Whole genome sequencing for the genetic diagnosis of heterogenous dystonia phenotypes

Kishore R. Kumar[a,b,c,d,e,*], Ryan L. Davis[a,b,c], Michel C. Tchan[c,f], G.M. Wali[g], Neil Mahant[h], Karl Ng[c,i], Katya Kotschet[j,k], Sue-Faye Siow[b,c,f], Jason Gu[l], Zachary Walls[m], Ce Kang[c], Gautam Wali[b,c], Stan Levy[n], Chung Sen Phua[n], Con Yiannikas[c,e,o], Paul Darveniza[p,q], Florence C.F. Chang[h], Hugo Morales-Briceño[h], Dominic B. Rowe[r], Alex Drew[a], Velimir Gayevskiy[a], Mark J. Cowley[a,s,t], Andre E. Minoche[a], Stephen Tisch[o], Michael Hayes[e], Sarah Kummerfeld[a], Victor S.C. Fung[h,1,**], Carolyn M. Sue[a,b,c,o,1,***]

[a] Kinghorn Centre for Clinical Genomics, Garvan Institute of Medical Research, Darlinghurst, NSW, 2010, Australia
[b] Department of Neurogenetics, Kolling Institute, University of Sydney and Royal North Shore Hospital, St Leonards, New South Wales, 2065, Australia
[c] Sydney Medical School, Faculty of Medicine and Health, University of Sydney, Camperdown, 2050, Australia
[d] Molecular Medicine Laboratory, Concord Hospital, 2139, Australia
[e] Department of Neurology, Concord Hospital, 2139, Australia
[f] Department of Genetic Medicine, Westmead Hospital, Westmead, NSW, 2145, Australia
[g] Neurospecialities Centre, Jawaharlal Nehru Medical College, Belgaum, India
[h] Movement Disorders Unit, Department of Neurology, Westmead Hospital, Sydney Medical School, University of Sydney, Sydney, 2145, Australia
[i] Department of Neurology and Neurophysiology, Royal North Shore Hospital, Reserve Road, St Leonards, New South Wales, 2065, Australia
[j] Florey Neuroscience Institute, University of Melbourne, Parkville, 3052, Australia
[k] Department of Neurology, St Vincent's Hospital, Fitzroy, 3065, Australia
[l] Department of Neurology, Wollongong Hospital, Wollongong, New South Wales, 2500, Australia
[m] Faculty of Engineering and Information Technologies, University of Sydney, Darlington, 2008, Australia
[n] Campbelltown Hospital, Campbelltown, 2560, Australia
[o] Department of Neurology, Royal North Shore Hospital, St Leonards, New South Wales, 2065, Australia
[p] School of Medicine, University of New South Wales, Sydney, Australia
[q] Department of Neurology, St Vincent's Hospital, Darlinghurst, 2010, Australia
[r] Department of Clinical Medicine, Faculty of Medicine and Health Sciences, Macquarie University, Macquarie Park, New South Wales, 2109, Australia
[s] Children's Cancer Institute, Kensington, 2750, Australia
[t] St Vincent's Clinical School, UNSW Sydney, Darlinghurst, 2010, Australia

ARTICLE INFO

ABSTRACT

Introduction: Dystonia is a clinically and genetically heterogeneous disorder and a genetic cause is often difficult to elucidate. This is the first study to use whole genome sequencing (WGS) to investigate dystonia in a large sample of affected individuals.
Methods: WGS was performed on 111 probands with heterogenous dystonia phenotypes. We performed analysis

* Corresponding author. Molecular Medicine Laboratory, Concord Hospital, Concord, NSW, 2139, Australia.
** Corresponding author.
*** Corresponding author. Department of Neurogenetics, Kolling Institute, University of Sydney and Royal North Shore Hospital, St Leonards, New South Wales, 2065, Australia.
E-mail addresses: kkum4618@uni.sydney.edu.au (K.R. Kumar), ryan.davis@sydney.edu.au (R.L. Davis), michel.tchan@health.nsw.gov.au (M.C. Tchan), walidoc@hotmail.com (G.M. Wali), nmahant@gmail.com (N. Mahant), karl.ng@health.nsw.gov.au (K. Ng), katya.kotschet@svha.org.au (K. Kotschet), suefaye.siow@health.nsw.gov.au (S.-F. Siow), gujason07@gmail.com (J. Gu), zac971@hotmail.com (Z. Walls), kkan1041@uni.sydney.edu.au (C. Kang), gautam.wali@sydney.edu.au (G. Wali), Stanley.Levy@sswahs.nsw.gov.au (S. Levy), chunsengphua@yahoo.com (C.S. Phua), y.con@bigpond.com (C. Yiannikas), recep702@stvincents.com.au (P. Darveniza), Florence.Chang@health.nsw.gov.au (F.C.F. Chang), Hugo.MoralesBriceno@health.nsw.gov.au (H. Morales-Briceño), dominicrowe@mac.com (D.B. Rowe), a.drew@garvan.org.au (A. Drew), vel@vel.nz (V. Gayevskiy), MCowley@ccia.org.au (M.J. Cowley), a.minoche@garvan.org.au (A.E. Minoche), stephen.tisch@svha.org.au (S. Tisch), michael.hayes1@health.nsw.gov.au (M. Hayes), s.kummerfeld@garvan.org.au (S. Kummerfeld), vscfung@ozemail.com.au (V.S.C. Fung), carolyn.sue@sydney.edu.au (C.M. Sue).

[1] These authors made an equal contribution to the manuscript.

for coding and non-coding variants, copy number variants (CNVs), and structural variants (SVs). We assessed for an association between dystonia and 10 known dystonia risk variants.

Results: A genetic diagnosis was obtained for 11.7% (13/111) of individuals. We found that a genetic diagnosis was more likely in those with an earlier age at onset, younger age at testing, and a combined dystonia phenotype. We identified pathogenic/likely-pathogenic variants in *ADCY5* (n = 1), *ATM* (n = 1), *GNAL* (n = 2), *GLB1* (n = 1), *KMT2B* (n = 2), *PRKN* (n = 2), *PRRT2* (n = 1), *SGCE* (n = 2), and *THAP1* (n = 1). CNVs were detected in 3 individuals. We found an association between the known risk variant *ARSG* rs11655081 and dystonia (p = 0.003).

Conclusion: A genetic diagnosis was found in 11.7% of individuals with dystonia. The diagnostic yield was higher in those with an earlier age of onset, younger age at testing, and a combined dystonia phenotype. WGS may be particularly relevant for dystonia given that it allows for the detection of CNVs, which accounted for 23% of the genetically diagnosed cases.

