

# Mutations in the Histone Methyltransferase Gene *KMT2B* Cause Complex Early Onset Dystonia

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116

117 **ABSTRACT**

118 Histone lysine methylation, mediated by mixed-lineage leukemia (MLL) proteins, is  
119 now known to be critical in the regulation of gene expression, genomic stability, cell  
120 cycle and nuclear architecture. Despite being postulated as essential for normal  
121 development, little is known about the specific functions of the different MLL lysine  
122 methyltransferases. Here we report heterozygous variants in the gene *KMT2B* (also  
123 known as *MLL4*) in 27 unrelated individuals with a complex progressive childhood-  
124 onset dystonia, often associated with a typical facial appearance and characteristic  
125 brain magnetic resonance imaging findings. Over time, the majority of affected  
126 individuals developed prominent cervical, cranial and laryngeal dystonia. Marked  
127 clinical benefit, including the restoration of independent ambulation in some cases,  
128 was observed following deep brain stimulation (DBS). These findings highlight a  
129 clinically recognizable and potentially treatable form of genetic dystonia,  
130 demonstrating the crucial role of *KMT2B* in the physiological control of voluntary  
131 movement.

132 **INTRODUCTION**

133 The control of voluntary movement is governed by interactive neural networks  
134 within the brain involving the basal ganglia, sensorimotor cortex, cerebellum and  
135 thalamus<sup>1</sup>. Disruption of these pathways can lead to a variety of movement  
136 disorders. Dystonia is characterized by sustained or intermittent muscle  
137 contractions causing abnormal, often repetitive, movements and postures affecting  
138 the limbs, trunk, neck and face. Dystonic movements are typically patterned,  
139 twisting, and may be tremulous, often initiated or worsened by voluntary action and  
140 associated with overflow muscle activation<sup>2</sup>.

141 Dystonia is described in a broad spectrum of genetic and acquired disorders, either  
142 in isolation or combined with other neurological and systemic features<sup>1-5</sup>. Despite  
143 genetic advances, the underlying cause remains elusive for a significant proportion  
144 of individuals with childhood-onset dystonia, hindering future prognostication and  
145 treatment strategies<sup>6</sup>. We report 27 individuals with an early-onset, complex,  
146 combined progressive dystonia associated with mono-allelic variants in *KMT2B*  
147 (*MLL4*, NM\_014727.2). *KMT2B* encodes a lysine histone methyltransferase,  
148 involved in H3K4 methylation, an important epigenetic modification associated with  
149 active gene transcription.

## 150 RESULTS

### 151 ***Chromosomal microdeletions and intragenic KMT2B sequence variants in*** 152 ***early-onset dystonia***

153 We identified 34 individuals with undiagnosed childhood-onset dystonia for  
154 molecular genetic investigation (**Online Methods, Supplementary Table 1,**  
155 **Supplementary Fig. 1**). On routine diagnostic testing, one case (Patient 1) was  
156 found to have a microdeletion at 19q13.12 of undetermined significance<sup>7</sup>.  
157 Diagnostic chromosomal microarray was performed in 23/34 individuals and  
158 overlapping microdeletions were detected in a further 5 cases (**Supplementary**  
159 **Table 1**, Patients 2-6). Using established external networks (**Online Methods,**  
160 **Supplementary Fig. 1**), 4 more cases (Patients 7-10) with microdeletions were  
161 identified. In total, 10 patients (Patients 1-10) had overlapping heterozygous  
162 interstitial microdeletions at 19q13.11-19q13.12 (**Table 1, Fig. 1a**). Microdeletions  
163 detected on diagnostic microarray were verified by established laboratory protocols  
164 and confirmed as *de novo* where parental testing was possible (**Supplementary**  
165 **Table 2**). The smallest region of overlap extended from 36,191,100-36,229,548bp  
166 (GRCh37/hg19), encompassing two HUGO Gene Nomenclature Committee  
167 curated genes, *ZBTB32* (zinc finger and BTB domain containing 32) and *KMT2B*  
168 (*MLL4*) (**Fig. 1a**).

169 For the remaining 28 cases without a 19q microdeletion, we performed either whole  
170 exome (n=6) or genome sequencing (n=9) in 15 (**Online Methods**). Heterozygous  
171 sequence variants within *KMT2B* were identified in 6/15 cases (Patients 13, 14, 17,  
172 21, 22, 27). Sanger sequencing of *KMT2B* in the other 13 individuals identified one  
173 additional mutation-positive case (Patient 16). Through national and international  
174 collaborations (**Online Methods, Supplementary Fig. 1**), a further 10 cases

175 (Patients 11, 12, 15, 18, 19, 20, 23, 24, 25, 26a) were subsequently ascertained.  
176 Overall, a total of 17 patients with intragenic heterozygous *KMT2B* variants were  
177 identified (**Table 1, Fig.1b**). These frameshift insertions (n=1), frameshift deletions  
178 (n=6), splice site (n=1), stop-gain (n=2) and missense (n=7) variants were  
179 confirmed by Sanger sequencing (**Supplementary Table 2, 3**). Whole exome and  
180 genome analysis did not identify pathogenic variants in (i) *ZBTB32*, (ii) known  
181 dystonia genes and (iii) genes causing other neurodevelopmental disorders. Where  
182 possible, mutations in *TOR1A* (NM\_000113.2), *THAP1* (NM\_018105.2) and *GNAL*  
183 (NM\_182978.3) were excluded by diagnostic single gene testing, next generation  
184 multiple gene panels and research Sanger sequencing (**Supplementary Table 4**).  
185 Parental DNA was available for 23/27 cases, and familial segregation studies  
186 verified that interstitial deletions or intragenic variants had arisen *de novo* in 20  
187 patients (**Supplementary Table 2, Supplementary Fig. 2**). Three patients had  
188 maternally inherited missense variants (Patient 22, 26a and 27). The *KMT2B*  
189 variant identified in Patient 26a had occurred *de novo* in his symptomatic mother  
190 (Patient 26b) (**Supplementary Table 2**).

### 191 ***Phenotypic characterization of patients with KMT2B variants***

192 We identified 27 patients (current age 6-40 years, 14 female, 13 male) with *KMT2B*  
193 variants, who presented with clinical symptoms in childhood (**Table 1, Table 2,**  
194 **Supplementary Table 5, Supplementary Videos 1-7**). Individuals presenting in  
195 early childhood (1-9 years, median age 4 years) had either limb or cranio-cervical  
196 symptoms. Clinical presentation for those with microdeletions, frameshift, splice-site  
197 and stop-gain mutations occurred significantly earlier (mean age 4.1 years) than for  
198 those with intragenic missense variants (mean age 6.4 years) (p-value 0.0223)

199 **(Supplementary Fig. 3)**. Most patients (21/27) had lower limb symptoms at  
200 disease onset, leading to foot posturing, toe-walking and gait disturbance (**Fig. 2a**).  
201 4/27 patients presented initially with upper limb symptoms associated with  
202 abnormal postures (**Fig. 2b,c**) and dystonic tremor, leading to reduced dexterity  
203 and handwriting difficulties (**Supplementary Fig. 4a,b**). With increasing age,  
204 cervical symptoms (torticollis, retrocollis) (**Fig. 2d,e**) and cranial involvement (facial  
205 dystonia, oromandibular involvement with dysarthria/anarthria and difficulties in  
206 chewing/swallowing) became prominent features in the majority of patients. In many  
207 patients, progressively severe dysphonia was suggestive of laryngeal involvement.  
208 None of the patients had airway compromise and videostroboscopy was not  
209 undertaken. Over time, most patients (24/27) developed progressive, generalized  
210 dystonia, 2-11 years after initial presentation (**Fig. 2f**). The dystonia was persistent  
211 in nature, absent in sleep, worsened by voluntary action and associated with  
212 overflow muscle activation. Some patients had dystonic tremor. Sudden, brief,  
213 involuntary muscle jerks, clinically consistent with myoclonus, were evident in 2  
214 cases (Patients 14 and 27). For a few subjects, dystonia was exacerbated when  
215 systemically unwell. Stepwise deterioration following intercurrent illness was  
216 particularly evident in Patient 14, and status dystonicus, triggered by a urinary tract  
217 infection, was reported in Patient 3.

218 Many patients with *KMT2B* variants had additional clinical findings, including  
219 microcephaly, seizures, spasticity and eye movement abnormalities (strabismus,  
220 saccade initiation failure and oculomotor apraxia) (**Table 2**). Dysmorphic features  
221 and characteristic facial appearance (elongated face and bulbous nasal tip) (**Fig.**  
222 **2g, Table 2**) were commonly reported. Developmental delay, intellectual disability,  
223 systemic (dermatological, renal, respiratory) features and psychiatric symptoms

224 were also present in some individuals (**Table 2, Supplementary Table 5,**  
225 **Supplementary Fig. 4c**). Malignancies were not reported in any patients.  
226 Cerebrospinal fluid (CSF) neurotransmitter analysis, undertaken in 13 patients  
227 revealed no major derangement of monoamine metabolites (**Supplementary Table**  
228 **6**). Magnetic resonance (MR) imaging revealed a characteristic signature in 17/22  
229 patients who had imaging sequences suitable for assessment (**Supplementary**  
230 **Table 7**). Subtle, symmetrical hypointensity of the globus pallidi (with a hypointense  
231 streak of bilateral globus pallidus externa) was evident on MR images known to  
232 demonstrate the magnetic resonance phenomenon of susceptibility (T2, T2\*-,  
233 susceptibility- and echo-planar imaging b0-diffusion-imaging datasets) (**Fig. 3**).  
234 Mean age at neuroimaging was significantly lower for patients with MR  
235 abnormalities (11.7 years) than for those with normal brain scans (19.0 years) (p-  
236 value 0.0167) (**Supplementary Fig. 5a-c**). Single positron emission tomography  
237 using  $^{123}\text{I}$  (DaTSCAN<sup>TM</sup>) and  $^{18}\text{F}$ -FDG-PET-CT glucose uptake studies, each  
238 undertaken in 3 patients, were normal (**Supplementary Table 7, Supplementary**  
239 **Fig. 5d**).

#### 240 ***Deep brain stimulation: clinical benefit in KMT2B-dystonia***

241 Overall, medical therapies were not clinically beneficial. None of the patients had a  
242 sustained response to levodopa treatment, nor other commonly used anti-dystonic  
243 agents (**Table 1**). Ten patients had symptomatic treatment with bilateral globus  
244 pallidus interna-deep brain stimulation (GPi-DBS) (**Table 1**). All showed clinical  
245 benefit, which was particularly striking in some of the younger patients. Patient 6  
246 showed significant reduction of torticollis and retrocollis, with improvements in  
247 motor function and gait. Patient 8 showed a sustained clinical response 6 years

248 after DBS insertion, with improvement of dystonia, even more evident after  
249 replacement of a faulty right DBS lead. Patient 9 had generalized dystonia and  
250 could not walk independently prior to DBS. Two weeks after DBS insertion, he  
251 dramatically regained independent ambulation with marked improvement of  
252 dystonic symptoms (**Supplementary Video 8**). Patient 17 and 21 were  
253 predominantly wheelchair-dependent prior to DBS insertion, but both patients  
254 showed restoration of independent walking and improvement of dystonia after DBS  
255 (**Supplementary Video 9, 10**). Patient 19 had amelioration of oromandibular  
256 symptoms with DBS. Patient 20 had DBS inserted at age 32 years and although  
257 most benefits were only transient, sustained improvement of foot posture was  
258 reported. Patient 23 had significant reduction of dystonic symptoms after DBS  
259 insertion. Patient 22, 9 months after DBS insertion (**Supplementary Video 11**) and  
260 Patient 25, 4 months after DBS insertion, have both shown significant gains in hand  
261 function and independent walking with improvement of dystonia. Five patients are  
262 now over three years post-DBS insertion, and all report a sustained reduction in  
263 dystonia, with restoration of function and prevention of progressive disability.

