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FREQUENCY AND PHENOTYPIC SPECTRUM OF *KMT2B* DYSTONIA IN CHILDHOOD: A SINGLE-CENTRE COHORT STUDY

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Abstract

Background: Childhood-onset dystonia is often genetically determined. Recently, *KMT2B* variants have been recognized as an important cause of childhood-onset dystonia.

Objective: To define the frequency of *KMT2B* mutations in a cohort of dystonic patients aged less than 18 years at onset, the associated clinical and radiological phenotype, and the natural history of disease.

Methods: Whole-exome sequencing or customized gene panels were used to screen a cohort of sixty-five patients who had previously tested negative for all other known dystonia-associated genes.

Results: We identified fourteen patients (21.5%) carrying *KMT2B* variants, of which one was classified as a Variant of Unknown Significance (VUS). We also identified two additional patients carrying pathogenic mutations in *GNAO1* and *ATM*. Overall, we established a definitive genetic diagnosis in 23% of cases. We observed a spectrum of clinical manifestations in *KMT2B* variant carriers, ranging from generalized dystonia to short stature or intellectual disability alone, even within the same family. In 78.5% of cases, dystonia involved the lower limbs at onset, with later caudo-cranial generalization. Eight patients underwent pallidal Deep Brain Stimulation with a median decrease of BFMDRS-M score of 38.5% in the long term. We also report four asymptomatic carriers, suggesting that some *KMT2B* mutations may be associated with incomplete disease penetrance.

Conclusions: *KMT2B* mutations are frequent in childhood-onset dystonia and cause a complex neurodevelopmental syndrome often featuring growth retardation and intellectual disability as additional phenotypic features. A dramatic and long-lasting response to Deep Brain Stimulation is characteristic of DYT-*KMT2B* dystonia.

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Introduction

Dystonia is a heterogeneous clinical and genetic entity[1]; since the discovery of DYT1 (*TOR1A*)[2], the list of genes underlying isolated and combined dystonia has rapidly grown, with a significant boost thanks to the advent of Next Generation Sequencing (NGS) techniques[3]. Nonetheless, a large number of pediatric and adult patients with dystonia remains without a definitive genetic diagnosis even after comprehensive genetic analyses[4].

In 2016, two research groups independently identified dominant mutations in *KMT2B* as a new cause of generalized childhood-onset dystonia[5,6]. *KMT2B* encodes a specific lysine methyltransferase that catalyzes the transfer of a methyl group to the fourth lysine (K4) of histone H3 (H3K4), an important post-translational mechanism that promotes gene transcription and expression with a key role in normal human development[7,8].

Data from the first two back-to-back papers suggested that mutations in *KMT2B* play a relevant role in the pathogenesis of early-onset dystonia and mutations in this gene were likely to be more frequent than other recently identified dystonia-related genes. However, the frequency of *KMT2B* mutations in childhood-onset dystonia has not been systematically assessed and the description of the phenotypic spectrum and natural history of the disease is still limited.

In this study, we screened a cohort of 65 patients with genetically undefined childhood-onset dystonia with whole-exome sequencing (WES) or a customized gene panels, with the aim of identifying pathogenic mutations in *KMT2B* and other dystonia-related genes and defining the relative prevalence, clinical findings and disease course of patients carrying *KMT2B* mutations.

Material and methods

Patients

Patients were enrolled at the Department of Child Neurology of Carlo Besta Neurological Institute in Milan, Italy, which is a tertiary referral center for the diagnosis and therapy of pediatric movement disorders.

Sixty-five subjects (30 females, 35 males) with childhood-onset dystonia, followed over a time-frame of about 30 years were included in the study. All patients were of Caucasian (Italian) ethnicity but one (Patient 14), who was born to Venezuelan parents of European descent. Inclusion criteria were: 1) onset of dystonia before 18 years of age, being the only or most relevant finding on examination; 2) exclusion of secondary causes of dystonia (traumatic brain injury, treatment with dopamine receptor blocking drugs, metabolic causes); 3) absence of pathogenic mutations in known dystonia-associated genes.

