT2B* and *KMT2B*-related neurodevelopmental disease without dystonia can occur even within the same family [22].

In summary, our review of clinical data suggests the existence of a phenotypic spectrum in *KMT2B*-related disease, ranging from neurodevelopmental disability, which can be un-accompanied by movement disorder, through to the predominant dystonia presentations, which are complicated by multisystem involvement or, less frequently, occur in apparent isolation [12, 14].

Genotype-phenotype correlations and differential diagnosis

Still, it remains difficult to reliably predict phenotypic consequences on the basis of the underlying *KMT2B* genotype. Meyer et al. found evidence that the age of symptom onset is influenced by mutation type [5]: in their cohort of 28 individuals with DYT-*KMT2B*, dystonia manifested significantly earlier in patients with PTVs and whole-gene deletions of *KMT2B* as compared to those with *KMT2B* missense mutations, but no relevant difference in overall dystonia severity was seen between the two groups. Additionally, Gorman et al. concluded in a recent literature review that complicating non-dystonic features are more common in the PTV/whole-gene deletion group [46]. Although the results of these early genotype-phenotype correlation studies are certainly important, investigations that advance our understanding of how specific *KMT2B* variants relate to clinical outcomes are desirable.

In clinical practice, a variety of genetically determined conditions need to be considered in the differential diagnosis of DYT-*KMT2B*. These include for example

hereditary dystonia type 1 and type 6, both of which also present with generalized dystonia involving muscles of the limbs or the cranio-cervical region. Regarding its multisystem involvement, DYT-*KMT2B* can resemble disease manifestations of mitochondriopathies, primary neurotransmitter defects, and inborn errors of metabolism. Finally, DYT-*KMT2B*`s features of symmetrical hypointensities in basal ganglia can be reminiscent of MRI changes seen in “neurodegeneration with brain iron accumulation” (NBIA) disorders [5, 15]. In fact, by reviewing genotype data in our cohort of 15,000 in-house sequenced exomes, we identified a pathogenic PTV in *KMT2B* in a disease subject who was classified clinically as having NBIA disorder (unpublished results). We anticipate that, with the growing availability of NGS-based genomic testing and array technologies, *KMT2B* mutations will be detectable across a wider range of neurological and neurodevelopmental indications. For a comprehensive catalog of DYT-*KMT2B*`s differential diagnoses and distinguishing features, we refer to GeneReviews® (entry “*KMT2B*-related dystonia”, https://www.ncbi.nlm.nih.gov/books/NBK493766/).

Management and treatment

A molecularly confirmed diagnosis of DYT-*KMT2B* should trigger patient-centered counselling, involving the expertise of geneticists, neuropediatricians, and neurologists. Importantly, the detection of a *KMT2B* mutation can guide preventative measures, supportive treatments, and specific therapeutic interventions.

First, evaluations following diagnosis should include assessment of swallowing function, examination of speech and language, developmental surveillance and screening for mental status changes, as well as monitoring of orthopedic or psychiatric sequelae. Further, to lessen the burden of disability on the patient, the use of adaptive

aids and early initiation of physiotherapy are crucial (GeneReviews®, https://www.ncbi.nlm.nih.gov/books/NBK493766/).

Second, pharmacotherapy appears to be effective in some patients with DYT-*KMT2B*. Different authors reported improvement of dystonic symptoms after treatment with anticholinergic drugs [14, 15]. In two patients from our in-house DYT-*KMT2B* patient cohort [4], we also noted satisfactory response to anticholinergics. Although certainly more studies are required, a trial with these agents should be considered reasonable.

Third, bilateral deep brain stimulation (DBS) of the globus pallidus internus is emerging as a highly beneficial therapeutic option in DYT-*KMT2B*. Altogether, 28 *KMT2B*-mutated dystonia patients who underwent DBS were identified upon systematic review of the literature (Tab. 2). The clinico-genetic details of these patients and their DBS response descriptions that were directly taken from the available tables and main text paragraphs within the original publications are presented in Table 2. Strikingly, sustained symptom relief and/or slowing down of disease progression have been pointed out in 26 of the DBS-implanted subjects. In many cases, DBS insertion led to the restoration of motor control, and seven patients were explicitly described as having regained a relevant degree of walking abilities (Tab. 2). Notably, the strongest reductions in motor disease severity were observed in patients in whom DBS was initiated at a young age.

We report in this review our own experience with DBS surgery after the detection of a *KMT2B* mutation: a p.Gln810*-bearing girl with generalized dystonia received DBS implantation at the age of 7 years, 6 months after the case had been published as part of our 2016 gene-identification study [4]. At 2 years follow-up, examination showed restoration from wheelchair-bound state to autonomous ambulation and marked improvement of fine manual dexterity (V. Pilshofer, personal communication) (Tab. 2).

We hypothesize that *KMT2B* mutational status could be established as a predictive molecular marker for DBS response. In this regard, there is a need for international collaboration in the aggregation of genomic screening results and *KMT2B*-related clinical data such that DBS response rates in larger DYT-*KMT2B* patient cohorts can be analyzed in a prospective manner.

On the basis of our review results, we recommend early referral for genetic testing in individuals suspected to have DYT-*KMT2B* in order to ensure timely diagnosis and allow for access to optimal management and treatment strategies.