264 ***KMT2B is constrained for missense and predicted protein truncating variants***  
265 Patient 13, 14, 17 and 21 had whole genome sequencing as part of the NIHR-  
266 funded BioResource-Rare Disease project. Enrichment analysis was undertaken in  
267 this cohort to determine whether predicted protein truncating variants (PPTVs) in  
268 *KMT2B* were observed more frequently in patients than would be expected by  
269 chance. Given the size and sequence context of *KMT2B*,  $5.73 \times 10^{-03}$  *de novo*  
270 *KMT2B* PPTVs would be expected to occur by chance in the subset of the NIHR  
271 BioResource-Rare Diseases cohort with pediatric onset neurological disease, but 3

272 PPTVs were observed. This represents a significant enrichment (p-value  $3.12 \times 10^{-}$   
273  $08$ ). Furthermore in ExAC, *KMT2B* is highly constrained for PPTVs (accessed July  
274 2016)<sup>8</sup> providing supportive evidence of its potential involvement in disease. 712  
275 *KMT2B* missense variants are reported in the ExAC database. Most of these are  
276 rare, as expected for a cohort of this size, and the median CADD score<sup>9</sup> for these  
277 variants is 22.9. The median CADD score for missense variants identified in our  
278 *KMT2B*-dystonia cohort is significantly higher at 29.1 (p-value 0.0001364;  
279 **Supplementary Table 3**). Given the size and sequence context of *KMT2B*, 956  
280 missense variants are predicted to occur by chance, suggesting that *KMT2B* may  
281 also be constrained for missense variation ( $z=4.06$ )<sup>8</sup>.

#### 282 ***KMT2B* variants are predicted to destabilize protein structure**

283 *In silico* homology modelling studies were undertaken to generate hypotheses  
284 regarding the predicted effects of sequence variants on *KMT2B* (NP\_055542.1)  
285 structure-function properties (**Supplementary Notes**). Based on Pfam domain  
286 assignments, *KMT2B* has a CXXC zinc finger domain, multiple PHD domains, an  
287 F/Y rich N-terminus (FYRN), FYRC (F/Y rich C-terminus) domain and a C-terminal  
288 SET domain (**Fig. 4a**). The modelled variants occurred in residues within the PHD-  
289 like, FYRN, SET and FYRC-SET linking domains (**Fig. 4b-d**). Evaluation of a  
290 number of variants using MAESTRO<sup>10</sup> and DUET<sup>11</sup> suggests a change in the free  
291 energy, with a predicted structure destabilizing effect (**Supplementary Notes**).

292 p.Phe1662Leu and p.Gly1652Asp occur within a PHD-like domain (residues 1574-  
293 1688), predicted to facilitate interaction with DNA, protein-protein interaction and  
294 recognition of methylated/unmethylated lysines<sup>12-14</sup>. Extensive hydrophobic  
295 interactions hold the globular structure of this region, which is important for its

296 function<sup>12</sup>. Phe1662 is fully buried at the core, stabilizing the structure of this PHD-  
297 like domain while Gly1652 is partially buried (**Fig. 4b,e,f**). Phe1662 is involved in  
298 multiple hydrophobic contacts at the core of the PHD domain, and exchange for  
299 leucine is predicted to cause loss of contacts at the core (**Fig. 4g**). Gly1652 is  
300 located on a loop (**Fig. 4e**) and substitution to aspartic acid is predicted to alter  
301 surface charge, with possible effects on the interaction network in the vicinity,  
302 involving a positively charged Arg1635 which is part of the helix  $\alpha 3$  implicated in  
303 DNA binding<sup>12</sup>. Arg1762 and Leu1781 occur in a FYRN domain. FYRN and FYRC  
304 regions, particularly common in MLL histone methyltransferases, interact to form a  
305 compact structural unit (**Fig. 4c,h**) important in maintaining the active structure<sup>15,16</sup>.  
306 Arg1762 forms hydrogen bonds with the backbone carboxyls of Arg2463 and  
307 Leu2464 of FYRC domain. Substitution of Arg1762 by cysteine is predicted to  
308 abolish these contacts and hence contribute to destabilization of FYRC-FYRN  
309 association. Leu1781, at the interface between FYRN and FYRC (**Fig. 4h,i**) is  
310 surface exposed and involved in backbone hydrogen bonds stabilizing the beta  
311 sheet formed together by the two domains. Substitution to proline (p.Leu1781Pro) is  
312 predicted to disrupt the backbone hydrogen bond at this position, because it lacks  
313 one hydrogen bond donor and its backbone torsion angles are not compatible with  
314 that of a beta sheet. This predicts a destabilizing effect on sheet structure,  
315 potentially affecting the normal association of FYRN and FYRC domains. Arg2517  
316 resides in the region linking FYRC and SET domains, known to bind WDR5, an  
317 effector required for trimethylation of histone H3<sup>17</sup>, presenting methylated histone  
318 H3 substrates to the MLL complex for further methylation<sup>18</sup>. Arg2517 is thought to  
319 be involved in a salt-bridge interaction with Asp172 of WDR5 (NP\_438172.1) (**Fig.**  
320 **4j**) and Arg2517Trp is predicted to lead to loss of this interaction. Ile2674, Tyr2688

321 and Ile2694 all occur in the catalytic methyltransferase SET domain common to  
322 histone lysine methyltransferases. Ile2674 is buried in the hydrophobic core,  
323 adjacent to the catalytic site (**Fig. 4d,k**). Substitution to threonine is predicted to  
324 lead to loss of contacts at the core of the domain (due to the shorter side chain) and  
325 also introduces a buried polar group (**Fig. 4l**). p.Tyr2688Thr occurs at the core of  
326 SET domain involving extensive hydrophobic interactions and a hydrogen bond  
327 interaction with Ser2661 (**Fig. 4m**). The frameshift mutation p.Tyr2688Thrfs\*50,  
328 with insertion of 50 additional residues, is predicted to destabilise the core and  
329 affect contacts due to the substitution with a shorter non-aromatic side-chain.  
330 Ile2694 is involved in the extensive hydrophobic contacts stabilizing the core of this  
331 domain. *In silico* analysis predicts that the frameshift mutation p.Ile2694Serfs\*44  
332 will disrupt the domain fold and affect methyltransferase activity.

### 333 ***KMT2B is ubiquitously expressed with reduced expression in KMT2B-*** 334 ***dystonia***

335 We confirmed widespread *KMT2B* expression in a variety of control fetal and adult  
336 human tissues (**Fig. 5a, Supplementary Fig. 6**). Moreover, *KMT2B* is ubiquitously  
337 expressed in the brain with higher expression in the cerebellum than in any other  
338 region (**Fig. 5b**). We ascertained fibroblasts from all patients consented for  
339 research testing (Patients 2, 13, 14, 16, all with microdeletions or PPTVs in *KMT2B*)  
340 and detected a statistically significant decrease in fibroblast *KMT2B* expression on  
341 quantitative RT-PCR when compared to control fibroblasts (**Fig. 5c**).

### 342 ***Histone H3K4 methylation is not globally reduced in KMT2B-dystonia***

343 To determine the effect of *KMT2B* variants on methylation of lysine 4 on histone H3  
344 (H3K4 methylation), we assayed tri-methylated H3K4 (H3K4me3) and di-

345 methylated H3K4 (H3K4me2). Immunoblotting of histones extracted from fibroblasts  
346 of Patients 14 and 16 showed no significant reduction in H3K4me3 or H3K4me2  
347 relative to control samples (**Fig. 5d, Supplementary Fig. 7a**). A *Dictyostelium*  
348 *discoideum* model was used to test the effect of SET domain variant p.Ile2647Thr  
349 on *in vivo* histone methyltransferase activity. The SET domain of KMT2B shares  
350 56% sequence identity with the *Dictyostelium* orthologue DdSet1, and Ile2647 is  
351 conserved (corresponding residue in *Dictyostelium* is Ile1447, XP\_636258.1)  
352 (**Supplementary Fig. 8f**). DdSet1 is the only H3K4 methyltransferase in  
353 *Dictyostelium* and targeted knockout of *DdSet1* (*set1*<sup>-</sup>) results in loss of all  
354 methylation at H3K4<sup>19</sup>. We constitutively expressed wild-type DdSet1 (WT-DdSet1)  
355 and mutant-DdSet1 (m-DdSet1), both with N-terminal GFP fusions, in *set1*<sup>-</sup>  
356 *Dictyostelium* cells and compared the resulting levels of H3K4 methylation.  
357 Expression of either GFP-WT-DdSet1 or GFP-mDdSet1 in *set1*<sup>-</sup> cells resulted in  
358 rescue of H3K4 tri-methylation to wild type levels (**Fig. 5e, Supplementary Fig.**  
359 **7b,c**).

### 360 ***Altered gene and protein expression in KMT2B-dystonia***

361 In order to determine whether KMT2B-dystonia is associated with dysregulation of  
362 specific genes and proteins, we investigated (i) gene and protein expression  
363 profiles for THAP1 and Torsin-1A in cultured patient fibroblasts from Patients 2, 13,  
364 14 and 16 and (ii) tyrosine hydroxylase and dopamine 2 receptor (D2R) protein  
365 levels in cerebrospinal fluid from Patients 2 and 16 (**Supplementary Notes,**  
366 **Supplementary Fig. 9, Supplementary Fig. 10**). We found significantly reduced  
367 transcript levels of *THAP1* and *TOR1A* when compared to control fibroblasts  
368 (**Supplementary Fig. 11a**). Fibroblast immunoblotting studies showed a statistically

369 significant reduction in THAP1 protein expression in all 4 patients when compared  
370 to control samples (**Supplementary Fig. 11b**). A statistically significant reduction in  
371 Torsin-1A was evident in Patient 14, though not in other patients (**Supplementary**  
372 **Fig. 11c**). CSF immunoblotting revealed significantly reduced levels of dopamine 2  
373 receptor (D2R) and increase in tyrosine hydroxylase (TH) levels (**Supplementary**  
374 **Fig. 11d**).

375

## 376 **DISCUSSION**

377 We report 27 individuals with heterozygous variants in the lysine methyltransferase  
378 gene, *KMT2B*, and define a new genetic movement disorder that, importantly, is  
379 amenable to treatment with DBS. Using the current classification system<sup>2</sup>, *KMT2B*-  
380 dystonia is defined as an inherited autosomal dominant, complex, combined  
381 dystonia usually of infantile or childhood-onset. In most patients, the dystonia is  
382 persistent and progressive in nature. Most individuals develop 4-limb dystonia with  
383 particularly prominent cervical, laryngeal and oromandibular symptoms. Whilst the  
384 majority of patients seem to follow this disease trajectory, we also report one young  
385 case (Patient 10, age 7 years) with developmental delay and intermittent toe-  
386 walking only. Furthermore, we describe atypical cases with mainly oromandibular  
387 features (Patient 18) or paroxysmal cervical dystonia (Patient 26a) and relatively  
388 little upper or lower limb involvement.

389 For many patients, *KMT2B*-dystonia is associated with a number of additional  
390 clinical features including other neurological symptoms, intellectual disability,  
391 psychiatric co-morbidity, dysmorphia, skin lesions and other systemic signs. Given  
392 the association with active gene expression, it is conceivable that *KMT2B* variants  
393 could account for these additional disease features. For Patients 1-10, other genes

394 within the 19q microdeletion may also contribute to aspects of their clinical  
395 phenotype<sup>20</sup>. *KMT2B* variants therefore cause a complex dystonia, and affected  
396 patients should have close surveillance of development during childhood, regular  
397 neurology assessments, routine dermatological review and formal neuropsychiatric  
398 testing.