1. Introduction

Dystonia is a movement disorder defined by 'sustained or intermittent muscle contractions causing abnormal, often repetitive, movements, postures, or both' [1]. The aetiology is complex but a monogenic cause may be identified. Genes implicated in dystonia include *ANO3*, *ATP1A3*, *GCH1*, *GNAL*, *KMT2B*, *SGCE, THAP1,* and *TOR1A*. Dystonia may follow a dominant (one copy of the gene affected), recessive (both copies affected) or X-linked (mutation on the X-chromosome) mode of inheritance.

Sanger sequencing, targeted gene panels, and whole exome sequencing (WES) have been performed in dystonia samples, with a diagnostic yield from less than 1% up to 37.5% [2–7]. The genetic cause for many individuals with dystonia has yet to be identified and this may be addressed by an unrestricted genome wide search.

Whole genome sequencing (WGS) offers several advantages over other targeted genetic sequencing approaches [8]. By querying the entire genome, it offers both uniform coverage of coding and noncoding regions of the genome and allows for accurate detection of copy number variants (CNVs) and structural variants (SV). These benefits may translate into a higher diagnostic yield when comparing WGS to WES [9].

Given the potential for a higher diagnostic yield from WGS, we used this method to screen individuals with idiopathic dystonia in order to determine the genetic contribution from known disease genes. To our knowledge, this is the first study to use WGS to investigate dystonia in a large sample of affected individuals.

2. Methods

We recruited consecutive individuals in whom dystonia was a prominent phenotypic feature between January 2015 and December 2018 from 6 movement disorder centres in Australia and India. We did not include individuals with a previous genetic diagnosis or a readily apparent acquired cause. The study received ethical approval and consent was obtained for experimentation with human subjects (RESP/15/314, HREC/13/RPAH/363). All persons visible on the videos have consented to the publication of these videos and this includes the online publication and dissemination of the videos. Participants were reviewed by neurologists and the diagnosis of idiopathic dystonia was made according to established criteria [1].

WGS was performed using the Illumina HiSeq X Ten platform at the Kinghorn Centre for Clinical Genomics, Garvan Institute of Medical Research, with alignment of reads to the GRCh37 reference genome, as previously described [10,11]. We searched a dystonia gene panel (Supplementary Table 1) and interrogated a TruSight One panel of over 4000 genes, which served as a 'clinical exome'. Variants were prioritised according to population frequency databases, variant impact, and

Table 1
Overall demographics and clinical manifestations of probands with dystonia.

Variables	Total	Genetic diagnosis identified	No genetic diagnosis identified	p value[a]
Gender (Male/Female)	37/74 (111)	8/5 (13)	29/69 (98)	0.058
Age at testing (years)[b]	48.9 ± 17.5 (8–84)	37.0 ± 16.1 (11–66)	50.4 ± 17.1 (8–84)	**0.009**
Age at onset (years)[b]	32.9 ± 21.4 (0–81)	15.1 ± 13.9 (2–50)	35.4 ± 21.2 (0–81)	**0.001**
Phenotypes (%)				
Isolated focal dystonia: Cervical (non-tremulous)	10 (9.0)	0 (0)	10 (10.2)	–
Isolated focal dystonia: Cervical (tremulous)	23 (20.7)	0 (0)	23 (23.5)	–
Combined dystonia	36 (32.4)	9 (69.2)	27 (2)	**0.004**
Isolated focal dystonia: upper limb	1 (0.9)	0 (0)	1 (1.0)	–
Isolated multifocal dystonia	5 (4.5)	0 (0)	5 (5.1)	–
Isolated segmental dystonia	26 (23.4)	2 (15.4)	24 (24.5)	0.729
Isolated generalized dystonia	10 (9.0)	2 (15.4)	8 (8.2)	0.331
Dystonia syndromes (%)				
Early-onset generalized isolated dystonia	9 (8.1)	2 (15.4)	7 (7.1)	0.200
Focal or segmental isolated dystonia with onset in adulthood	51 (45.9)	1 (7.7)	50 (51.0)	**0.003**
Dystonia-parkinsonism	10 (9.0)	2 (15.4)	8 (8.2)	0.238
Myoclonus-dystonia	4 (3.6)	2 (15.4)	2 (2.0)	0.042
Family History (%)	47 (42.3)	5 (38.5)	42 (42.9)	0.999
Abnormalities on MRI (%)	13 (11.7)	2 (15.4)	11 (11.2)	0.648

[a] Analysis comparing probands with and without a genetic diagnosis.

[b] Mean ± SD (range). IBM SPSS Version 24 (2016) was used for statistical analysis. Gender, Phenotype, Family History, MRI were compared using Fisher's exact test. Age at testing and age at onset were compared using a 2-sample *t*-test. A Bonferroni correction was applied. Subjects were recruited from 6 tertiary centres: Royal North Shore Hospital (Sydney), Concord Repatriation General Hospital (Sydney), Westmead Hospital (Sydney), St Vincent's Hospital (Sydney), St Vincent's Hospital (Melbourne), Neurospecialties Centre (Belgaum). Of the probands, 12/111 were recorded to have had previous genetic testing, which included DYT1 testing in 5/12 individuals. The ethnic background of the probands included European/Caucasian (n = 82, 73.9%), South Asian (n = 14, 12.6%), Southwest Asian (n = 6, 5.4%), East Asian (n = 5, 4.5%), Southeast Asian (n = 2, 1.8%), African (n = 1, 0.9%) and Pacific Islander (n = 1, 0.9%).

in silico prediction tools, as described [10]. We assessed for CNVs and SVs using a clinically accredited detection pipeline (ClinSV, Minoche et al. in preparation, and as described [12]).

We used a software tool (VarSome [13]) for standard variant interpretation. We assessed non-coding regions of our dystonia gene set using an in-house bioinformatics tool, (*Introme*, Gayevskiy et al., manuscript in preparation, https://github.com/KCCG/introme), that identified all non-coding variants from a vcf file and provided inference of their potential to disrupt known canonical splice sites and branch-points and to introduce potential new canonical splice sites. Sequencing variants were confirmed using Sanger sequencing, CNVs were confirmed using multiplex ligation-dependent probe amplification or PCR. We considered a genetic diagnosis if the variant was classified as likely pathogenic or pathogenic according to ACMG criteria [14]. Variants falling just short of 'likely pathogenic' were considered as 'variants of uncertain significance (VUS) (favour pathogenic)', as described [15].