Conclusions

DYT-*KMT2B* is caused by a heterogeneous collection of *KMT2B*-disrupting protein-truncating, missense, and deletion variants, which can be most accurately detected via modern NGS and array testing approaches. De novo status of the mutations is observed frequently and can be regarded as an important criterion for variant pathogenicity. DYT-*KMT2B* appears to have a characteristic phenotype that presents in childhood with lower limb onset and subsequently generalizes, usually involving the bulbar and cranio-cervical muscles. Intellectual disability and minor facial dysmorphism are the most common non-dystonic abnormalities seen in DYT-*KMT2B*. The available literature suggests that DBS therapy shows high efficacy in patients with DYT-*KMT2B*. Assuming that the success of DBS in the treatment of DYT-*KMT2B* is related to the presence of *KMT2B* mutation, we propose that the implementation of genomic testing in routine diagnostic algorithms for childhood dystonia is justified. Prospectively designed studies will be necessary to formally prove that an identified *KMT2B* mutation can serve as a predictor for positive DBS response. Moreover, additional research will be essential to gain more insight into the brain transcriptomic

alterations induced by compromised *KMT2B* dosage or function and understand how they relate to dystonia pathobiology.

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Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with animal subjects performed by any of the authors. Signed informed consent for publication of clinical and genetic findings was obtained in accordance with institutional review board regulations and protocols from all the patients (or their legal representatives) who were investigated by the authors and their cooperation partners.

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individuals with DYT-*KMT2B*, show that *KMT2B* mutations result in impaired expression of the dystonia-linked genes *TOR1A* and *THAP1*, and highlight deep brain stimulation as a promising treatment option for DYT-*KMT2B*. In addition, they elaborate on the broad spectrum of non-dystonic *KMT2B*-related symptoms and demonstrate that hypointense basal ganglia lesions can be seen on brain MRI scans of patients with DYT-*KMT2B*.

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Figure legend

Figure 1 Synopsis of 66 different *KMT2B* variants in 76 individuals with dystonia, as summarized from 20 publications and the DECIPHER database (2016 December 1 – 2019 June 30), and gene-disease associations in the KMT2 family. (A) Distribution of reported variants along the schematically represented 2715-amino acid KMT2B protein (color code for known functional domains is given: AT-hook, AT-hook DNA-binding domain; CXXC, CXXC zinc-finger domain; PHD1-3, plant homodomain finger 1-3; PHD-like, plant homodomain-like domain; FYR-N/C, N-terminal/C-terminal FY-rich domain; SET, su[var]3-9 enhancer-of-zeste trithorax domain). Protein-truncating variants (PTVs) are indicated by black circles. Non-truncating variants are shown as red (pathogenic/likely pathogenic [P/LP] missense variants), yellow (missense variants of uncertain clinical significance [VUS]), and orange (in-frame deletions) circles. Two splicing mutations (c.3528+2T>A [p.?], c.5198-4_5206del [p.?]) are not illustrated because their effect at the protein level was not determined [5, 17]. Blue rectangles symbolize *KMT2B*-involving gross deletions at 19q13.11-19q13.12 (GRCh37/hg19). The 19q13.12 segment and the deletions are enlarged for visualization purposes and the size of each deletion is provided. (B) Diagram showing the numbers and percentages of different *KMT2B* variants reported to date. Note the significant contribution of PTVs and whole-gene deletions of *KMT2B* to DYT-*KMT2B*. (C) *KMT2B* Missense Tolerance Ratio (MTR) plot (ExAC version-2 [27]) [26] illustrating that the missense (and in-frame deletion) variants reported in dystonia preferentially affect the variant-intolerant residues of the protein. In particular, all

missense variants reported as de novo occurrences (P/LP, red circles) are situated in the invariant sequence. MTR data were obtained from the MTR Gene Viewer (http://mtr-viewer.mdhs.unimelb.edu.au/). “Neutrality” indicates that the same number of variants is observed as expected based on the underlying sequence context (dashed blue line). The *KMT2B*-specifc median MTR is indicated by a black dashed line. The *KMT2B*-specific 25th and 5th centile MTRs are also shown (green and orange dashed line, respectively). ExAC, Exome Aggregation Consortium. (D) List of the six members of the lysine *N*-methyltransferase 2 (KMT2) family. In humans, mutations in any of these haploinsufficient genes are linked to neuropsychiatric phenotypes, underscoring the importance of the KMT2 family for normal brain development and function. pLI, probability of being loss-of-function intolerant; OMIM, Online Mendelian Inheritance in Man; AD, autosomal-dominant; HI, haploinsufficiency; ID, intellectual disability; ASD, autism spectrum disorder; DS, dysmorphic signs; DYS, dystonia; EPI, epilepsy; MCA, multiple congenital anomalies; SCHIZ, schizophrenia. [a]OMIM numbers are as follows: *KMT2A*, 159555; *KMT2B*, 606834; *KMT2C*, 606833; *KMT2D*, 602113; *KMT2E*, 608444; *KMT2F* (also known as *SETD1A*), 611052. [b]as reported in the original gene identification studies [33-37].