399 In *KMT2B*-dystonia, the majority of patients had a characteristic pattern on MR  
400 imaging, with subtle, low pallidal signal on T2<sup>\*</sup>-, diffusion- and susceptibility-  
401 weighted sequences, particularly affecting the lateral aspect of the globus pallidus  
402 externa (**Fig. 3**). Genotype did not appear to influence MR findings. However, those  
403 with abnormal imaging had scans undertaken at a significantly younger age than  
404 those with normal imaging. MR abnormalities may possibly be an age-dependent  
405 phenomenon, perhaps becoming less apparent with increasing age, as evident in  
406 serial imaging from Patient 22 (**Supplementary Table 7, Supplementary Fig.**  
407 **5b,c**). The overall significance of these neuroradiological abnormalities remains  
408 unclear. Such findings are reminiscent of, but subtly different to, those reported in  
409 Neurodegeneration with Brain Iron Accumulation (NBIA) syndromes<sup>21,22</sup>. Similar  
410 non-specific features of T2<sup>\*</sup>-weighted hypointensity are increasingly recognized in  
411 other neurological conditions, including Huntington's disease, *TUBB4A*-related  
412 disorders, GM1 gangliosidosis, alpha-fucosidosis and mitochondriopathies.

413 *KMT2B* variants were identified in 13/34 (38%) individuals with a relatively  
414 homogenous phenotype of early onset progressive dystonia. For externally  
415 screened cohorts, detection rates varied from 1.3-30% according to the phenotypic  
416 focus of the cohort (**Supplementary Fig. 1**). For cases where *KMT2B* mutations  
417 were not detected, it is likely that another etiology accounts for their symptoms.  
418 However, it is possible that *KMT2B* mutations may have been missed as (i)

419 single/multiple exon *KMT2B* deletions and duplications may not be detected on  
420 microarray, Sanger sequencing and whole exome/genome sequencing and (ii)  
421 promoter mutations and intronic *KMT2B* variants may not have been identified by  
422 whole exome and Sanger sequencing.

423 The majority of individuals with *KMT2B* variants (Patients 1-20) had either  
424 heterozygous interstitial microdeletions leading to *KMT2B* haploinsufficiency or  
425 variants predicted to cause protein truncation, protein elongation, splicing defects or  
426 nonsense-mediated mRNA decay. The remaining 7 patients (Patients 21-27) had  
427 non-synonymous variants of *KMT2B*. Although a degree of caution must be  
428 exercised for missense variants, those identified in our cohort are (i) described in  
429 patients with a compatible phenotype, (ii) predicted to affect conserved residues  
430 within key protein domains for 5/7 cases (**Supplementary Fig. 8, Supplementary**  
431 **Fig. 12**) and (iii) predicted by *in silico* tools to be deleterious with a destabilizing  
432 effect on protein structure (**Supplementary Table 3**). Initial disease presentation  
433 was significantly earlier in those with missense variants (**Supplementary Fig. 3**)  
434 though genotype did not seem to influence subsequent rate of symptom evolution,  
435 disease severity or DBS response.

436 For the majority of patients, *KMT2B* variants were confirmed as *de novo* where  
437 parental testing could be undertaken. In our cohort, 3 patients had missense  
438 changes that were maternally inherited (Patient 22, 26a, 27). The possibility of  
439 imprinting at the disease locus was considered, but deemed unlikely, given that (i)  
440 *de novo* microdeletions in Patients 2 and 10 occurred on paternally inherited alleles  
441 and (ii) there is bi-allelic expression of *KMT2B* single nucleotide polymorphisms in  
442 human tissues, including brain (**Supplementary Fig. 13**). Importantly, whole exome  
443 sequence analysis undertaken in Patients 22, 26a and 27 did not identify other rare

444 or *de novo* variants to account for disease. Interestingly, Patient 26a inherited  
445 p.Arg2517Trp from his symptomatic mother (26b) in whom the change occurred *de*  
446 *novo* (**Supplementary Fig. 2**). She was more mildly affected, with onset of  
447 symptoms in early adulthood, reporting gait abnormalities, progressive inability to  
448 run and periodic paroxysmal upper limb and neck dystonia. Both had similar facial  
449 appearances to others in the cohort (**Fig. 2g**). In contrast, the mothers of Patients  
450 22 and 27 were clinically examined and neither had evidence of a motor phenotype,  
451 intellectual disability, other neurological features, neuropsychiatric symptoms, facial  
452 dysmorphism, skin lesions or other systemic signs. The identification of both  
453 symptomatic and asymptomatic carriers suggests either 'apparent' incomplete  
454 penetrance, due to parental mosaicism, or true incomplete disease penetrance, a  
455 phenomenon commonly reported in other autosomal dominant genetic  
456 dystonias<sup>23,24</sup>. Other genetic, epigenetic and environmental modifiers may also  
457 influence disease penetrance and phenotypic presentation in KMT2B-dystonia.

458 *KMT2B* encodes a ubiquitously expressed lysine methyltransferase specifically  
459 involved in H3K4 methylation<sup>25,26</sup>, an important epigenetic modification associated  
460 with active transcription. H3K4me3 is enriched at promoters, marking transcription  
461 start sites of actively transcribed genes, whereas H3K4me1 is associated with  
462 active enhancer sequences<sup>27</sup>. H3K4me2 is less specifically localized, but may be  
463 enriched at transcription factor binding sites<sup>28</sup>. Members of the SET/MLL protein  
464 family, including KMT2B, are responsible for the generation of H3K4me1,  
465 H3K4me2, and H3K4me3 which are essential for gene activation in normal  
466 development<sup>29</sup>. Using patient-derived fibroblasts and a *Dictyostelium discoideum*  
467 model, we demonstrated that *KMT2B* variants are not associated with widespread  
468 alterations in overall levels of H3K4 methylation. This is not surprising, given that

469 haploinsufficiency of other MLL family members have not been convincingly shown  
470 to affect global H3K4 levels. The fundamental physiological role of MLL proteins is  
471 affirmed by the observation that loss-of-function heterozygous mutations in MLL-  
472 encoding genes are reported in a number of human developmental disorders<sup>30</sup>,  
473 namely Wiedemann Steiner (*KMT2A*, *MLL1*)<sup>31</sup>, Kleefstra-like (*KMT2C*, *MLL3*)<sup>32</sup> and  
474 Kabuki (*KMT2D*, *MLL2*)<sup>33</sup> syndromes, and most recently *SETD1A*-related disease  
475 (*KMT2F*)<sup>34</sup>. Although physiological functions of MLL proteins are yet to be fully  
476 characterized, the observation that mutations in different *MLL* genes cause  
477 phenotypically distinct syndromes (**Supplementary Table 8**) suggests that each  
478 MLL protein has a unique role, regulating the expression of a specific set of  
479 genes<sup>35,36</sup>.

480 Amongst the previously reported *MLL*-gene disorders, dystonia appears fairly  
481 specific to *KMT2B*-related disease and is not commonly described in other *MLL*  
482 syndromes (**Supplementary Table 8**), providing further evidence that different *MLL*  
483 proteins mediate the activation and transcription of a specific set of genes, with  
484 temporal and cellular context<sup>37</sup>. In order to determine downstream effects of *KMT2B*  
485 mutations, we investigated expression profiles of specific genes and proteins  
486 implicated in the pathogenesis of dystonia using patient-derived fibroblasts and  
487 CSF (**Supplementary Notes; Supplementary Fig. 9-11**). We detected a  
488 statistically significant reduction of *THAP1* and *TOR1A* gene expression and  
489 decreased THAP1 protein expression in fibroblasts. CSF immunoblotting studies  
490 revealed reduction of D2R protein and increase in TH levels in two patients with  
491 *KMT2B*-dystonia when compared to control CSF samples. The mechanisms  
492 causing such alterations in *KMT2B*-dystonia remain yet to be elucidated. Whilst  
493 H3K4 methylation is clearly associated with the process of active transcription,

494 several studies have shown that H3K4 methylation is required, not for absolute  
495 transcriptional output, but rather for transcription stability or consistency<sup>38,39</sup>, so the  
496 effects of *KMT2B* haploinsufficiency could conceivably operate via an intermediary  
497 sensitive to stochastic fluctuations. It is highly likely that dysregulation of other  
498 genes and proteins are also involved in the disease pathophysiology of KMT2B-  
499 dystonia. Further studies will determine whether expression profiles of other genes  
500 and proteins are affected in KMT2B-dystonia and contributory to the phenotype.

501 In conclusion, we report *KMT2B* variants in 27 patients with a clinically recognizable  
502 form of dystonia. To date, the underlying genetic etiology is only resolved in a  
503 minority of childhood-onset cases of dystonia, which precludes confirmatory  
504 diagnosis, accurate disease prognostication and selection of appropriate treatment  
505 strategies. We have shown that many patients with KMT2B-dystonia have  
506 significant, sustained clinical improvement with DBS. Referral for DBS assessment  
507 should therefore be considered for this group. Identification of additional cases will  
508 allow further characterization of the full phenotypic disease spectrum. Our report  
509 highlights mutations in *KMT2B* as a new and important cause of complex early-  
510 onset dystonia, emphasizing the crucial role of KMT2B in the control of voluntary  
511 movement.

512 **URLs:**

513 Exome Aggregation Consortium (ExAC) database (accessed July 2016)

514 <http://exac.broadinstitute.org>

515 DECIPHER

516 <http://decipher.sanger.ac.uk>

517 UK10K Project

518 <http://www.uk10k.org>

519 Deciphering Developmental Disorders (DDD) study

520 <http://www.ddduk.org/>

521 1000 Genomes

522 <http://browser.1000genomes.org/index.html>

523 NHLBI GO Exome Sequencing Project (release 20130513)

524 <http://evs.gs.washington.edu/EVS/>

525 Ensembl genome browser

526 <http://www.ensembl.org/index.html>

527 Primer3

528 <http://bioinfo.ut.ee/primer3/>

529 Chromas Sequencing software

530 <http://www.technelysium.com.au/chromas.html>

531 Clustal Omega

532 <http://www.ebi.ac.uk/Tools/msa/clustalo/>

533 SIFT

534 <http://sift.jcvi.org/>

535 PolyPhen-2

536 <http://genetics.bwh.harvard.edu/pph2/>

537 Mutation Taster

538 <http://www.mutationtaster.org/>

539 Combined Annotation Dependent Depletion (CADD)

540 <http://cadd.gs.washington.edu/>

541 BRAINEAC

542 <http://www.braineac.org>.

543 **Methods:**

544 Methods and any associated references are available in the online version of the  
545 paper.

546 **Accession codes:**

547 **Chromosomal microarray data:** Microarray data for Patient 1 (Ref: 326759),  
548 Patient 2 (Ref: 326749), Patient 3 (Ref: 326748), Patient 4 (Ref: 326751), Patient 5  
549 (Ref: 326750), Patient 6 (Ref: 326752), Patient 7 (Ref: 285035) and Patient 8 (Ref:  
550 280902) are deposited in DECIPHER. The data from Patient 1 (Ref: 326759) and  
551 Patient 8 (Ref: 280902) is publically available. The remaining patients did not  
552 consent for their data to be publicly released.