Mutation negative cases had been previously studied by targeted re-sequencing using a customized gene panel including 67 genes associated with dystonia and other movement disorders (full list of genes available upon request: disturbimovimento@istituto-besta.it).

Parental consent to perform genetic analyses and for video recordings was obtained in all cases; this included consent to perform additional genetic analyses for any newly discovered movement disorder-related gene. DNA was stored in the movement disorder biobank located at the Molecular Neurogenetics Unit of Carlo Besta Institute.

For most patients included in the study, videos from previous clinical assessments allowed to evaluate the disease course over several years of follow-up.

Genetic Analyses

DNA was extracted from peripheral white blood cells according to standard procedures.

52 patients were analyzed by WES. Exomes were captured using Illumina's Nextera Rapid Capture according to the manufacturer's recommendations. Indexed and pooled libraries were then sequenced on Illumina's HiSeq3000 (100 bp, paired-end). Bioinformatics analysis of WES data was performed as previously described[9]. In brief, reads were aligned using BWA[10]. Duplicate read removal, format conversion, and indexing were performed with Picard (http://broadinstitute.github.io/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, according to GATK good practice[11]. Annotated variant files were generated using ANNOVAR[12].

After the identification of *KMT2B* mutations in childhood-onset dystonia, we included this gene in our customized gene panel for movement disorders.

13 additional patients were then screened for *KMT2B* mutations with this updated version of the panel. Target regions of interest (coding sequence + UTR) were amplified and the amplicons generated were sequenced through the MiSeq platform (Illumina) as previously described[4].

Detected *KMT2B* variants were considered only if coding or affecting canonical splice-sites and if they had minor allele frequency (MAF) < 0.001 in gnomAD browser (https://gnomad.broadinstitute.org)[13].

Copy Number Variations (CNVs) were analyzed using Excavator[14] and Cn MOPS tool[15] for WES and targeted resequencing data, respectively. Multiple Ligation Probe Amplification (MLPA) technique could not be used to detect *KMT2B* CNVs as no kit is commercially available at present.

All variants were confirmed by Sanger sequencing in probands and segregation analysis was performed in available relatives. Prediction of pathogenicity of missense variants was assessed based on *in silico* prediction programs (Polyphen2, SIFT, Mutation Taster, Combined Annotation Dependent Depletion (CADD) Phred score)[16]. Conservation of altered amino acids and nucleotides was investigated with BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and PhyloP (http://compgen.cshl.edu/phast)[17], respectively.

In silico modelling

In silico modelling was performed to better characterize the potential pathogenic effects of some missense variants on KMT2B structure and function. Known protein domains were assigned to the full-length KMT2B sequence using the Pfam database[18]. Proteins of known structure sharing similar sequence and structural properties were identified using HHpred[19]. The selected templates had more than 99% probability (based on HHpred alignment score) of being structurally related to

specific domain segments of KMT2B. MODELLER[20] was used to model different regions of KMT2B based on the structures of templates identified and HHpred alignments were used to guide the modeling. 150 models were generated for each domain region using MODELLER loop optimization protocol and the best model was selected based on the normalized DOPE score[21].

The structure of the PHD-like domain (residues 1574-1688) was modelled using the second extended PHD domain in Plant Homeodomain Finger-6 (PHF6) protein as template[22] (PDB id: 4NN2). The sequence identity between the two homologous domains is ~25% but the zinc finger motifs are well conserved. The modeled domain is an extended PHD with three zinc-binding motifs, where a zinc finger (pre-PHD) precedes the PHD domain.

Some missense variants were located in regions of KMT2B that could not be modeled due to the lack of known structural information on the specific protein sequence.