We performed statistical analysis with IBM SPSS Statistics (Version 24, 2016) software. Individuals with and without a genetic diagnosis were compared. We analysed the association between genetic diagnosis and age at testing and age at onset using a 2-sample *t*-test (p = 0.05). Gender, phenotype, family history, and MRI findings were compared using a Fisher's exact test (Bonferroni correction applied). We also tested 10 previously identified genetic risk variants for dystonia [16] (rs11655081, rs61999318, rs6265, rs3759664, rs10483639, rs12147422, rs71521601, rs1182, rs1801968 and rs35153737), using a Fisher exact test (p value less than 0.005, Bonferroni correction applied). We analysed the entire dystonia sample as a homogenous group and compared to the Medical Genome Reference Bank (MGRB) database of healthy elderly individuals with a predominant European background (97% non-Finnish European) [17].

3. Results

3.1. Sample characteristics

We recruited 111 dystonic probands with no previous genetic diagnosis (Table 1). We also included 9 affected and 16 unaffected family members for family studies. Of the probands, there were 37 males and 74 females. The ethnic background of the probands was predominantly European/Caucasian (n = 82, 73.9%, Table 1).

3.2. Coverage of disease-relevant variants in dystonia genes: WGS versus WES

In order to compare WGS and WES, we assessed coverage of pathogenetic/likely pathogenic variants from ClinVar for genes from the dystonia panel using our inhouse WGS and WES datasets of the NA12878 cell line (see Minoche et al. [12] for methods). We considered a cut-off of 15 reads for the reliable detection of variants [18]. Using this criterion, we found that WGS covered 99.7% of clinically relevant variants; this compared favourably to WES, which only covered 94.6% and 91.8% for the 2 WES datasets tested (benefit persists at higher coverage, Supplementary Fig. 1).

3.3. Clinico-genetic findings

A genetic diagnosis was obtained in 11.7% (13/111) of probands (Table 1, Supplementary Table 2, Fig. 1). We identified pathogenic/likely-pathogenic variants in *ADCY5* (n = 1), *ATM* (n = 1), *GNAL* (n = 2), *GLB1* (n = 1), *KMT2B* (n = 2), *PRKN* (n = 2), *PRRT2* (n = 1), *SGCE* (n = 2), and *THAP1* (n = 1), but not in any of the other > 4000 genes in the 'clinical exome'. All individuals with a genetic diagnosis
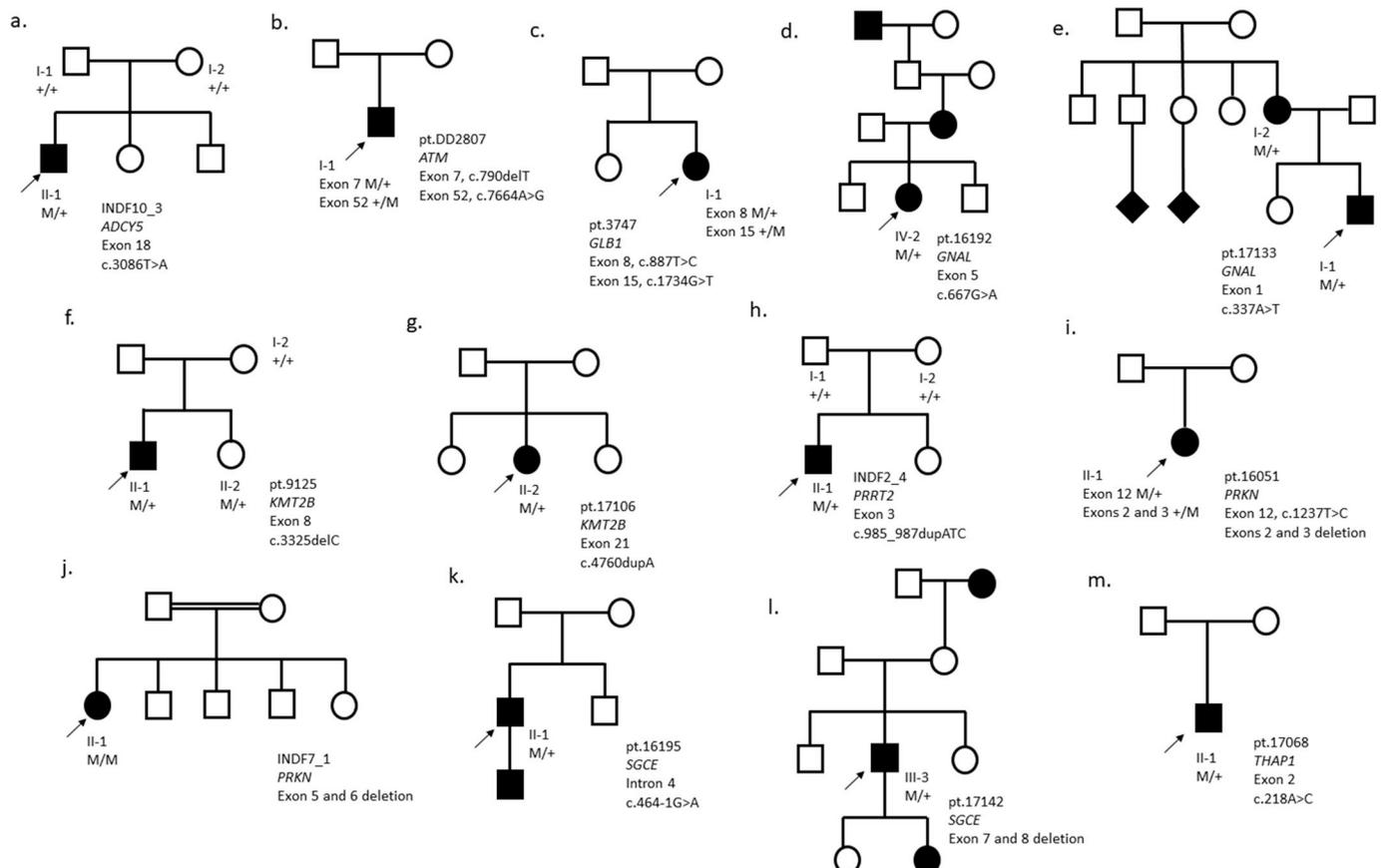
Fig. 1. Pedigrees of probands with pathogenic/likely pathogenic variants identified on WGS. Family members were not available to confirm compound heterozygosity for probands pt.DD2807, pt.3747, and pt.16051.

had segmental, generalized or combined dystonia. A genetic diagnosis was more likely in those with an earlier age at onset (p = 0.001), younger age at testing (p = 0.009) and a combined dystonia phenotype (p = 0.009). In comparison, probands with focal or segmental isolated dystonia with onset in adulthood were less likely to have a genetic diagnosis (p = 0.003). We compared our results to other studies (Supplementary Tables 3) and a brief description of individuals with a genetic diagnosis follows.