553 <https://decipher.sanger.ac.uk/search?q=326759#consented-patients/results>

554 <https://decipher.sanger.ac.uk/search?q=280902#consented-patients/results>

555 **NIHR BioResource-Rare Diseases (NIHRBR-RD) Study:** Whole genome  
556 sequencing data is deposited in the NIHR BioResource Rare Diseases BRIDGE  
557 consortium sequencing projects (short name: NIHR-BR-RD). Accession code:  
558 EGAS00001001012. Title of dataset: SPEED childhood dystonia KMT2B dataset:  
559 EGAD00001002730. Data is deposited for Patient 1 (Ref: EGAR00001314765);

560 Patient 13 (Ref: EGAR00001320121); Patient 14 (Ref: EGAR00001314777);  
561 Patient 17 (Ref: EGAR00001314751) and Patient 21 (Ref: EGAR00001314767).

562 <https://www.ebi.ac.uk/ega/home>

563 **UK10K Project:** UK10K whole exome sequencing data has been deposited under  
564 the name UK10K\_RARE\_FIND. Accession code: EGAS00001000128. Title of  
565 dataset: UK10K\_RARE\_FIND REL-2013-10-31 variant calling: EGAD00001000750  
566 Data is deposited for Patients 22 (Ref: UK10K\_FIND5536224) and 27 (Ref:  
567 UK10K\_FIND5536279).

568 <https://www.ebi.ac.uk/ega/studies/EGAS00001000128>

569 **Deciphering Developmental Disorders (DDD) study:** Exome sequencing data is  
570 accessible via the European Genome-phenome Archive (EGA) under accession  
571 EGAS00001000775.

572 <https://www.ebi.ac.uk/ega/studies/EGAS00001000775>

573 **National Institutes of Health, Bethesda; Institute of Human Genetics,**  
574 **Erlangen; Radboud University Medical Center, Nijmegen, UCL-Institute of**  
575 **Neurology, London:** Whole exome sequencing data has not been deposited since  
576 participating patients have not consented for the data to be publicly released.

577

578 **Note:**

579 Any Supplementary Information and Source Data files are available in the online  
580 version of the paper.

581

582 **Acknowledgements:**

583 We would like to thank all our patients and their families for taking part in this study  
584 and encouraging international collaboration to seek out similar cases. Thank you to

585 Dr Miho Ishida for kindly providing the fetal cDNA, Dr Karin Tuschl for kindly  
586 providing the human cDNA panel, Dr Lorenzo Bassioni for kindly selecting  
587 DaTSCAN images for the Supplementary manuscript, Dr Matthew Adams for  
588 reviewing the imaging of the NHNN patients and Rowdy Meijer for helping with the  
589 sequencing analysis at the Department of Human Genetics (Nijmegen). We would  
590 like to thank Professors Gudrun Moore and Philip Stanier for proof-reading the  
591 manuscript. Many thanks to Aybeniz Panahian-Jand for excellent administrative  
592 support. M.A.K. has a Wellcome Intermediate Clinical Fellowship (WT098524MA).  
593 E.M. and M.A.K. received funding from the Rosetrees Trust, Great Ormond Street  
594 Hospital Children's Charity and Gracious Heart Foundation. N.E.M. receives  
595 support from the Department of Health's National Institute for Health Research  
596 Biomedical Research Centers funding streams. A.P. has a joint Action Medical  
597 Research/ British Paediatric Neurology Association Research Training Fellowship.  
598 J.N. has a Medical Research Council Research Training Fellowship. A.N. has an  
599 Action Medical Research Training Fellowship. H.B.P. has a DBS training travel  
600 grant from the Daniel Turnberg Trust Fund. H.H. is funded by MRC and Wellcome  
601 Trust (Synaptopathies award). D.A. is supported by the Prusiner-Abramsky Award.  
602 H.P. has received grant support from The Dystonia Society (UK). K.J.P. has an  
603 Academy of Medical Sciences Clinical Starter Grant. B.P.D. received funding from  
604 grants 20143130-La Marató de TV3 and PI15/00287-Ministerio Español de  
605 Economía y Competitividad. J-P.L. has been supported by a Guy's and St Thomas  
606 Charity New Services and Innovation Grant: G060708; The Dystonia Society (UK):  
607 Grants 01/2011 and 07/2013 and an Action Medical Research: AMR - GN2097.  
608 This research was supported by the National Institute for Health Research  
609 Biomedical Research Centre at Great Ormond Street Hospital for Children NHS

610 Foundation Trust, University College London, University of Cambridge and from the  
611 NIHR for the BioResource for Rare Diseases (grant number RG65966). This study  
612 makes use of data generated by the DECIPHER community. A full list of centres  
613 contributing to the generation of the data is available from  
614 <http://decipher.sanger.ac.uk> and via email from [decipher@sanger.ac.uk](mailto:decipher@sanger.ac.uk). Funding  
615 for the project was provided by the Wellcome Trust for UK10K (WT091310) and  
616 DDD Study. The DDD study presents independent research commissioned by the  
617 Health Innovation Challenge Fund [grant number HICF-1009-003] - see  
618 [www.ddduk.org/access.html](http://www.ddduk.org/access.html) for full acknowledgement. This work was supported in  
619 part by the Intramural Research Program of the National Human Genome Research  
620 Institute and the Common Fund, NIH Office of the Director. This work was  
621 supported in part by the German Ministry of Research and Education (grant nos.  
622 01GS08160 and 01GS08167; German Mental Retardation Network) as part of the  
623 National Genome Research Network to A.R. and D.W. and by the Deutsche  
624 Forschungsgemeinschaft (AB393/2-2) to A.R. Brain expression data was provided  
625 by the UK Human Brain Expression Consortium (UKBEC), which comprises John  
626 A. Hardy, Mina Ryten, Michael Weale, Daniah Trabzuni, Adaikalavan Ramasamy,  
627 Colin Smith and Robert Walker, affiliated with UCL Institute of Neurology (J.H.,  
628 M.R., D.T.), King's College London (M.R., M.W., A.R.) and the University of  
629 Edinburgh (C.S., R.W.).

630

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632 E.M., K.J.C., J.M.E.N., J.R.C., F.L.R. and M.A.K. conceived and designed  
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634 N.F., N.G., A.H., H.H., J.A.H., Z.I., M.K., P.L., D.L., S.Mc., S.M., S.S.M., V.N., J.Ni.,

635 M.N., H.P., K.J.P., G.B.P., P.P., M.S.R., P.R., R.S., M.Si., M.Sm., P.T., S.M.W.,  
636 D.W., B.T.W., G.W., UK10K Consortium, DDD study, NIHRBR-RD study, L.J.C.,  
637 B.P-D., J-P.L., A.R., W.A.G., C.T., K.P.B., N.W.W., E-J.K., P.G., R.C.D., F.L.R. and  
638 M.A.K. ascertained patients, contributed clinical information, photographs, videos  
639 and neuroimaging studies. M.A.K. performed phenotypic characterization of all  
640 patients. W.K.C. and M.A.K. reviewed patient neuroimaging. A.P. and M.A.K. edited  
641 patient videos. A.Bo., C.W. and D.M. undertook chromosomal microarray analysis.  
642 E.M., K.J.C., D.G., N.E.M., S.W., A.Pi., UK10K Consortium, DDD study, NIHRBR-  
643 RD study, A.R., W.A.G., C.T., E-J.K. and M.A.K. carried out whole exome/genome  
644 sequencing analysis. E.M. and A.N. performed variant validation by direct Sanger  
645 Sequencing. K.J.C. performed enrichment analysis (and corresponding statistical  
646 analysis). S.P. and S.J.H.H. analyzed CSF neurotransmitters. A.P.J. and M.T.  
647 undertook comparative homology modelling. J.M.E.N. and J.R.C. undertook the  
648 histone methylation assay (and corresponding statistical analysis) and cloning of  
649 Set1 Point Substitution in Dictyostelium. S.B. generated dopaminergic neurons,  
650 collected RNA and cDNA samples and undertook quantitative RT-PCR  
651 experiments. E.M. maintained fibroblast cultures, collected RNA, cDNA and protein  
652 samples, performed fibroblast immunoblotting analysis (and corresponding  
653 statistical analysis) and CSF immunoblotting (and corresponding statistical  
654 analysis). J.N. carried out CSF immunoblotting analysis. P.G. and F.L.R.  
655 contributed critical suggestions for experimental work. E.M. and M.A.K. wrote the  
656 manuscript. K.J.C., J.R., J.M.E.N., D.G., A.P.J., N.E.M., A.R., W.A.G., C.T., E-J.K.,  
657 W.K.C., M.T., J.R.C. and F.L.R. contributed written sections for manuscript. M.A.K.  
658 oversaw the overall project. All authors critically reviewed manuscript.

659

660 **Competing financial interests**

661 H.P. has unrestricted support for Educational Activity from Medtronic.

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## 770 **Figure legends**

### 771 **Figure 1:**

#### 772 **Molecular Genetics Findings in Patients with *KMT2B* Variants**

773 (a) Top panel: Schematic representation of chromosome 19. Middle panel: Ten  
774 microdeletions on 19q13.11-19q13.12 (GRCh37/hg19). Lower panel: The smallest region  
775 of overlap comprising two genes, *ZBTB32* and *KMT2B*. (b) Schematic exon-intron  
776 structure of *KMT2B* (NCBI Reference Sequence: NM\_014727.2) indicating 7 frameshift  
777 insertions and deletions, 2 stop-gain mutations, 1 splice site variant and 7 missense  
778 changes.

779

### 780 **Figure 2:**

#### 781 **Clinical Features of Patients with *KMT2B* Variants**

782 (a) Patient 17, age 13 years: gait disturbance with dystonic posturing of the four limbs. (b)  
783 Patient 27, age 19 years and (c) Patient 14, age 18 years: bilateral upper limb dystonic  
784 posturing. (d,e) Patient 23, age 8 years: retrocollis. (f) Patient 12, age 6 years: generalized  
785 dystonia, with jaw-opening dystonia and 4-limb posturing. (g) Montage of patient faces:  
786 Top row (left to right) Patients 1, 2, 3, 4, 8, 9; middle row (left to right) Patients 11, 12, 13,  
787 14, 16, 17 and bottom row (left to right) Patients 21, 23, 25, 26a, 26b. Consent to publish  
788 patient photographs has been obtained. Facial elongation, broad nasal base and bulbous  
789 nasal tip evident in some patients.

790

### 791 **Figure 3:**

#### 792 **Radiological Features of Patients with *KMT2B* Variants**

793 Magnetic resonance imaging (MRI) with T2\*-weighted (a,d) and T2-weighted images (b,c),  
794 echo-planar technique diffusion-imaging datasets images with b-value of zero (e-h) and  
795 susceptibility weighted sequences (i-l). Abnormal findings indicated by yellow arrows.

796 (a,e,i) Representative MRI from control subjects for T2\*-weighted sequences (a: age  
797 10y2m), diffusion-weighted sequences (e: age 10y4m) and susceptibility weighted  
798 sequences (i: age 10y8m) indicating normal appearances of basal ganglia. Patient 1, age  
799 9y5m (b,f,j), Patient 13, age 11y3m (c,g,k), Patient 9, age 15y1m (d), Patient 22, age  
800 13y1m (h) and Patient 25, age 16y (l): evidence of bilateral subtle hypointensity of the  
801 globus pallidus with hypointense lateral streak of globus pallidus externa.