Results

Molecular characterization of *KMT2B* variants

The full list of identified genomic *KMT2B* variants is shown in **Table 1** and their location along the protein structure in **Fig. 1**. All variants identified in this study were not reported in gnomAD (last access August 2018). 12 out of 14 variants were novel, whereas two (p.Arg1705Gln and p.Lys553Glnfs*46) were previously reported in affected subjects[6,23-24].

Three patients carried frameshift mutations causing a premature stop codon and a truncated protein. p.Ser2070Argfs*20 and p.Ala2139Glyfs*6, located in *KMT2B* exon 28, arose *de novo*. p.Lys553Glnfs*46[23,24] was absent in the proband's unaffected mother and brother; the patient's unaffected father was not available for genetic testing. One patient carried a *de novo*, in-frame 24-nucleotide deletion within exon 26 (p.Gln1802_Ala1808del) causing a partially truncated protein lacking eight amino acids outside known functional domains.

Nine patients carried *KMT2B* missense variants. The CADD Phred score was >23 for 8 out 9 variants, supporting a deleterious effect (**Table 1**).

Segregation analysis confirmed that three of these variants, p.Ala1632Val, p.Arg1705Gln and p.Leu1753Pro had arisen *de novo*. The p.Ala1632Val variant (located within the PHD-like domain) and the p.Arg1705Gln variant (located outside known functional domains) affect highly-conserved amino acids based on BLAST and PhyloP scores and have a CADD Phred score of 32. The p.Ala1632Val is predicted to affect the correct folding of this protein region destabilizing the whole structure of KMT2B by *in silico* modeling. Thp.Leu1753Pro missense mutation causes the substitution of a highly-conserved leucine located within the FYRN domain of KMT2B and is predicted to be pathogenic *in silico* (CADD Phred 33).

For two variants, p.Arg1777Pro and p.Ser1615Leu, segregation analysis was incomplete. However, they both involve highly conserved amino acids located in functional domains of the protein and have CADD Phred score higher than 23, suggesting a disease-causing effect. The p.Ser1615Leu is predicted to have a destabilizing effect on the folding of PHD domain by *in silico* modeling. Furthermore, both variants were absent in all healthy relatives available for testing (**Fig. 2**)

Four additional *KMT2B* missense variants were inherited from an unaffected parent, which may suggest incomplete penetrance of the variants.

The p.Arg1003Gln variant affects a highly-conserved Arginine located in the CXXC zinc-finger domain of KMT2B and is predicted to be pathogenic (CADD Phred 32). *In silico* modeling predicts that substitution of Arg1003 to Gln is has a destabilizing effect on DNA binding in this protein domain. The proband (**Video 1**) inherited the missense substitution from his unaffected father; his two sisters did not show dystonia but one had short stature and carried the same variant, whereas the other was wild type and had a normal neurological examination and somatic development.

The p.Leu2431Ser variant is predicted to be disease-causing (CADD Phred 28.7) and affects a moderately conserved amino acid (down *to mus musculus*) within the FYRC domain. The

proband's motor phenotype was strongly similar to previously reported cases. *In silico* modelling predicted a destabilizing effect of this variant on the protein structure affecting the normal association of FYRN and FYRC domains. Segregation analysis demonstrated that two out of three siblings carried the same missense variant and both had mild to moderate intellectual disability without dystonia; one healthy brother and the unaffected father were wild type. The proband's mother, reported to be unaffected, had deceased at the time genetic testing was performed.

The p.Asp1144Val variant, located in exon 10, is predicted to be disease-causing (CADD Phred 28.2), is absent in gnomAD and affects an amino acid falling out of functional protein domains, but fully conserved in mammalian species. The patient inherited the variant from her unaffected mother.

The p.Gln747Arg substitution, located outside of KMT2B functional domains, was predicted to be tolerated *in silico* (CADD Phred 18.42), but was not found in gnomAD. Although the patient's phenotype was consistent with *KMT2B* dystonia, this variant can at present be classified as a VUS according to the ACMG guidelines[25], thus caution must be used in the interpretation of its pathogenicity.