3.3.1. ADCY5 mutation

An 18 year old man from India (INDF10_3) had a history of continuous fidgety movements of the limbs and episodes of ballistic movements occurring 10–15 times per day lasting 10–15 minutes each time from 1 year of age, consistent with myoclonic dystonia. Seventeen years later, he was restricted to a bed and anarthric with flexed posturing of the limbs. Using WGS, we detected a *de novo* missense variant (NM_183357.2:c.3086T > A (p.Met1029Lys)), previously reported as a cause of ADCY5-related dyskinesias [19].

3.3.2. ATM mutations

A 48 year old man (pt.DD2807) had myoclonic dystonia, with age at onset of 13 years, with jerky movements of the neck, face and limbs and blepharospasm. There was no nystagmus or upper limb ataxia, but tandem gait was abnormal. There was no cerebellar atrophy on brain MRI. On WGS, we identified a known mutation in the *ATM* gene (NM_000051.3:c.790delT (p.Tyr264Ilefs)), as well as a VUS (NM_000051.3:c.7664A > G (p.His2555Arg)). This prompted a clinical re-evaluation, leading to the identification of tortuous conjunctival vessels (Fig. 2) and an elevated alpha-foetoprotein (40 IU/mL, normal < 8), confirming ataxia telangiectasia. Serum immunoglobulin levels were normal. He was subsequently instituted on a cancer screening programme.

3.3.3. GLB1 mutations

A 40 year old Indonesian woman (pt.3747) had a normal birth and development until the age of 19 years, when she developed generalized dystonia, impaired mobility, anarthria, and an asymmetrical/dysmorphic face. WGS revealed heterogenous variants in the *GLB1* gene, a previously reported variant (NM_000404.2:c.1734G > T, p. (Lys578Asn)) [20] and a novel variant (NM_000404.2:c.887T > C, p. (Ile296Thr)). The diagnosis of GM1 gangliosidosis was confirmed on biochemical testing (beta-galactocerebrosidase activity < 0.1, range 0.1–6 nmol/min/mg). An MRI brain showed changes consistent with GM1 gangliosidosis (Supplementary Fig. 2).

3.3.4. GNAL mutations

The first individual with *GNAL*-related dystonia (pt.16192) was a 42 year old woman whose symptoms began at age 32 years with difficulty pronouncing vowels. She also developed neck dystonia (Video 1), blepharospasm and writer's cramp. Her family history was consistent with autosomal dominant inheritance (Fig. 1). Using WGS, we identified a variant in *GNAL* (NM_182978.3:c.667G > A (p.Val223Met)), which has previously been reported [4].

Supplementary video related to this article can be found at https://doi.org/10.1016/j.parkreldis.2019.11.004.

The other individual (pt. 17133) was a 25 year old man with generalized dystonia and an autosomal dominant pattern of inheritance. He had onset of writer's cramp as a teenager, associated with hand tremor, and later developed tremulous cervical dystonia. He was found to have a novel loss of function mutation in *GNAL* (NM_182978.3:c.337A > T (p.Lys113Ter)).

3.3.5. KMT2B mutations

We identified two individuals with *KMT2B* variants.

The first individual (pt. 9125) was a 23 year old man who was well until he developed dystonia of the left foot and laryngeal dystonia by five years of age. He stopped walking at 11 years old, with acute worsening of dystonia following a lumbar puncture at 15 years of age. He had generalized dystonia with a tremulous and twisting pattern (Video 2). Brain MRI showed pallidal hypointensity and external segment atrophy (Fig. 2). Deep brain stimulation (DBS) at the age of 20 years resulted in a good improvement in jaw opening, cervical and upper limb dystonia, but only mild improvement in the hip flexion dystonia (Video 3). We identified a novel loss of function variant in *KMT2B* (NM_014727.2:c.3325delC) on WGS. His 28 year old sister also carried the variant. On neurological examination she did not have dystonia but did have mild intellectual impairment and short stature; a brain MRI was not performed.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.parkreldis.2019.11.004.

Another novel loss of function *KMT2B* variant (NM_014727.2:c.4760dupA) was found in a woman (pt.17106), with a
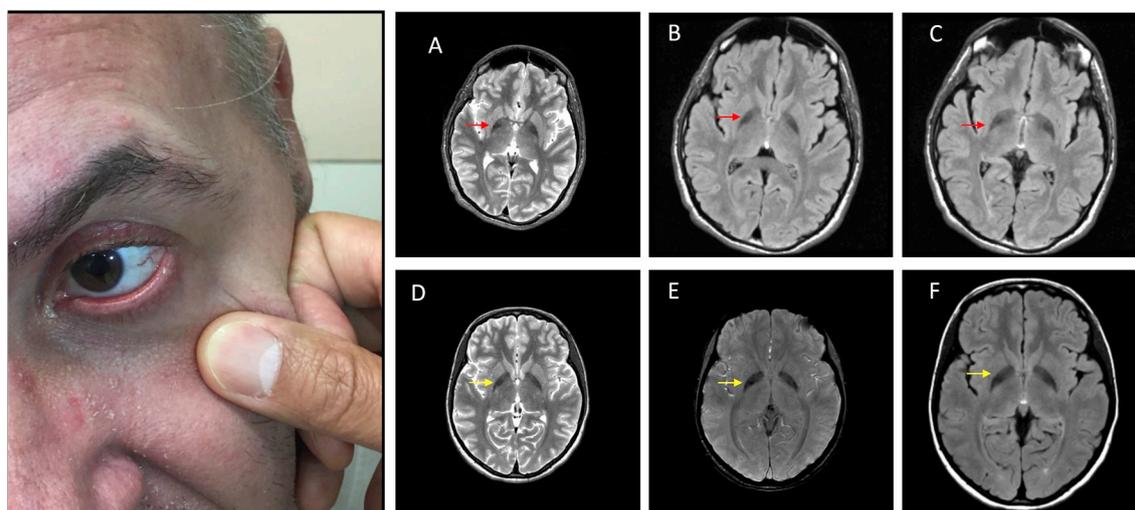
Fig. 2. Left Panel: Telangiectasia was detected on clinical re-evaluation of a man (pt.DD2807) with myoclonic dystonia after the findings of variants in the *ATM* gene on WGS. Right Panels: Radiologic MRI features of patients with *KMT2B* variants. Representative images from pt.9125, T2 (a), fluid-attenuated inversion recovery (FLAIR, b, c) showing bilateral pallidal hypointensity and external segment atrophy (red arrow). Representative images from pt.17106 (BL), T2 (e), gradient echo (f) and FLAIR (g), showing mild bilateral pallidal hypointensity in pt.17106 (yellow arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