802

803 **Figure 4:**

#### 804 **Comparative Modelling of KMT2B Protein Structure**

805 (a) Schematic domain architecture of KMT2B. (b-d) Degree of amino conservation is  
806 displayed in the structural models for different domains. Red to blue indicates increasing  
807 conservation. (b) Model of PHD-like domain shows Gly1652 and Phe1662. (c) Model of  
808 FYRN domain presents position and conservation of Arg1762 and Leu1781. (d) Model of  
809 the SET methyltransferase domain indicates position and conservation of Ile2674,  
810 Tyr2688 and Ile2694. (e) Location of Gly1652 in the PHD-like domain model and the  
811 hydrogen bond network in the vicinity ( $\alpha$ 3 helix is indicated). (f) Hydrophobic packing  
812 involving Phe1662. (g) Change to leucine at 1662 is predicted to cause loss of contacts  
813 within the hydrophobic core. Residue side chains are presented as spheres highlighting  
814 van der Waals contacts. (h) Interactions involving Arg1762 from FYRN with Arg2463 and  
815 Leu2464 of FYRC. The hydrogen bond interactions and distances are highlighted. (i)  
816 Leu1781 shown at the interface of FYRN (orange)/FYRC (magenta) domains. The  
817 backbone hydrogen bonds stabilizing the sheet structure are highlighted. (j) Interactions  
818 involving Arg2517 and WDR5 (beige). The salt bridge interaction between Arg2517 of  
819 KMT2B and Asp172 of WDR5 is highlighted. (k) Location and contacts involving Ile2674 in  
820 the hydrophobic core of the SET domain (SAH is indicated). (l) Substitution with threonine  
821 at 2674 is predicted to result in loss of contacts in the hydrophobic core. (m) Interactions

822 involving Tyr2688 and Ile2694 in the core of the SET domain. The hydrogen bond  
823 between Tyr2688 and Ser2661 is highlighted.

824

825 **Figure 5:**

826 ***KMT2B* Expression and Effects on Histone H3K4 Methylation**

827 (a) PCR analysis of human fetal and adult cDNA for expression of *KMT2B* (cropped gel  
828 image; for uncropped image see **Supplementary Fig. 6**). *KMT2B* is widely expressed in  
829 human tissues, including fibroblasts, brain tissue and midbrain dopaminergic neurons. (b)  
830 Box plots of *KMT2B* mRNA expression levels in 10 adult brain regions (source:  
831 BRAINEAC; <http://www.braineac.org/>). Expression levels are based on exon array  
832 experiments as previously described and plotted on a log<sub>2</sub> scale (y axis)<sup>40</sup>. *KMT2B* is  
833 ubiquitously expressed across all 10 brain regions analyzed, with expression highest in the  
834 cerebellum. Putamen (PUTM), frontal cortex (FCTX), temporal cortex (TCTX), occipital  
835 cortex (OCTX), hippocampus (HIPPO), substantia nigra (SNIG), medulla (specifically inferior  
836 olivary nucleus, MEDU), intralobular white matter (WHMT), thalamus (THAL), and  
837 cerebellar cortex (CRBL). “N” indicates the number of brain samples analyzed to generate  
838 the results for each brain region. Whiskers extend from the box to 1.53 the interquartile  
839 range. (c) Quantitative RT-PCR indicates that patients with *KMT2B* mutations (n = 4) have  
840 significantly decreased fibroblast mRNA levels of *KMT2B* when compared to controls (n =  
841 2) (Controls = 1.01±0.16SD; Patients = 0.57±0.12SD). n = 3 technical replicates were  
842 analyzed per sample. Data were analyzed by two-tailed unpaired t-test: \*P = 0.0182 (t =  
843 3.856, df = 4). No significant difference in variances between the groups was detected by  
844 F-test. (d) Histone methylation was assayed independently in three samples (n = 3;  
845 technical replicates) taken from each patient-derived fibroblast cell line (n = 2; Patient 14  
846 and 16) on different days, and compared with control cell lines (n = 2). Methylation values  
847 are normalized to pan-histone H3 levels. Individual data-points are plotted with center bar

848 showing mean and error bars showing standard deviation. Differences between control  
849 and patient-derived samples are not significant (H3K4me3 (left): Controls =  
850 96.63±19.98SD; Patient 16 = 104.1±40.31SD; Patient 14 = 94.75±38.36SD; H3K4me2  
851 (right): Controls = 94.33±19.25SD; Patient 16 = 127.8±20.79SD; Patient 14 =  
852 80.23±31.09SD). Data were analyzed by one-way ANOVA: H3K4me3: P = 0.9196 (F =  
853 0.08462, DF<sub>n</sub> = 2, DF<sub>d</sub> = 9); H3K4me2: P = 0.0727 (F = 3.557, DF<sub>n</sub> = 2, DF<sub>d</sub> = 9). (e)  
854 Quantification of immunoblotting of tri-methyl H3K4 in *Dictyostelium* cell lysates. Tri-methyl  
855 H3K4 intensity values are normalized against levels of total histone H3. H3K4 tri-  
856 methylation is impaired in set1<sup>-</sup> cells compared to wild type. Expression of GFP-DdSet1 or  
857 GFP-DdSet1(I1447T) in set1<sup>-</sup> cells rescues levels of H3K4Me3. Three independent point-  
858 mutant cell lines (GFP-DdSet1(I1447T) 1-3) were created using the same point-mutant  
859 DNA construct. Individual data-points (three independently prepared samples taken from  
860 each cell line; n= 3, technical replicates) are plotted with center bar showing mean and  
861 error bars showing standard deviation (Wild type = 115±48.25SD; set1<sup>-</sup> = 5.94±9.37SD;  
862 set1<sup>-</sup> GFP-DdSet1(I1447T) 1 = 133.7±38.11SD; set1<sup>-</sup> GFP-DdSet1(I1447T) 2 =  
863 129.8±42.34SD; set1<sup>-</sup> GFP-DdSet1(I1447T) 3 = 96.07±31.82SD; set1<sup>-</sup> GFP-DdSet1 =  
864 110.5±12.02SD). No statistical testing was applied.

365 **Table 1: KMT2B Variants and Evolution of Motor Phenotype in KMT2B-dystonia**

Pat	Age (y) Sex M/F	KMT2B variants <sup>(a)</sup>	Symptoms at presentation: Body distribution & motor features	Onset of symptoms (y)	Bilateral LL involvement (y)	Bilateral UL involvement (y)	Onset of cranial, cervical, laryngeal dystonia (y)	Symptoms of cranial, cervical, laryngeal dystonia	Trial of medication and clinical response	Deep brain stimulation (DBS)
1	14 M	Deletion: Chr19: 35,608,666- 36,233,508	RLL Right foot posturing Gait disturbance	4	6	6-11	5	Dysarthria Dysphonia Swallowing difficulties	L-dopa trial – no benefit	No
2	14 F	Deletion: Chr19: 35,197,252- 38,140,100	Bilateral LL Limping Gait disturbance	7	7	8-11	8	Dysarthria Dysphonia Drooling	L-dopa trial – no benefit BLF – no benefit	No
3	9 M	Deletion: Chr19: 34,697,740- 37,084,510	RLL Right foot posturing Gait disturbance	2.5	3	6-7	4	Dysarthria Dysphonia Swallowing difficulties Drooling	GBP – some reduction in tone	No
4	11 F	Deletion: Chr19: 36,191,100- 36,376,860	LLL Left toe walking Gait disturbance	4	8	9-12	5	Dysarthria Dysphonia Swallowing difficulties Drooling	L-dopa trial – minimal benefit THP – minimal benefit	Planned for 2016
5	20 M	Deletion: Chr19: 31,725,360- 36,229,548	Developmental delay Gait disturbance	Present but age of onset not known	Present but age of onset not known	Present but age of onset not known	Not known	Nasal voice	None	No
6	10 F	Deletion: Chr19: 35,017,972- 36,307,788	RLL Right foot inversion	2.5	4	4	4-7	Dysarthria/anarthria Jaw-opening dystonia Swallowing difficulties NGF 6y PEG 8y Torticollis Severe retrocollis	L-dopa trial – no benefit THP – no benefit	Inserted age 7y Sustained excellent clinical benefits 3y post-DBS, marked improvement in torticollis, retrocollis, manual abilities and left leg dystonia. Loss of efficacy when 'DBS off' for almost a year and functional recovery when switched on again.
7	21 M	Deletion: Chr19: 35,414,997- 37,579,142	RLL Right foot dragging Gait disturbance	7	7-8	13	13	Dysarthria Dysphonia Swallowing difficulties	L-dopa trial – no benefit BLF – no benefit	No

Pat	Age (y) Sex M/F	KMT2B variants <sup>(a)</sup>	Symptoms at presentation: Body distribution & motor features	Onset of symptoms (y)	Bilateral LL involvement (y)	Bilateral UL involvement (y)	Onset of cranial, cervical, laryngeal dystonia (y)	Symptoms of cranial, cervical, laryngeal dystonia	Trial of medication and clinical response	Deep brain stimulation (DBS)
8	17 F	Deletion: Chr19: 35,414,997- 37,579,142	RLL Right foot posturing	4	6	4-12	2.5	Dysarthria Dysphonia Drooling Torticollis	L-dopa trial – no benefit	Inserted age 10y Good response over 6 years, particularly evident after replacement of faulty right DBS lead
9	14 M	Deletion: Chr19: 35,967,904- 37,928,373	Bilateral LL Gait disturbance	4	4	9-13	9	Dysarthria Dysphonia	L-dopa trial – possible initial benefit but not sustained	Inserted age 14y Very good clinical response at 4m post-DBS with restoration of independent ambulation
10	7 F	Deletion: Chr19: 35,794,775- 38,765,822	Bilateral LL Intermittent toe walking Gait disturbance	4	4	-	-	-	None	No
11	25 F	c.402dup p.Ser135Glnfs*23	RUL Right hand cramps and posturing	6	12	12	14 <sup>(b)</sup>	Anarthria Orolingual dystonia Tongue thrusting Swallowing difficulties PEG	L-dopa trial – poorly tolerated, no benefit	Being considered
12	6 F	c.1690C>T p.Arg564*	Bilateral LL Toe walking	4	5	6	5	Dysarthria Swallowing difficulties	L-dopa trial – no benefit	No
13	11 M	c.3026_3027del p.Glu1009Glyfs*9	Bilateral UL Posturing, tremor Difficulty handwriting	8	9-10	8	9	Dysarthria Dysphonia	L-dopa trial – no benefit	No
14	18 M	c.3143_3149del p.Gly1048Glufs*132	Bilateral UL Posturing of hands Myoclonic jerks	8	13	8	13	Dysarthria Dysphonia Swallowing difficulties	L-dopa trial – no benefit	No
15	20 F	c.4545C>A p.Tyr1515*	Bilateral LL Toe Walking Clumsy	2	9	9	8.5	Dysarthria Dysphonia Oromandibular dystonia Swallowing difficulties PEG 18y	Moderate responses to (and currently taking) THP CLZ L-dopa BLF	No