Patient 14 carried a *de novo* 1.3 Mb deletion at 19q13.2 spanning 46 genes, including *KMT2B*, that was confirmed by CGH array.

In silico modeling

In silico modeling was performed to better characterize the potential pathogenic effects of some missense variants on KMT2B structure and function.

According to Pfam domain assignments[18], KMT2B has a CXXC zinc finger domain, followed by multiple PHD/PHD-like domains and a FYRN domain. The FYRC region and SET catalytic domain are located within the C-terminus. The FYRN (F/Y rich N-terminus) and FYRC domains of KMT2B (residues 1730-1807 and 2415-2494) are particularly common in histone H3K4

methyltransferases, especially in the family of proteins that include Mixed Lineage Leukemia (MLL). The structures of these domains were modeled using the crystal structures of transforming growth factor beta regulator 1 (TBRG1) domains (PDB id:2WZO) as templates. The FYRN and FYRC domains are separated by about 600 amino acids but they are likely to interact together to form a compact structural unit (**Fig. 3A-C**). This is observed in MLL as well, where these two domains interact to maintain the active structure after the proteolytic cleavage between FYRN and FYRC domains, by taspase1[26,27].

The amino acid contacts involving the residues Leu2431, Arg1777 and Leu1753 are thought to stabilize the association between FYRN and FYRC domains. Leu1753 forms inter-domain contacts to support the bound conformation of these domains (**Fig. 3A**) while Arg1777 and Leu2431 are involved in intra-domain interactions (**Fig. 3B-C**).

p.Leu1753Pro (Patient 8) is expected to result in the loss of hydrophobic interactions involving Leu1753 (**Fig. 3A**). Arg1777 forms a salt bridge with Asp1738 in the FYRN-FYRC complex (**Fig. 3B**). It is also involved in backbone hydrogen bonds that stabilize the beta sheet in the FYRN domain. Substitution to Proline (p.Arg1777Pro; Patient 7) is likely to disrupt the backbone hydrogen bond at this position as it lacks one hydrogen bond donor. The mutation is thus predicted to have a destabilizing effect on the sheet structure and potentially affects the association of FYRN and FYRC domains. p.Leu2431Ser (Patient 11) results in a shorter side chain at this position and causes loss of hydrophobic contacts involving Leu2431 that stabilizes the bound form of FYRC (**Fig. 3D**).

Arg1003 is part of a zinc finger motif that selectively binds DNA, with a known crystal structure (PDB ID: 4PZI). Arg1003 forms a salt-bridge with Asp977 (**Fig 3E**), stabilizing the DNA-bound form of the domain. Mutation of Arg1003 to Gln (Patient 12) will eliminate these interactions stabilizing DNA binding.

The fold of the PHD-like domain (residues 1574-1688) is stabilized by a hydrogen-bond interaction between Ser1615 and Thr1650 (**Fig 3F**). Mutation of this Ser1615 to Leu (Patient 9) will eliminate this interaction and Leu having a relatively longer and branched side-chain, can affect the residue packing in this region destabilizing the fold of this domain and possibly the entire protein. Ala1632 is also at the core of the PHD-like domain and forms van der Waals contacts with Ser1615 (**Fig 3G**). Mutation of Ala1632 to Val (Patient 13) adds a branched side-chain that is likely to affect the conformations of neighboring residues, including Ser1615 and Glu1617, and the interactions involving these residues. Ser1615 forms a hydrogen bond with Thr1650, while Glu1617 forms a hydrogen bond with the backbone of Ala1632. Disruption of these two interactions is likely to destabilize the fold of this domain and the entire protein.