phenotype of childhood onset dystonia that later evolved to severe dysarthria with oromandibular dystonia, and an elongated face (Video 4). Brain MRI showed mild pallidal hypointensity (Fig. 2). She had bilateral globus pallidus interna (GPi) DBS at age 21 years with a moderate response although with progression of generalized dystonia by examination age 26 years (Video 5).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.parkreldis.2019.11.004.

3.3.6. PRKN mutations

An individual (pt. 16051) with *PRKN* mutations and a parkinsonism-dystonia phenotype developed a tremor of the left hand at the age of 21 years and a gait disturbance. She also had torticollis to the right side that improved with touching of her chin. WGS revealed disease relevant variants in *PRKN*: a heterozygous missense variant (NM_013987.2:c.1237T > C (p.Cys413Arg)), which has been previously reported [21], in addition to a heterozygous deletion encompassing exons 2 and 3, that was detected on analysis for CNVs/SVs using the ClinSV tool (Fig. 3).

We also found a homozygous (~380 kb) deletion of *PRKN* encompassing exons 5 and 6 (Fig. 3, Supplementary Fig. 3) in a consanguineous 52 year old woman (INDF7_1) with a 2 years history of gait disturbance. Examination revealed rigidity of all limbs with mild dystonic involvement of the limbs and neck.

3.3.7. PRRT2 mutation

We detected a *de novo* in frame duplication in *PRRT2* (NM_001256442.1:c.985_987dupATC) in a boy (INDF2_4) who developed paroxysmal kinesigenic dyskinesia from the age of 3 years, occurring 1–2 times per day and lasted 1–2 min each time. He had a complete response to phenytoin, 100 mg twice daily. The variant is located in the second transmembrane domain in close proximity to the following reported mutations including c.972delA (p.Val325SerfsTer12) [22], c.970G > A (p.Gly324Arg) [23], and c.971G > A (p.Gly324Glu) [23]. Although the CADD score was only 2.2, we scored the variant as likely pathogenic on ACMG criteria (PM1, PM2, PM4, PM6).

3.3.8. SGCE mutations

There were two individuals identified with *SGCE* mutations. The first, a 66 year old man (pt.16195), had onset of vocal stuttering and a writer's cramp from the age of 9 years, which he noticed after a tonsillectomy and adenoidectomy procedure. At the age of 40 he was diagnosed with spasmodic dysphonia and treated with botulinum toxin injections with good effect. On examination, he had occasional myoclonic jerks of the shoulders and mild writer's cramp. His son had spasmodic dysphonia, segmental dystonia and writer's cramp, but did not wish to proceed with genetic testing. WGS revealed a novel canonical splice variant in *SGCE* (NM_001099400.1:c.464-1G > A) as the cause of myoclonus dystonia in the proband.

In the second individual with myoclonus dystonia (pt.17142), we found a heterozygous deletion of exons 7 and 8 in *SGCE* (Fig. 3). This 45 year old man developed jerking of the head and shoulders and a head tilt to the right (Video 6) following a tonsillectomy at age 21 years which responded to alcohol.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.parkreldis.2019.11.004.

3.3.9. THAP1 mutation

One individual was identified with a *THAP1* mutation. This 54 year old man (pt.17068) had onset of right hand writer's cramp at 15 years of age and later developed left hand involvement and tremulous cervical dystonia. We detected a novel, heterozygous missense variant in *THAP1* that was classified as likely pathogenic (NM_018105.2:c.218A > C (p.Lys73Thr)) according to the ACMG guidelines (PM1, PM2, PP2, PP3). There was no family history and the parents were unavailable for clinical or genetic studies.

3.4. Variants of uncertain significance, genes of uncertain significance

We identified 32 individuals with VUS, of which 5 were classified as VUS (favour pathogenic) (Supplementary Table 4). Of note, analysis for non-coding variants using *Introme* included potential cryptic splice sites in *GNAL* (NM_182978.3:c.377–1338C > G in pt.17108) and *SLC20A2* (ENST00000342228.3:c.-265 + 25727A > G in pt.14135).

3.5. Assessment of known risk variants

We identified a significant p value for the risk variant *ARSG* rs11655081 (p value = 0.003, Supplementary Table 5). The *ARSG*
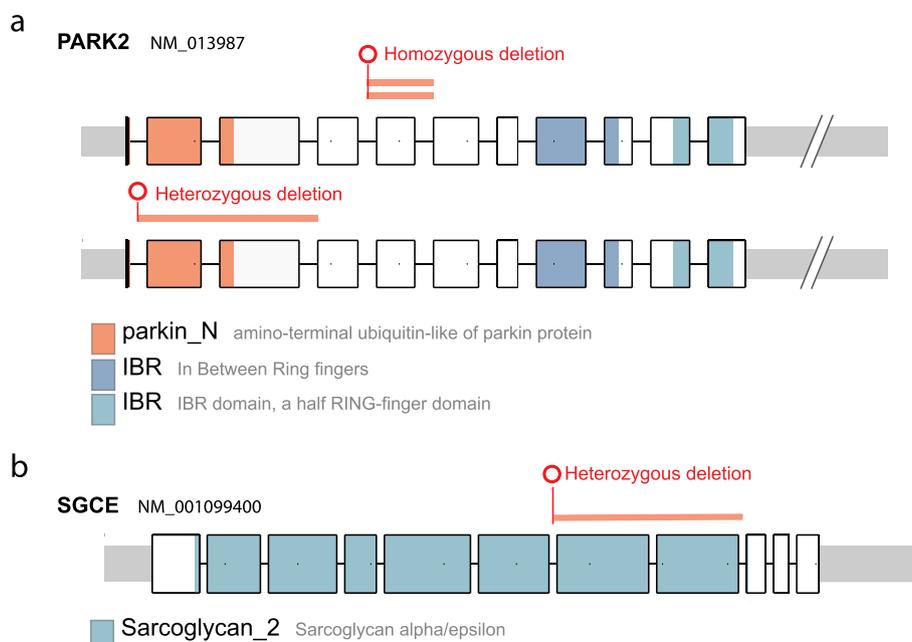

Fig. 3. Schematic showing deletions detected in the *PRKN/PARK2* (A) and *SGCE* (B) gene from WGS data in probands INDF7_1, 16051, and 17142 respectively.

rs11655081 variant was found in individuals with the following phenotypes: cervical (n = 1), tremulous cervical (n = 3), combined (n = 7), multifocal (n = 1), segmental (n = 2), and generalized (n = 2). The other risk variants did not make the level of significance.