Pat	Age (y) Sex M/F	KMT2B variants <sup>(a)</sup>	Symptoms at presentation: Body distribution & motor features	Onset of symptoms (y)	Bilateral LL involvement (y)	Bilateral UL involvement (y)	Onset of cranial, cervical, laryngeal dystonia (y)	Symptoms of cranial, cervical, laryngeal dystonia	Trial of medication and clinical response	Deep brain stimulation (DBS)
16	6 F	c.4688del p.Ala1563Aspfs*83	Bilateral LL Increasing falls Gait disturbance	3	3	5	6	Dysarthria Dysphonia	L-dopa trial – no benefit THP – initial benefit, not sustained	No
17	17 M	c.6515_6518delinsC CCAA p.Val2172Alafs*11	Bilateral LL Toe walking Gait disturbance	1	1	8	12	Dysarthria Dysphonia Swallowing difficulties	L-dopa trial – no benefit TBZ – no benefit BLF and THP – mild benefit	Inserted age 16y Very good clinical response 4m post-DBS with restoration of independent ambulation
18	20 F	c.8061del p.Tyr2688Thrfs*50	Clumsy movements Difficulties with speech articulation	1	-	-	Infancy	Dysarthria Dysphonia Swallowing and chewing difficulties	No	No
19	28 M	c.8079del p.Ile2694Serfs*44	Bilateral LL Toe walking Severe speech delay	2	3	4 (L>R)	7	Anarthria Jaw opening dystonia Tongue protrusion Swallowing difficulties PEG 8y L torticollis, R laterocollis	L-dopa trial – no benefit THP and TBZ reduced tongue protrusion	Inserted age 27y Improvement of jaw opening dystonia and tongue protrusion
20	40 M	c.3528+2T>A	LLL Gait disturbance L foot dragging Clumsiness	4	5	8	10	Severe dysarthria Dysphonia L torticollis	L-dopa trial – no benefit TBZ, THP, SUL – no benefit	Inserted age 32y – no benefit. Electrode replaced in 2009 with sustained improvement in foot posture but only transient benefit to cervical, UL and LL dystonia
21	18 M	c.4955G>A p.Gly1652Asp	RLL Right leg posturing	6	8	12	5	Dysarthria Dysphonia Swallowing difficulties	L-dopa trial – no benefit THP – not tolerated	Inserted age 15y Sustained clinical benefit 3y post-DBS, improved dystonia and independent walking
22	20 F	c.4986C>A p.Phe1662Leu	RLL Right foot posturing Abnormal gait	5	8	5-13	5-6	Dysarthria Dysphonia Swallowing difficulties Torticollis	L-dopa trial – no benefit BLF – no benefit THP – low dose, mild benefit BTX neck – reduction in pain, no functional benefit	Inserted age 20y Very good clinical response 9m post-DBS with improved dystonia and independent walking

Pat	Age (y) Sex M/F	KMT2B variants <sup>(a)</sup>	Symptoms at presentation: Body distribution & motor features	Onset of symptoms (y)	Bilateral LL involvement (y)	Bilateral UL involvement (y)	Onset of cranial, cervical, laryngeal dystonia (y)	Symptoms of cranial, cervical, laryngeal dystonia	Trial of medication and clinical response	Deep brain stimulation (DBS)
23	8 M	c.5114G>A p.Arg1705Gln	Bilateral LL Toe-walking	3	3	6	6.5	Dysarthria Torticollis	L-dopa trial – no benefit CLZ, THP, IT BLF – some benefit	Inserted age 7y with considerable benefit
24	27 F	c.5284C>T p.Arg1762Cys	LLL Tiptoe walking and in-turning of L foot	6	6	7	7	Dysarthria Anarthria from 14-15y Reduced tongue movements Swallowing preserved	L-dopa trial – no benefit THP- no benefit	No
25	19 F	c.5342T>C p.Leu1781Pro	RLL Right foot posturing Gait disturbance	8	12	13	10	Dysarthria Dysphonia Swallowing difficulties Torticollis	L-dopa trial – no benefit LVT – mild benefit	Inserted age 19y Very good clinical response 4m post-DBS with improved dystonia and ambulation <sup>(c)</sup>
26a	8 M	c.7549C>T p.Arg2517Trp	Delayed speech Delayed motor development	8	-	-	8	Severe paroxysmal retrocollis and jaw dystonia	-	No
26b	46 F	c.7549C>T p.Arg2517Trp	Bilateral UL UL posturing Torticollis Inability to walk long distances and run	23	26	23	23	Dysphonia Torticollis	None	No
27	19 F	c.8021T>C p.Ile2674Thr	RUL Posturing, tremor Difficulty handwriting Myoclonic jerks	9	11-13	10	9-10	Dysphonia	L-dopa trial – no benefit THP – no benefit LVT – no benefit CBZ – initial benefit, not sustained CLZ – not tolerated	No

BLF: baclofen; BTX: botulinum toxin; CLZ: clonazepam; GBP: gabapentin; IT: intrathecal; L: left; LL: lower limbs; LLL: left lower limb; LVT: levetiracetam; m: months; NGF: nasogastric feeding; Pat: patient; PEG: percutaneous endoscopic gastrostomy; R: right; RLL: right lower limb; RUL: right upper limb; SUL: sulphiride; UL: upper limbs; TBZ: tetrabenzine; THP: trihexyphenidyl; y: years

<sup>(a)</sup> based on NCBI Reference Sequence: NM\_014727.2

<sup>(b)</sup> onset shortly after being fitted with orthodontic braces

<sup>(c)</sup> had undergone 2 posterior cranial fossa explorations and palatal surgery before DBS

375 Table 2: Additional Clinical Features in Patient with *KMT2B* Variants

Patient	<i>KMT2B</i> variants	Number of genes in microdeletion	Intellectual disability	Dysmorphic features	Additional neurological features	Psychiatric features	Abnormal skin features	Other systemic manifestations
1	Deletion: Chr19: 35,608,666-36,233,508	38	Mild	Elongated face	Not reported	Not reported	Not reported	Not reported
2	Deletion: Chr19: 35,197,252-38,140,100	124	No	Elongated face Bulbous nasal tip	Not reported	Not reported	Not reported	Not reported
3	Deletion: Chr19: 34,697,740-37,084,510	109	Moderate	Elongated face	Not reported	Not reported	Cutis aplasia <sup>(a)</sup>	Retinal dystrophy
4	Deletion: Chr19: 36,191,100-36,376,860	14	V mild - subtle memory problems	Elongated face Broad nasal bridge Bulbous nasal tip	Not reported	Prone to anxiety <sup>(b)</sup>	Not reported	Not reported
5	Deletion: Chr19: 31,725,360-36,229,548	110	Moderate	Sparse hair Blepharophimosis Absent eyelashes of lower eyelids Low set, posteriorly rotated ears Epicanthic folds Narrow nasal bridge, ridge and point Largely bifid tongue Micrognathia Teeth overcrowding Finger contractures 5 <sup>th</sup> finger clinodactyly Toe over-riding Dysplastic toenails	Microcephaly	Not reported	Occipital cutis aplasia	Small echogenic kidneys with low GFR, required renal transplant at 17 years
6	Deletion: Chr19: 35,017,97-36,307,788	69	No	Not reported	Microcephaly	Not reported	Not reported	Not reported
7	Deletion: Chr19: 35,414,997-37,579,142	99	Mild	Elongated face	Absence seizures	Not reported	Not reported	Absent right testis
8	Deletion: Chr19: 35,414,997-37,579,142	99	Mild	5 <sup>th</sup> finger clinodactyly	Not reported	Not reported	Ectodermal dysplasia	Not reported
9	Deletion: Chr19: 35,967,904-37,928,373	79	Mild	Elongated face	Strabismus	Not reported	Not reported	Cleft palate
10	Deletion: Chr19: 35,794,775-38,765,822	111	Moderate	Not reported	Strabismus	Not reported	Not reported	Short stature Bronchiectasis

Patient	KMT2B variants	Number of genes in microdeletion	Intellectual disability	Dysmorphic features	Additional neurological features	Psychiatric features	Abnormal skin features	Other systemic manifestations
11	c.402dup p.Ser135Glnfs*23	-	No	Bulbous nasal tip	Not reported	Not reported	Not reported	Not reported
12	c.1690C>T p.Arg564*	-	Moderate	Elongated face Bulbous nasal tip, short nasal root, Hypertelorism, large mouth with full lower lip	Epilepsy	Not reported	Not reported	Not reported
13	c.3026_3027del p.Glu1009Glyfs*9	-	V mild - difficulties with attention	Elongated face	Not reported	Not reported	Not reported	Not reported
14	c.3143_3149del p.Gly1048Glufs*132	-	No	Elongated face Bulbous nasal tip	Not reported	Not reported	Not reported	Not reported
15	c.4545C>A p.Tyr1515*	-	No	Elongated face Bulbous nasal tip	Not reported	Not reported	Not reported	Not reported
16	c.4688del p.Ala1563Aspfs*83	-	No	Elongated face	Not reported	Not reported	Not reported	Not reported
17	c.6515_6518delinsCCCAA p.Val2172Alafs*11	-	No	Elongated face	Not reported	Not reported	Phimosos	Short stature
18	c.8061del p.Tyr2688Thrfs*50	-	Mild	Micrognathia Atrophic tongue Bulbous nasal tip 5 <sup>th</sup> finger clinodactyly	Not reported	Not reported	Not reported	Not reported
19	c.8079del p.Ile2694Serfs*44	-	No	Short stature	Delay in saccade initiation and hypometric vertical saccades	ADHD <sup>(3)</sup> with no response to Ritalin	Not reported	Not reported
20	c.3528+2T>A	-	Moderate 6y- verbal IQ 74 Performance IQ 87 No cognitive decline	Not reported	Not reported	Not reported	Not reported	Not reported
21	c.4955G>A p.Gly1652Asp	-	Mild	Elongated face	Not reported	Not reported	Not reported	Short stature Hypertrichosis
22	c.4986C>A p.Phe1662Leu	-	No	Elongated face Bulbous nasal tip	Not reported	Not reported	Not reported	Not reported

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Patient	KMT2B variants	Number of genes in microdeletion	Intellectual disability	Dysmorphic features	Additional neurological features	Psychiatric features	Abnormal skin features	Other systemic manifestations
23	c.5114G>A p.Arg1705Gln	-	Mild-moderate 6y WISC-IV 50-60	Elongated face Bulbous nasal tip Broad philtrum, Upslanted eyes, epicanthus, low-set ears, periorbital fullness, gap between front teeth	Spasticity in lower limbs from 6y	Not reported	Ichthyotic skin lesions with criss-cross pattern under the feet and at knees, broad scarring after operation	Episodic vomiting
24	c.5284C>T p.Arg1762Cys	-	No	Short stature	Oculomotor apraxia with difficulty initiating saccades. Mild spasticity	No	Not reported	Not reported
25	c.5342T>C p.Leu1781Pro	-	No	Elongated face Bulbous nasal tip	Not reported	Not reported	Not reported	Not reported
26a	c.7549C>T p.Arg2517Trp	-	No	Bulbous nasal tip	None	ADHD <sup>(c)</sup> Currently on methyphenidate, oxazepam, risperidone	Not reported	Not reported
26b	c.7549C>T p.Arg2517Trp	-	No	Bulbous nasal tip	Idiopathic intracranial hypertension – on acetazolamide	None	Not reported	Not reported
27	c.8021T>C p.Ile2674Thr	-	V subtle mild learning difficulties	Bulbous nasal tip	Not reported	Anxiety Self-harm behavior Depression Obsessive- compulsive traits <sup>(d)</sup>	Not reported	Not reported

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(a) Supplementary Figure 4c

(b) Identified on formal psychology review

(c) Diagnosed by psychiatrist and under regular psychiatry review

(d) Under regular review with psychiatrist (ICD-10-CM F06.30; ICD-10-CM F42)

ADHD: attention deficit hyperactivity disorder; GFR: glomerular filtration rate; V: very; y: years

885 **ONLINE METHODS**

886 **(1) Case Ascertainment**

887 Case ascertainment is summarized in **Supplementary Table 1** and  
888 **Supplementary Fig. 1**. At Great Ormond Street-Institute of Child Health (GOS-  
889 ICH), we identified 34 patients referred to our center with undiagnosed dystonia  
890 (**Supplementary Table 1**). All patients (median age 13.5 years), presented with  
891 progressive dystonia, with disease onset in childhood. None had a clinical history or  
892 neuroimaging compatible with acquired dystonia, nor blood, urine or CSF biomarker  
893 evidence of an underlying neurometabolic disorder. We used established national  
894 and international clinical genetic and pediatric neurology networks to identify further  
895 patients with microdeletions similar to those detected in the GOS-ICH cohort  
896 (**Supplementary Fig. 1**). We also collaborated with research groups undertaking  
897 whole exome sequencing in patients with early-onset dystonia (**Supplementary**  
898 **Fig. 1**).