Clinical characterization of *KMT2B*-mutation carriers

We identified 14 out of 65 patients (21.5%; 8 females, 6 males; **Table 2**) carrying *KMT2B* variants, of which 13 were predicted to be pathogenic based on available evidence, thus indicating an overall mutational frequency of 20%. The median age of onset of dystonia was 6 years (range 3-13 years); median age at last examination was 23 years (range 10-52 years), with a median disease duration of 18 years (range 3-42 years). No family history of dystonia was reported in any of these patients. Motor milestones were normally achieved in all patients, whereas delayed language was reported in three cases. Lower limbs were the site of onset of dystonia in 11/14 patients (78.5%), presenting as foot in-turning, abnormal plantar flexion or tip-toe walking. Generalization of dystonia occurred in all but one patient (93%) over a time frame of 2-4 years after onset. The only subject showing persistent focal distribution of dystonia (torticollis) has the shortest disease duration (3 years) in our series, thus we cannot rule out generalization in the future. Laryngeal dystonia, either of an abductor or adductor type became evident early during disease course in 11/14 cases (78.5%), progressing to scarcely intelligible speech or even complete anarthria in four patients (Patients 2, 8, 9, 11; **Video**

2). Oromandibular dystonia presented in 8/14 patients (57%) as clenched jaw associated with hypomobility of the tongue and reduced jaw opening that worsened speech quality. When generalization occurred, the upper body and axial involvement was particularly severe in all cases, whereas lower limb dystonia was not the major source of disability in any patient, even several years after disease onset. Worsening of dystonia lasting hours to days was triggered in some patients (11/14) by fever, infections, emotional stress or menstruation. None of mutation carriers experienced status dystonicus during disease course.

Additional neurological signs included myoclonus (with electrophysiological features consistent with subcortical origin) in the neck and upper limbs in one case, mild palpebral ptosis in two, microcephaly in two and asymmetric akinetic-rigid parkinsonism in one, that developed some years after DBS. Pyramidal signs in the lower limbs with normal muscular strength were relatively frequent (6/14 patients, 43%).

A formal psychometric assessment (WISC-R or Raven's Progressive Matrices) was available in 13 patients and mild intellectual disability was diagnosed in 6 cases, whereas 3 patients tested in the low-average range of intelligence (IQ $\leq$ 77). Cognitive decline was not documented in any patient during the follow-up either clinically or by formal testing.

Short stature with harmonic somatic development recurred in more than half of *KMT2B* variant carriers (9/14; 64%). Short stature was defined as height below the expected genetic target calculated from parents' height or two standard deviations or more below the mean height for individuals of the same sex and age. Psychiatric disturbances were not diagnosed in any patient.

Minor facial dysmorphic features were observed in 9 out of 14 patients (64%), and included bulbous nasal tips, low-set ears, thin upper lip, mild palpebral ptosis, broad nasal bridge and elongated face.

Brain MRI was performed in all patients at different disease stages and no abnormalities were reported. In particular, basal ganglia showed normal signal in all sequences (including Diffusion

Weighted- and Susceptibility Weighted Images) in all patients. In only one case (Patient 5) mild, static hypoplasia of cerebellar vermis was observed.

Pharmacological treatment and Deep Brain Stimulation efficacy

All patients were treated with different combinations of trihexyphenidyl, benzodiazepines, baclofen, levodopa and pimozide with various clinical responses. High doses of trihexyphenidyl (30-40 mg/day) produced mild to excellent clinical improvement in some cases (**Video 3**). A combination of trihexyphenidyl and clonazepam was the most effective pharmacological strategy in most patients. Overall, laryngeal dystonia was not improved by pharmacological treatment. All patients were given a trial of levodopa/carbidopa for at least three months, but a dramatic and sustained response to low doses (3 mg/kg/day) was observed only in Patient 10.