4. Discussion

In this study we screened a large sample of probands presenting clinically with dystonia using WGS. We confirmed a genetic diagnosis in 11.7% (13/111) of the cohort. A genetic diagnosis was more likely in individuals with a younger age at onset and earlier age at testing. This could be due to a greater proportion of these cases being monogenic, rather than accumulating environmental factors over time. Furthermore, probands with a combined dystonia phenotype were also more likely to have a genetic diagnosis, whereas those with focal or segmental isolated dystonia with onset in adulthood were less likely.

WGS served as a comprehensive technique, allowing us to screen coding regions of all known disease genes, associated non-coding sequences, and to interrogate for CNVs and SVs. The diagnostic rate is relatively high compared to other studies [4,7]. The detection of CNVs may be particularly relevant in dystonia given that this mutation type accounted for 23% of genetically diagnosed cases. This is consistent with other studies highlighting the importance of CNVs in dystonia [24]. WGS CNV detection is robust and has no lower size limit; it has been demonstrated to perform well compared to CNV detection by WES [25]. Our coverage data indicates that we will miss fewer clinically relevant variants with WGS in comparison to WES (Supplementary Fig. 1). We did not identify mutations in *TOR1A* or *GCH1*; these individuals may have been previously identified, and so were not recruited to our study.

Reasons for a genetic cause not being identified include mutations in genes that have yet to be discovered or repeat expansion disorders that were not specifically targeted in this study. Other non-mendelian etiological factors may also have a role, including environmental influences, polygenic interactions and epigenetic modifications. Our study provides support that the rs11655081 in *ARSG*, previously linked with musician's dystonia [26], is associated with dystonia, although it is possible that population stratification may have impacted this result. Additional risk factors may be validated using a larger sample. Furthermore, although individuals were examined by experienced neurologists, there is a possibility of alternative causes of dystonia, such as psychogenic or drug-induced dystonia.

We detected several VUS in known dystonia genes (32/111 probands). If all VUS were proven to be pathogenic, the total number of probands diagnosed would be 45, providing a diagnostic yield of 40.5%, more in keeping with other movement disorders [2]. It seems likely that as further genomic information and understanding becomes available in the future, the status of these VUS will become clear. For example, RNA sequencing studies may validate candidate splice-disrupting variants in both coding and non-coding regions, which may be particularly useful for the VUS in *GNAL* and *SLC20A2* found in subjects pt.17108 and pt.14135, respectively.

Our findings may have management implications. Individuals with *KMT2B* mutations show a good though incomplete response to DBS [27]. Therefore, a genetic diagnosis should be considered prior to DBS to help inform expectations. An individual with *ATM* mutations was instituted on monitoring for early detection of cancer, a known manifestation of this disorder. The individual with an *SGCE* mutation will be considered for drug treatment specific for myoclonus dystonia, including zonisamide.

Many of these diagnoses were not suspected (i.e. unanticipated findings), which may be the case in atypical presentations, although clinicians should be alert to subtle but characteristic diagnostic clues. Given the unexpected finding of ATM-associated dystonia, we suggest screening for AFP, immunoglobulin levels and regularly check for telangiectasias in individuals with young onset dystonia, especially if jerky or myoclonic. Additional evidence supporting *KMT2B* pathogenesis includes childhood onset dystonia with relatively rapid progression and characteristic changes on MRI [27]. Isolated dystonia with an autosomal dominant family history may indicate a *GNAL* mutation. Dystonia parkinsonism is suggestive of mutations in *PRKN* and paroxysmal kinesigenic dyskinesia is suggestive of a *PRRT2* mutation [28]. Furthermore, the syndrome of dystonia with anarthria may have a broad range of causes, including GM1 gangliosidosis, as previously described [29].

In this study, we identified a *KMT2B* mutation carrier unaffected by dystonia (the sibling of pt.9125), but with short stature and mild intellectual impairment. This is in keeping with reports that *KMT2B* mutation carriers may be unaffected by dystonia but have other isolated clinical features [30].

5. Conclusion

While WGS is more expensive than other technologies [9], our study suggests that WGS may be advantageous in dystonia, due to i) superior detection of clinically-relevant sequencing variants in dystonia genes in comparison to WES, ii) reliable detection of disease-causing CNVs, and iii) identification of candidate deep-intronic variants. However, further studies are required to demonstrate an unequivocal benefit of WGS over other NGS technologies, and careful clinical characterisation may allow for more targeted, economical genetic testing in dystonia.

Funding sources

Documentation of author roles

Author	1. Research Project			2. Statistical analysis			3. Manuscript preparation	
	A. Conception	B. Organization	C. Execution	A. Design	B. Execution	C. Review and critique	A. Writing of the first draft	B. Review and critique
Kishore R. Kumar	✓	✓	✓	✓	✓	✓	✓	✓
Ryan L. Davis		✓	✓			✓		✓
Michel Tchan		✓	✓			✓		✓
G.M. Wali		✓	✓			✓		✓
Neil Mahant		✓	✓			✓		✓
Karl Ng		✓	✓			✓		✓
Katya Kotschet		✓	✓			✓		✓
Sue-Faye Siow		✓	✓			✓		✓
Jason Gu		✓	✓			✓		✓
Zachary Walls		✓	✓			✓		✓

Ce Kiang	✓	✓	✓	✓	✓	✓
Gautam Wali	✓	✓			✓	✓
Stan Levy	✓	✓			✓	✓
Chung Sen Phua	✓	✓			✓	✓
Con Yiannikas	✓	✓			✓	✓
Paul Darveniza	✓	✓			✓	✓
Florence C·F. Chan	✓	✓			✓	✓
Hugo Morales	✓	✓			✓	✓
Dominic B. Rowe	✓	✓			✓	✓
Alex Drew	✓	✓			✓	✓
Velimir Gayevskiy	✓	✓			✓	✓
Mark Cowley	✓	✓			✓	✓
Andre Minoche	✓	✓			✓	✓
Stephen Tisch	✓	✓			✓	✓
Michael Hayes	✓	✓			✓	✓
Sarah Kummerfeld	✓	✓			✓	✓
Victor S·C. Fung	✓	✓			✓	✓
Carolyn M. Sue	✓	✓	✓		✓	✓