899 **(2) Molecular Genetic Analysis**

900 Genomic DNA was extracted from peripheral lymphocytes by standard techniques.  
901 Written informed consent was obtained from participants, and all studies approved  
902 by local ethics committees: National Research Ethics Service (NRES), London  
903 Bloomsbury REC:13/LO/0168, Cambridge South REC:10/H0305/83; Republic of  
904 Ireland REC:GEN/284/12; Human Research Ethics Committee (HREC),  
905 HREC:10/CHW/114, 10/CHW/45; National Human Genome Research Institute  
906 Institutional Research Board 76-HG-0238; Universities of Essen-Duisburg and  
907 Erlangen-Nürnberg ethics committees Ref.3769; Medical Review Ethics Committee  
908 Region Arnhem-Nijmegen, Ref:2011/188; UCL ethics committee, UCLH 06/N076.

909 Research was performed in accordance with the Declaration of Helsinki. Additional  
910 consent for publication of photographs and videos was provided.

### 911 **Chromosomal Microarray**

912 Patients were analyzed for copy number variants using chromosomal microarray by  
913 standard diagnostic techniques (**Supplementary Table 2**). Data is presented as  
914 minimum coordinates in GRCh37/hg19.

### 915 **Whole Exome and Genome Sequencing (WES/WGS)**

916 WES/WGS was undertaken using center-specific protocols (see below). Reads  
917 were aligned to the reference genome GRCh37/hg19. Detailed variant analysis was  
918 performed for single nucleotide variants (SNVs) and small insertion/deletions  
919 (indels) that (i) passed standard local quality filters, (ii) were predicted to alter  
920 protein sequence in conserved residues, (iii) were predicted deleterious by  
921 bioinformatics tools (including SIFT, PolyPhen-2, LRT, Mutation Taster, CADD), (iv)  
922 had an allele frequency <0.01 in 1000 Genomes<sup>41</sup>, NHLBI GO Exome Sequencing  
923 Project, UK10K<sup>42</sup>, Exome Aggregation Consortium (ExAC) database<sup>8</sup> and internal  
924 control exomes/genomes. Data analysis was initially undertaken for known disease-  
925 causing genes prior to analysis for autosomal recessive and dominant inheritance  
926 models.

927 NIHR BioResource-Rare Diseases (NIHRBR-RD) Study: WGS was undertaken  
928 using the Illumina TruSeq DNA PCR-Free Sample Preparation kit (Illumina Inc.,  
929 San Diego, CA, USA) on Illumina HiSeq 2500, generating minimum coverage of  
930 15X for ~95% of the genome, and average coverage of ~30X. Reads were aligned  
931 using Isaac aligner (version 01.14) (Illumina Inc, Great Chesterford, UK)<sup>43</sup>. SNVs  
932 and indels were identified using Isaac variant caller (version 2.0.17).

933 Wellcome Trust UK10K Rare Diseases project: DNA samples were captured using  
934 Agilent SureSelect Target Enrichment V5 (Agilent Technologies, Santa Clara, CA,  
935 USA) pull-down array. WES was performed on Illumina HiSeq 2000 platform.  
936 Reads were aligned using the Burrows-Wheeler Alignment tool. SNVs and indels  
937 were identified with SAMtools<sup>44,45</sup>. Variants were identified for each sample using  
938 the Genome Analysis Toolkit (GATK) Unified Genotyper<sup>46</sup> and annotated with vcf-  
939 annotate<sup>47</sup> and Ensembl Variant Effect Predictor v73 (VEP)<sup>48</sup>.

940 National Institutes of Health, Bethesda: Exome sequencing was completed using  
941 the TruSeqV2 exome capture kit. Data was aligned and processed as previously  
942 described<sup>49-51</sup>.

943 Institute of Human Genetics, Erlangen: Exome sequencing was performed on a  
944 HiSeq 2500 (Illumina) platform with 125 bp paired-end sequencing using  
945 SureSelect v.5 capturing reagents (Agilent).

946 Radboud University Medical Center, Nijmegen: Exome sequencing was undertaken  
947 using Agilent SureSelectXT Human All Exon 50 Mb Kit, with sequencing on SOLiD  
948 5500XL, producing an average sequence depth of 91X and average coverage of at  
949 least 20X for 89% of targets. For calling and annotation of variants, a custom in-  
950 house diagnostic pipeline was deployed<sup>52</sup>.

951 UCL-Institute of Neurology, London: Exome sequencing was performed using  
952 Illumina's Nextera Rapid Capture. Indexed and pooled libraries were sequenced on  
953 Illumina's HiSeq3000 (100bp, paired-end). Reads were aligned with Novoalign.  
954 Duplicate read removal, format conversion, and indexing were performed with  
955 Picard. GATK was used to recalibrate base quality scores, perform local  
956 realignments around possible indels, and to call (HaplotypeCaller) and filter (VQSR)  
957 variants<sup>46</sup>. Annotated variant files were generated using ANNOVAR<sup>53</sup>.

958 Deciphering Developmental Disorders study: Exome sequencing of family triomes  
959 was performed using Agilent SureSelect Exome bait design (Agilent Human All-  
960 Exon V3 Plus with custom ELID C0338371 and Agilent Human All-Exon V5 Plus  
961 with custom ELID C0338371) on a Illumina HiSeq at the Wellcome Trust Sanger  
962 Institute as previously described<sup>54,55</sup>. Data is currently available on 4,295 triomes  
963 which were interrogated via a DDD complementary research proposal (CAP#120).

#### 964 **Sanger Sequencing for Variant Validation and Gene Screening**

965 Direct sequencing was undertaken to (i) screen the entire coding region of *KMT2B*  
966 for 13 cases from the GOS-ICH cohort (**Supplementary Table 1**), (ii) confirm  
967 variants identified on next generation sequencing and (iii) establish familial  
968 segregation (**Supplementary Fig. 2**). Additionally, cDNA from fibroblasts and  
969 patient derived dopaminergic neurons were sequenced for a common SNP in exon  
970 30 (rs231591). Primer pairs for all 37 coding exons and exon/intron boundaries of  
971 *KMT2B* (Ensembl ENSG00000272333, transcript ENST00000420124) were  
972 designed with Primer3 (**Supplementary Table 9**)<sup>56,57</sup>. PCR conditions can be  
973 provided on request. PCR products were cleaned up (MicroCLEAN, Web Scientific)  
974 and sequenced using the Big Dye Terminator Cycle Sequencing System (Applied  
975 Biosystems Inc.). Sequencing reactions were run on an ABI PRISM 3730 DNA  
976 Analyzer (Applied Biosystems Inc.) and analyzed using Chromas.

#### 977 **Enrichment Analysis**

978 The number of *de novo* predicted protein truncating variants (PPTVs) in *KMT2B*  
979 expected to be seen by chance in a subset of the NIHR BioResource–Rare  
980 Diseases cohort with pediatric onset neurological disease (n=272), was calculated  
981 using published gene-specific mutation rates<sup>58</sup> and scaled to account for frameshift,  
982 nonsense and essential splice site variants<sup>58</sup>. To assess significance, the expected

983 number of *de novo* PPTVs were compared to the observed number, assuming a  
984 Poisson distribution.

### 985 **(3) CSF Neurotransmitter Analysis**

986 CSF was collected by lumbar puncture and diagnostically analyzed for  
987 neurotransmitter monoamine metabolites in specialist laboratories (London,  
988 Barcelona, Sydney, Jerusalem) by high performance liquid chromatography<sup>59,60</sup>

### 989 **(4) Comparative Modelling**

990 *In silico* homology modeling was utilized to predict putative effects of *KMT2B*  
991 variants. The Pfam database<sup>61</sup> was used to assign known domains to the full-length  
992 sequence of *KMT2B*. Evolutionary conservation of residues in the sequence was  
993 quantified using ConSurf server<sup>62</sup>, based on alignment with a set of homologous  
994 sequences, which share 35%-95% sequence identity with *KMT2B*. HHpred<sup>63</sup> was  
995 utilized to identify proteins or domains with known structure that have similar  
996 sequence and structural features to *KMT2B*. Selected templates had more than  
997 99% probability (based on HHpred alignment score) of being related structurally to  
998 specific domain segments of *KMT2B*. MODELLER<sup>64</sup> was employed for different  
999 regions of *KMT2B* and HHpred alignments were used to dictate residue  
1000 equivalences with the template. For each domain, 150 models were generated with  
1001 MODELLER loop optimization protocol and the best model was selected based on  
1002 the normalized DOPE score<sup>65</sup>. The effect of a point substitution on the stability of  
1003 the domain structure was evaluated using DUET<sup>11</sup> and MAESTRO<sup>10</sup>. Visualization  
1004 and analysis of amino acid interactions and generation of mutant models were done  
1005 with UCSF Chimera<sup>66</sup>.

## 1006 **(5) Histone Methylation Assays**

### 1007 **H3K4 Methylation**

1008 Histones were extracted from fibroblasts using a modified version of a published  
1009 protocol<sup>67</sup>. Cells were lysed by rotating at 4°C in hypotonic lysis buffer (10mM Tris-  
1010 Cl pH8.0, 1mM KCl, 1.5mM MgCl<sub>2</sub>, 1mM DTT, Roche complete protease inhibitors).  
1011 Intact nuclei were pelleted by centrifugation and resuspended in 0.2N HCl.  
1012 Following overnight histone extraction by rotating at 4°C, nuclear debris was  
1013 removed by centrifugation and soluble histones precipitated by dropwise addition of  
1014 TCA to a final concentration of 33%. Following one hour precipitation on ice,  
1015 histones were pelleted by centrifugation and washed with acetone before  
1016 resuspension in Milli-Q water.

### 1017 **Expression of p.Ile1447Thr Set1 Point Substitution in *Dictyostelium***

1018 *Dictyostelium discoideum* cells were grown as previously described<sup>68</sup>. They are not  
1019 listed in the database of commonly misidentified cell lines maintained by ICLAC.  
1020 *Dictyostelium* strains<sup>69</sup> included wild type—AX2 (DBS0238015) and *set1*-KO—*set1*-  
1021 (DBS0236928). DdSet1 has Dictybase gene ID DDB\_G0289257 and Uniprot ID  
1022 Q54HS3. p.Ile1447Thr was created by substituting ATT for ACT in a *DdSet1*  
1023 genomic clone by PCR (**Supplementary Table 10**). The product containing this  
1024 substitution was cloned as a ClaI/EcoRI fragment replacing the equivalent region of  
1025 a wild type *DdSet1* genomic clone. This region was subsequently subcloned as a  
1026 ClaI/AccI fragment into a pDEXH<sup>70</sup> based integrating plasmid containing *GFP*-  
1027 *DdSet1* under control of the *DdAct15* promoter and a G418 selection marker –  
1028 replacing the same region of the wild type *DdSet1* cDNA sequence. The presence  
1029 of p.Ile1447Thr in the resulting plasmid, pJN106, was confirmed by Sanger  
1030 sequencing. Constructs for expression of GFP-DdSet1(p.Ile1447Thr) (pJN106) and

1031 wild type GFP-DdSet1 (pJRC18) were transformed into *set1<sup>-</sup> Dictyostelium* cells<sup>71</sup>  
1032 as previously described<sup>68</sup>. Transformants were selected by addition of 10ug/ml  
1033 Geneticin (Gibco) to growth medium. Expression of full-length wild type and point  
1034 mutant GFP-DdSet1 was confirmed by anti-GFP immunoblotting.