Eight patients presenting unsatisfactory response to multiple antidystonic medications underwent stereotactic bilateral DBS targeting the somatosensory portion of Internal Globus Pallidus. At the time of surgery, median disease duration was 6.5 years (range 4-31 years) and patients' median age was 10 years (range 8-38 years). Clinicians were blind to patients' genetic status when performing the BFMDRS-M scale pre-operatively, since all patients were operated before *KMT2B* discovery. At the last post-operative follow-up, raters were still blind to the genetic diagnosis in most cases but not blind to the DBS status (on/off/no DBS).

In one case (Patient 5) DBS implant was removed 2.5 years after surgery because of recurrent infections unresponsive to antibiotics. Excluding this patient, the median post-operative follow-up time was 12 years (range 8-17 years). Due to left electrode fracture, Patient 13 had it replaced 3 years after DBS and she experienced intracranial bleeding during this procedure, causing a mild right-sided hemiparesis and expressive aphasia.

In all cases clinical and functional improvement was immediately evident after surgery, with progressive amelioration in the following 3-12 months. The median decrease of BFMDRS-M score

at the last follow-up was 38.5% (range 81% -2.4%). In only one case (Patient 8), after a substantial clinical improvement following DBS (BFMDRS-M score -38% after one year) a slow but constant worsening was observed, with the BFMDRS-M score returning comparable to the pre-operative levels 12 years after surgery (-2.4% at last follow-up). Before the implant removal, also Patient 5 showed a substantial improvement of dystonia following DBS, and Patient 13 experienced significant amelioration of dystonia for three years after the initial DBS procedure (BFMDRS -62%); at last follow-up, BFMDRS was not applicable but dystonia appeared to be still substantially improved in the trunk and cervical region. Reduction of dystonia severity was documented in all anatomical districts (**Videos 4** and **5**), but laryngeal dystonia did not show relevant improvement regardless of stimulation parameters, and some patients even developed laryngeal dystonia after DBS.

Discussion

In this study, we screened a cohort of 65 patients with genetically undefined childhood-onset dystonia with whole-exome sequencing (WES) or a customized gene panel, with the aim of identifying pathogenic mutations in *KMT2B* and other dystonia-related genes and defining the relative prevalence, clinical findings and disease course of patients carrying *KMT2B* mutations. Previous work indicated a highly variable *KMT2B* mutational frequency in childhood-onset dystonia, from 1.3 to 38%[6]. In our series, 21.5% of patients with genetically undefined early-onset dystonia carried *KMT2B* variants; 13 (20%) carried variants classified as pathogenic based on available evidence. We acknowledge that the overall *KMT2B* mutational frequency in our study might be have been underestimated since the methods used to detect CNVs are suboptimal for detection of small single or multiple exonic deletions or duplications.

We also identified bi-allelic *ATM* mutations and a *de novo GNAO1* mutation in two additional patients (**Table 2; Supplementary material**), allowing 23% of patients to receive a definitive genetic diagnosis.

47 *KMT2B* variants have been reported to date, including microdeletions encompassing *KMT2B* as well as missense, nonsense, frameshift and splice-site mutations[5-6,23-24,28-36]. Of these, 35 (74.5%) arose *de novo*, 5 (10.5%) were dominantly inherited and 7 (15%) had an unknown inheritance pattern. With our series, the number of *KMT2B* variants reported increases up to 61.

Childhood-onset dystonia is an almost universal clinical manifestation in *KMT2B* mutation carriers, as reviewed by Gorman *et* al.[37] However, a small number of patients is asymptomatic[5,28] or present with moderate-to-severe intellectual disability without dystonia[24,31-32]. This suggests incomplete penetrance of some *KMT2B* mutations and a phenotypic spectrum extending beyond movement disorders.

Our results highlight that mutations in *KMT2B* are a frequent cause of generalized childhood-onset dystonia; however, it is of note that a substantial proportion of patients are left without a genetic diagnosis even after extensive screening, indicating the likely presence of pathogenic mutations in other unknown genes causing dystonia.