Full financial disclosure for the previous 12 months

Author	Financial Disclosures
Kishore R. Kumar	KRK was supported by a NHMRC Early Career Fellowship.
Ryan L. Davis	RLD is supported by a NSW Health Early-Mid Career Fellowship.
Michel Tchan	Nothing to disclose
G.M. Wali	Nothing to disclose
Neil Mahant	Nothing to disclose
Karl Ng	Nothing to disclose
Katya Kotschet	Nothing to disclose
Sue-Faye Siow	Nothing to disclose
Jason Gu	Nothing to disclose
Zachary Walls	Nothing to disclose
Ce Kiang	Nothing to disclose
Gautam Wali	GW was supported by grant funding from Spastic Paraplegia Foundation, INC.
Con Yiannikas	Nothing to disclose
Paul Darveniza	Nothing to disclose
Florence F·C. Chang	Nothing to disclose
Hugo Morales	Nothing to disclose
Dominic B. Rowe	Nothing to disclose
Alex Drew	Nothing to disclose
Velimir Gayevskiy	Nothing to disclose
Mark Cowley	MC is supported by an NSW Health Early-Mid Career Fellowship.
Andre Minoche	Nothing to disclose
Stephen Tisch	Nothing to disclose
Michael Hayes	Nothing to disclose
Sarah Kummerfeld	Nothing to disclose
Victor S·C. Fung	Nothing to disclose
Carolyn M. Sue	CMS is supported by a National Health and Medical Research Council Practitioner Fellowship.

Declaration of competing interest

None.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.11.004.

References

[1] A. Albanese, K. Bhatia, S.B. Bressman, M.R. Delong, S. Fahn, V.S. Fung, M. Hallett, J. Jankovic, H.A. Jinnah, C. Klein, A.E. Lang, J.W. Mink, J.K. Teller, Phenomenology and classification of dystonia: a consensus update, Mov. Disord. 28 (7) (2013) 863–873.

[2] S. Montaut, C. Tranchant, N. Drouot, G. Rudolf, C. Guissart, J. Tarabeux, T. Stemmelen, A. Velt, C. Fourrage, P. Nitschke, B. Gerard, J.L. Mandel, M. Koenig, J. Chelly, M. Anheim, P.s. French, C. Movement Disorders, Assessment of a targeted gene panel for identification of genes associated with movement disorders, JAMA Neurol 75 (10) (2018) 1234–1245.

[3] J. Ma, L. Wang, Y.M. Yang, X.H. Wan, Targeted gene capture sequencing in diagnosis of dystonia patients, J. Neurol. Sci. 390 (2018) 36–41.

[4] M. Zech, N. Gross, A. Jochim, F. Castrop, M. Kaffe, C. Dresel, P. Lichtner, A. Peters, C. Gieger, T. Meitinger, B. Haslinger, J. Winkelmann, Rare sequence variants in ANO3 and GNAL in a primary torsion dystonia series and controls, Mov. Disord. 29 (1) (2014) 143–147.

[5] M. Zech, S. Boesch, A. Jochim, S. Weber, T. Meindl, B. Schormair, T. Wieland, C. Lunetta, V. Sansone, M. Messner, J. Mueller, A. Ceballos-Baumann, T.M. Strom, R. Colombo, W. Poewe, B. Haslinger, J. Winkelmann, Clinical exome sequencing in early-onset generalized dystonia and large-scale resequencing follow-up, Mov. Disord. 32 (4) (2017) 549–559.

[6] M.E. van Egmond, C.H.A. Lugtenberg, O.F. Brouwer, M.F. Contarino, V.S.C. Fung, M.R. Heiner-Fokkema, J.J. van Hilten, A.H. van der Hout, K.J. Peall, R.J. Sinke, E. Roze, C.C. Verschuuren-Bemelmans, M.A. Willemsen, N.I. Wolf, M.A. Tijssen, T.J. de Koning, A post hoc study on gene panel analysis for the diagnosis of dystonia,

Mov. Disord. 32 (4) (2017) 569–575.

[7] K.R. Kumar, K. Lohmann, I. Masuho, R. Miyamoto, A. Ferbert, T. Lohnau, M. Kasten, J. Hagenah, N. Bruggemann, J. Graf, A. Munchau, V.S. Kostic, C.M. Sue, A.R. Domingo, R.L. Rosales, L.V. Lee, K. Freimann, A. Westenberger, Y. Mukai, T. Kawarai, R. Kaji, C. Klein, K.A. Martemyanov, A. Schmidt, Mutations in GNAL: a novel cause of craniocervical dystonia, JAMA Neurol 71 (4) (2014) 490–494.

[8] K.R. Kumar, M.J. Cowley, R.L. Davis, Next-generation sequencing and emerging technologies, Semin. Thromb. Hemost. 45 (7) (2019) 661–673.

[9] J.S. Mattick, M. Dinger, N. Schonrock, M. Cowley, Whole genome sequencing provides better diagnostic yield and future value than whole exome sequencing, Med. J. Aust. 209 (5) (2018) 197–199.

[10] K.R. Kumar, G.M. Wali, M. Kamate, G. Wali, A.E. Minoche, C. Puttick, M. Pinese, V. Gayevskiy, M.E. Dinger, T. Roscioli, C.M. Sue, M.J. Cowley, Defining the genetic basis of early onset hereditary spastic paraplegia using whole genome sequencing, Neurogenetics 17 (4) (2016) 265–270.

[11] A. Kim, K.R. Kumar, R.L. Davis, A.C. Mallawaarachchi, V. Gayevskiy, A.E. Minoche, Z. Walls, H.J. Kim, M. Jang, M.J. Cowley, J.H. Choi, C. Shin, C.M. Sue, B. Jeon, Increased diagnostic yield of spastic paraplegia with or without cerebellar ataxia through whole-genome sequencing, Cerebellum 18 (4) (2019) 781–790.