### 1035 **Immunoblot Analysis of Histone Methylation**

1036 Fibroblast histone samples were diluted in SDS sample buffer (Bio-Rad) containing  
1037 5% [v/v]  $\beta$ -mercaptoethanol and protease inhibitors (Roche Complete), separated  
1038 by SDS-PAGE, then blotted onto nitrocellulose. Histone H3 and methylated K4  
1039 variants were detected using rabbit polyclonal anti-histone H3 (Abcam ab1791,  
1040 1:1000 dilution), rabbit polyclonal anti-histone H3 tri-methyl K4 (Abcam ab8580,  
1041 1:1000 dilution), rabbit polyclonal anti-histone H3 di-methyl K4 (Millipore 07-030,  
1042 1:2000 dilution). Secondary antibody used was donkey anti-rabbit IgG HRP-  
1043 conjugated (GE Healthcare NA934V; for Histone H3 and tri-methyl K4 detection  
1044 1:30000 dilution, for di-methyl K4 detection 1:20000 dilution). Following detection  
1045 using Supersignal West Pico chemiluminescent substrate (Thermo) and CL-  
1046 Xposure film (Thermo), densitometry was performed using ImageJ<sup>72</sup>.

1047 *Dictyostelium* cells were collected by centrifugation and resuspended in KK<sub>2</sub>  
1048 buffer<sup>68</sup> before lysis in SDS sample buffer (Bio-Rad) containing 5% [v/v]  $\beta$ -  
1049 mercaptoethanol and protease inhibitors (Roche Complete). Immunoblotting for  
1050 GFP-DdSet1 expression was assayed as above, using a mouse IgG monoclonal  
1051 anti-GFP primary (Roche 11814460001, 1:500 dilution) and anti-mouse IgG HRP-  
1052 conjugated secondary antibody (BioRad 170-6516, 1:20000 dilution).  
1053 Immunoblotting for Histone H3 and tri-methyl H3K4 was conducted as for  
1054 fibroblasts (with the modification: anti-histone H3 tri-methyl K4 dilution 1:3000).

1055 **(6) RNA and Protein Measurements**

1056 **Fibroblast RNA Extraction and cDNA Synthesis**

1057 Skin biopsies from Patients 2, 13, 14 and 16 were taken for fibroblast culture, and  
1058 grown in Dulbecco's Modified Eagle's Medium (DMEM, Sigma) with 4.5g/L glucose,  
1059 4mM L-glutamine, and 10% heat inactivated fetal bovine serum (Life Technologies)  
1060 and maintained in an incubator at 37°C and 5% CO<sub>2</sub>. Fibroblasts from two age-  
1061 matched controls were supplied by the Dubowitz Neuromuscular Centre Biobank  
1062 (GOS-ICH). Cultures were checked for mycoplasma contamination (MycoAlert  
1063 Mycoplasma Detection Kit, Lonza). As fibroblast cultures were derived from human  
1064 skin biopsies, no authentication was undertaken. Furthermore these cell lines do  
1065 not belong to the commonly misidentified cell lines listed in the database  
1066 maintained by ICLAC. RNA was extracted from fibroblasts of T75 cell culture flasks  
1067 using the RNeasy Mini Kit from QIAGEN. First-Strand cDNA synthesis was carried  
1068 out with SuperScript® III Reverse Transcriptase (Invitrogen) using 500ng total RNA  
1069 per reaction and Oligo (dT) primers (Thermo Fisher Scientific).

1070 **Generation of Dopaminergic Neurons, RNA Extraction, cDNA Synthesis**

1071 Fibroblasts from a KMT2B-negative individual were reprogrammed into induced  
1072 pluripotent stem cells (iPSC) using an established Sendai virus protocol  
1073 (CytoTune®-iPS Reprogramming Kit, Invitrogen)<sup>73</sup>. iPSC lines were stringently  
1074 tested for pluripotency using established methods<sup>74</sup> before differentiation into  
1075 dopaminergic neurons<sup>75</sup>. After 60 days of differentiation, dopaminergic identity was  
1076 confirmed by immunofluorescence for neuronal marker, MAP2, (mouse monoclonal  
1077 anti-MAP2, Sigma, M9942, 1:400 dilution) and dopaminergic marker, TH (chicken  
1078 polyclonal anti-TH, Aves labs, TYH, 1;400 dilution). Nuclei were contrasted with  
1079 DAPI. Microscopic images were captured (Zeiss LSM710 Confocal) and analyzed

1080 using ImageJ<sup>72</sup>. Neuronal differentiation efficiency was determined by calculating  
1081 the number of MAP2/TH positive cells relative to MAP2-positive cells  
1082 (**Supplementary Fig. 14**). RNA extraction and cDNA synthesis was carried out as  
1083 described for fibroblasts.

#### 1084 **PCR Analysis**

1085 We investigated tissue expression of *KMT2B* in (i) human fetal cDNA samples  
1086 (Moore fetal tissue cohort)<sup>76</sup>, (ii) a human cDNA panel (Clontech), (iii) human  
1087 fibroblasts and (iv) dopaminergic neurons differentiated from human iPSC. PCR  
1088 amplification of cDNA (**Supplementary Table 11**) was performed with BioMix™  
1089 Red (Bioline Ltd, conditions available on request). PCR products were separated on  
1090 a 2% agarose gel containing Ethidium bromide (Sigma) and visualized with Gel  
1091 Doc™ XR+ System (Bio Rad).

1092 Changes in relative expression of *KMT2B*, *THAP1* and *TOR1A*, were measured by  
1093 quantitative RT-PCR on a StepOnePlus™ Real-Time PCR System (Applied  
1094 Biosystems). RT-PCR reactions comprised 1x MESA Blue qPCR MasterMix Plus  
1095 for SYBR® Assay (Eurogentec), 0.1µl ROX Reference Dye (Invitrogen), 9µL cDNA  
1096 (of a dilution 1:25) and 500nM of each primer (**Supplementary Table 11**). RT-PCR  
1097 conditions are available on request. Relative quantification of gene expression was  
1098 determined using the  $2^{-\Delta\Delta Ct}$  method<sup>77</sup>, with glyceraldehyde-3-phosphate  
1099 dehydrogenase (*GAPDH*) as a reference gene.

#### 1100 **Fibroblast Protein Preparation and Immunoblot Analysis**

1101 Fibroblasts grown in T25 cell culture flasks were washed with cold PBS and  
1102 incubated with lysis buffer [150mM NaCl, 50mM Tris pH8, 1% NP40 and 1x  
1103 cOmplete™ Mini Protease Inhibitor Cocktail (Roche)] for 30 minutes on ice. Lysed  
1104 cells were centrifuged at 13,000 rpm for 15 minutes to remove cell debris. Protein

1105 concentrations of the cell lysates were measured with the Pierce™ BCA Protein  
1106 Assay Kit (Thermo Fisher Scientific). A total of 5-10ng protein were prepared with  
1107 1x Laemmli buffer and 0.5M DTT and boiled for 5 minutes at 100°C for denaturing.  
1108 Proteins were separated by electrophoresis on 4–20% Mini-PROTEAN® TGX  
1109 Stain-Free™ Protein Gels (Bio Rad) by applying 300V for ~17 minutes. Proteins  
1110 were transferred to polyvinylidene difluoride (PVDF) membranes (Bio Rad) using  
1111 the Trans-Blot® Turbo™ Transfer System (Bio Rad). Membranes were incubated  
1112 for 1 hour at room temperature in blocking solution (5% nonfat dry milk in  
1113 Phosphate-buffered saline-Tween 20, PBS-T) and then probed with polyclonal  
1114 rabbit anti-THAP1 (Cambridge Bioscience [Supplier: Proteintech], 12584-1-AP,  
1115 1:1500 dilution) and monoclonal mouse anti-TorsinA (Cell Signaling, D-M2A8,  
1116 1:1000 dilution), respectively, in blocking buffer (1% nonfat dry milk in PBS-T;  
1117 except THAP1 antibody which was diluted in 5% nonfat dry milk in PBS-T) for  
1118 approximately 16 hours at 4°C. Following three washing steps with PBS-T,  
1119 membranes were incubated for 1 hour at room temperature with horseradish  
1120 peroxidase (HRP)-conjugated goat anti-rabbit IgG antibody (Cell Signaling, #7074,  
1121 1:3000 dilution) and HRP-conjugated horse anti-mouse IgG antibody (Cell  
1122 Signaling, #7076, 1:3000 dilution), respectively. Afterwards the blot was washed  
1123 three times with PBS-T and signals were visualized with Clarity™ ECL Western  
1124 Blotting Substrate (Bio Rad) on a Gel Doc™ XR+ System (Bio Rad). To confirm  
1125 equivalent loading, blots were stripped at 37°C for 15 minutes in Restore™ Western  
1126 blot Stripping buffer (Thermo Fisher Scientific), blocked for 1 hour, and reprobed  
1127 with HRP-conjugated rabbit anti-β-Tubulin (Cell Signaling, 9F3, 1:1000 dilution). For  
1128 quantification, intensity values of control and patient bands were determined using

1129 Fiji software<sup>78</sup> and normalized against the intensity value of the reference protein  
1130 band.

### 1131 **CSF Immunoblotting**

1132 CSF protein levels of tyrosine hydroxylase (TH) and dopamine receptor D2 (D2R)  
1133 were analyzed. CSF samples were available from two patients (Patients 2 and 16)  
1134 and four gender and age-matched controls (patients with no history of movement  
1135 disorder, on no medication). Immunoblotting was carried out as described above.  
1136 For the detection of TH and D2R the membranes were incubated with polyclonal  
1137 rabbit anti-TH (Millipore, AB152, 1:1000 dilution) and polyclonal rabbit anti-D2R  
1138 (Millipore, AB5084P, 1:1000 dilution), respectively, followed by 2 hours incubation  
1139 with HRP-conjugated goat anti-rabbit IgG antibody (Cell Signaling, #7074, 1:3000  
1140 dilution). As an internal control for loading monoclonal mouse anti-Transferrin  
1141 (Santa Cruz, E-8, 1:1000 dilution) followed by HRP-conjugated horse anti-mouse  
1142 IgG antibody (Cell Signaling, #7076, 1:3000 dilution) were used.

### 1143 **(7) Statistics**

1144 The statistical analyses for the histone methylation assays were conducted using  
1145 GraphPad Prism v7.01 and for the analyses of the fibroblast cell lines and CSF  
1146 immunoblotting using GraphPad v5. The final data are represented with the mean  
1147 and the standard deviation as error bars. For multiple comparisons one-way  
1148 ANOVA was performed whereas for dual comparisons unpaired two-tailed  
1149 Student's t test were employed.  $P < 0.05$  was considered significant: \* $P < 0.05$ , \*\* $P$   
1150  $< 0.01$ , \*\*\* $P < 0.001$ . The F test was utilized to compare the variances between the  
1151 groups in dual comparisons.

1152 We assume that technical replicates of immunoblot assays using the same cell  
1153 lines will be normally distributed. For the fibroblast histone methylation assay the

1154 Brown-Forsythe test was used to check differences in variance between the groups  
1155 compared, and no significant differences was found in standard deviation  
1156 (H3K4Me3:  $p = 0.7567$  [ $F = 0.2877$ ,  $DFn = 2$ ,  $DFd = 9$ ]; H3K4me2:  $p=0.8446$  [ $F =$   
1157  $0.1721$ ,  $DFn = 2$ ,  $DFd = 9$ ]). For the remaining experiments data distribution was  
1158 not tested but was assumed to be normal. Blinding was not applied for data  
1159 collection and analysis.

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