With regard to the patients' phenotype, DYT-*KMT2B* dystonia appeared similar to DYT-*TOR1A* dystonia for age at onset and initial lower limb involvement[41]; however, unlike DYT1 dystonia, the larynx and oromandibular region were almost universally affected during the generalization process, that occurred 2-4 years after onset with a body distribution that mimicked DYT-*THAP1* dystonia. Dystonia was severely disabling in most patients, making surgical treatment the preferred option in more than half of our cases. We calculated a median long-term decrease of BFMDRS-M score of 38.5% following DBS, providing a quantification of motor improvement after surgery, with a median post-operative follow-up of 12 years. Our data confirm that carriers of *KMT2B* mutations show an excellent, long-lasting motor response to pallidal DBS, and that improvement is rapidly

observed particularly in the limbs and trunk, but not in the larynx. Different genetic causes of isolated and combined dystonia are emerging as poor or favorable prognostic factors for DBS outcome[39,40]; our series fully supports *KMT2B* mutations as a positive prognostic factor for a good functional outcome after DBS, similarly to DYT-*TOR1A* patients. Aside from dystonia, *KMT2B* variants were frequently associated with additional neurological features such as mild intellectual disability, short stature, brisk reflexes in the lower limbs and minor facial dysmorphic features. Consistently, among six mutation carriers reported by Zech *et al*., short stature was observed in three, and intellectual disability in four[5], whereas Meyer *et al*. reported these features in 55% and 11% of cases, respectively[6].

Unlike previously reported mutation carriers[6,37], MRI basal ganglia alterations were not observed at any disease stage, thus our data do not confirm a characteristic radiological signature in *KMT2B* mutation carriers. Six probands' family members were found to carry *KMT2B* missense variants, and three had normal examination (**Fig. 2**). Four asymptomatic carriers have been previously reported[5,33,37], suggesting incomplete disease penetrance of some *KMT2B* mutations, a well-known mechanism described for other dystonia-related genes, such as *DYT1* and *THAP1*[41,42]. Somatic mosaicism may explain incomplete disease penetrance in asymptomatic carriers; however, no cases of *KMT2B* somatic mosaicism has been reported so far. Additional biological samples from unaffected mutation carriers were not available and alternative techniques to detect somatic mosaicism were not performed in our study, thus we cannot rule out that mosaicism was detectable in any of these subjects.

In our cohort, three family members carrying *KMT2B* missense variants showed mild to moderate intellectual disability or isolated short stature in absence of dystonia (**Fig. 3**). Similarly, *de novo* or dominantly inherited *KMT2B* mutations have been previously found in few patients with developmental delay and moderate-to-severe intellectual disability with or without a family history of dystonia[24,31-32].

These observations suggest a role of *KMT2B* not only in the pathogenesis of dystonia, but also in growth retardation and intellectual disability, supporting the theory of dystonia as a neurodevelopmental disorder[43,44]. Heterozygous mutations in genes belonging to the KMT2 family underlie different human disorders all sharing various degrees of intellectual disability and developmental delay as a core feature, associated with other disease-specific neurological and non-neurological abnormalities, such as Wiedmann-Steiner syndrome (MIM 605130, linked to *KMT2A* mutations[45]), Kabuki syndrome (MIM 147920, due to *KMT2D* mutations[46]), and Kleefstra syndrome (MIM 610253, due to *KMT2C* mutations[47]). A possible genotype-phenotype correlation explaining the variety of clinical features observed in *KMT2B* mutation carriers might emerge in the future, although no definite conclusions in this regard can be drawn so far.

It is conceivable that the abundant expression of KMT2B in areas responsible for motor control during embryonic development and in the adult brain may be related to the pathogenesis of dystonia in mutation carriers, and additional genetic and environmental factors might contribute to the disease penetrance and clinical expressivity of *KMT2B* mutations.

Our series also further expands the *GNAO1* phenotypic spectrum to include childhood-onset myoclonus-dystonia with predominant upper body distribution and psychiatric disturbances in adulthood, a phenotype highly resembling DYT-*SGCE* myoclonus-dystonia. Unlike previously reported *GNAO1* patients[48], our case had no intellectual disability, recurrent status dystonicus, epilepsy or brain MRI alterations.

In conclusion, *KMT2B* variants are a relevant cause of childhood-onset dystonia, with a 21.5% prevalence in our series. We observed a variety of clinical manifestations in mutation carriers ranging from severe generalized isolated dystonia to intellectual disability or short stature in isolation, thereby expanding the phenotypic spectrum of *KMT2B* mutations beyond movement

disorders. Different phenotypes were observed even within the same family, in line with other recent reports[24].

We recommend *KMT2B* genetic screening for patients with childhood-onset dystonia without DYT1-mutations, in particular when (1) dystonia first presents in the lower limbs with subsequent generalization and severe involvement of the truncal, oro-mandibular and laryngeal districts (leading to complete anarthria in some patients); and (2) dystonia is accompanied by either microcephaly, short stature, intellectual disability or minor dysmorphic facial traits.

In line with previous reports, we observed a dramatic and long-lasting response to pallidal DBS in patients with *KMT2B* mutations. Hence, reaching a molecular diagnosis of DYT-*KMT2B* dystonia has critical prognostic and therapeutic implications. In cases with unsatisfactory response to drugs, an early surgical treatment may in fact prevent the severe and disabling generalization characteristic of DYT-*KMT2B* dystonia and improve patients' motor performances and quality of life.

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Video is part of ms

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Video legend

Video 1. Patient 12. Generalized dystonia with severe laryngeal involvement associated with microcephaly, minor facial dysmorphic traits, short stature (calculated as genetic target) and brisk reflexes in the lower limbs.

Video 2. Patient 9 and 11. Examples of severe dysarthria/anarthria in *KMT2B* mutation carriers.

Video 3. Patient 3. Long-term positive motor outcome after administration of high doses of trihexyphenidyl, with persistence of speech dystonia.

Video 4. Patient 1: disease course before and after DBS. Severe generalized dystonia with excellent long-term motor outcome after pallidal DBS. The early laryngeal involvement (whispering dysphonia) persisted after surgery.

Video 5. Patient 7: disease course before and after DBS. Severe generalized dystonia significantly improved after DBS; the patient slowly regained the ability to walk unassisted. Oromandibular and laryngeal dystonia persisted in the long-term.

Figure legend

Figure 1: Position of *KMT2B* variants identified in the study along the protein structure.

Figure 2: Family trees of patients carrying *KMT2B* variants. Black symbols indicate patients affected by dystonia; grey symbols indicate subjects with other isolated clinical features (short stature and/or intellectual disability). n.i.: not investigated.

Figure 3: Predicted effect of *KMT2B* variants on structure-function properties. The FYRN and FYRC domains are shown in orange and pink respectively. (**A**) Hydrophobic packing involving Leu1753 (blue), at the interface of FYRN/FYRC domains. Residue side chains are shown as spheres highlighting van der Waals contacts. (**B**) The salt bridge interaction between Arg1777 and Asp1738 in the FYRN domain is shown. (**C**) Hydrophobic contacts involving Leu2431 are shown and (**D**) the loss of contacts as a result of Leu2431Ser variant is highlighted (yellow circle). (**E**) The

zinc finger domain of KMT2B bound to DNA. The salt bridge between the Arg1003 (cyan) and Asp977 is shown. The DNA backbone phosphate is shown in orange. (**F**) PHD-like domain of KMT2B (purple) showing the location of Ser1615 (cyan) and its hydrogen bond between with Thr1650. (**G**) The contact involving Ala1632 (cyan) are shown and Ser1615 (both shown with sphere representation of atoms). Hydrogen bonds involving the neighboring residues Ser1615 and Glu1617 are also shown.