[12] A.E. Minoche, C. Horvat, R. Johnson, V. Gayevskiy, S.U. Morton, A.P. Drew, K. Woo, A.L. Statham, B. Lundie, R.D. Bagnall, J. Ingles, C. Semsarian, J.G. Seidman, C.E. Seidman, M.E. Dinger, M.J. Cowley, D. Fatkin, Genome sequencing as a first-line genetic test in familial dilated cardiomyopathy, Genet. Med. 21 (3) (2018) 650–662.

[13] C. Kopanos, V. Tsiolkas, A. Kouris, C.E. Chapple, M. Albarca Aguilera, R. Meyer, A. Massouras, VarSome: the human genomic variant search engine, Bioinformatics 35 (11) (2018) 1978–1980.

[14] S. Richards, N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, W.W. Grody, M. Hegde, E. Lyon, E. Spector, K. Voelkerding, H.L. Rehm, A.L.Q.A. Committee, Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology, Genet. Med. 17 (5) (2015) 405–424.

[15] D.C. Hoskinson, A.M. Dubuc, H. Mason-Suares, The current state of clinical interpretation of sequence variants, Curr. Opin. Genet. Dev. 42 (2017) 33–39.

[16] V. Siokas, A.M. Aloizou, Z. Tsouris, A. Michalopoulou, A.A. Mentis, E. Dardiotis, Risk factor genes in patients with dystonia: a comprehensive review, Tremor Other Hyperkinet Mov (N Y) 8 (2018) 559.

[17] M. Pinese, P. Lacaze, E.M. Rath, A. Stone, M.-J. Brion, A. Ameur, S. Nagpal, C. Puttick, S. Husson, D. Degrave, T.N. Cristina, V.F.S. Kahl, A.L. Statham, R.L. Woods, J.J. McNeil, M. Riaz, M. Barr, M.R. Nelson, C.M. Reid, A.M. Murray, R.C. Shah, R. Wolfe, J.R. Atkins, C. Fitzsimmons, H.M. Cairns, M.J. Green, V.J. Carr, M.J. Cowley, H.A. Pickett, P.A. James, J.E. Powell, W. Kaplan, G. Gibson, U. Gyllensten, M.J. Cairns, M. McNamara, M.E. Dinger, D.M. Thomas, The Medical Genome Reference Bank: Whole Genomes and Phenotype of 2,570 Healthy Elderly, bioRxiv, 2018, p. 473348.

[18] A.M. Meynert, M. Ansari, D.R. FitzPatrick, M.S. Taylor, Variant detection sensitivity and biases in whole genome and exome sequencing, BMC Bioinf. 15 (2014) 247.

[19] C. Shaw, F. Hisama, J. Friedman, T.D. Bird, ADCY5-Related dyskinesia, in: M.P. Adam, H.H. Ardinger, R.A. Pagon, S.E. Wallace, L.J.H. Bean, K. Stephens, A. Amemiya (Eds.), GeneReviews((R)), Seattle (WA), 1993.

[20] Y. Feng, Y. Huang, X. Zhao, H. Sheng, Y. Feng, W. Zhang, L. Liu, Clinical and molecular characteristics of 11 Chinese probands with GM1 gangliosidosis, Metab. Brain Dis. 33 (6) (2018) 2051–2057.

[21] D.K. Chan, V. Mok, P.W. Ng, J. Yeung, J.B. Kwok, Z.M. Fang, R. Clarke, L. Wong, P.R. Schofield, N. Hattori, PARK2 mutations and clinical features in a Chinese population with early-onset Parkinson's disease, J. Neural Transm. 115 (5) (2008) 715–719.

[22] W.J. Chen, Y. Lin, Z.Q. Xiong, W. Wei, W. Ni, G.H. Tan, S.L. Guo, J. He, Y.F. Chen, Q.J. Zhang, H.F. Li, Y. Lin, S.X. Murong, J. Xu, N. Wang, Z.Y. Wu, Exome sequencing identifies truncating mutations in PRRT2 that cause paroxysmal kinesigenic dyskinesia, Nat. Genet. 43 (12) (2011) 1252–1255.

[23] C. Marini, V. Conti, D. Mei, D. Battaglia, D. Lettori, E. Losito, G. Bruccini, G. Tortorella, R. Guerrini, PRRT2 mutations in familial infantile seizures, paroxysmal dyskinesia, and hemiplegic migraine, Neurology 79 (21) (2012) 2109–2114.

[24] A. Grunewald, A. Djarmati, K. Lohmann-Hedrich, K. Farrell, J.A. Zeller, N. Allert, F. Papengut, B. Petersen, V. Fung, C.M. Sue, D. O'Sullivan, N. Mahant, A. Kupsch, R.S. Chuang, K. Wiegers, H. Pawlack, J. Hagenah, L.J. Ozelius, U. Stephani, R. Schuit, A.E. Lang, J. Volkmann, A. Munchau, C. Klein, Myoclonus-dystonia: significance of large SGCE deletions, Hum. Mutat. 29 (2) (2008) 331–332.

[25] R. Tan, Y. Wang, S.E. Kleinstein, Y. Liu, X. Zhu, H. Guo, Q. Jiang, A.S. Allen, M. Zhu, An evaluation of copy number variation detection tools from whole-exome sequencing data, Hum. Mutat. 35 (7) (2014) 899–907.

[26] K. Lohmann, A. Schmidt, A. Schillert, S. Winkler, A. Albanese, F. Baas, A.R. Bentivoglio, F. Borngraber, N. Bruggemann, G. Defazio, F. Del Sorbo, G. Deuschl, M.J. Edwards, T. Gasser, P. Gomez-Garre, J. Graf, J.L. Groen, A. Grunewald, J. Hagenah, C. Hemmelmann, H.C. Jabusch, R. Kaji, M. Kasten, H. Kawakami, V.S. Kostic, M. Liguori, P. Mir, A. Munchau, F. Ricchiuti, S. Schreiber, K. Siegesmund, M. Svetel, M.A. Tijssen, E.M. Valente, A. Westenberger, K.E. Zeuner, S. Zittel, E. Altenmuller, A. Ziegler, C. Klein, Genome-wide association study in musician's dystonia: a risk variant at the arylsulfatase G locus? Mov. Disord. 29 (7) (2014) 921–927.

[27] E. Meyer, K.J. Carss, J. Rankin, J.M. Nichols, D. Grozeva, A.P. Joseph, N.E. Mencacci, A. Papandreou, J. Ng, S. Barral, A. Ngoh, H. Ben-Pazi, M.A. Willemsen, D. Arkadir, A. Barnicoat, H. Bergman, S. Bhate, A. Boys, N. Darin, N. Foulds, N. Gutowski, A. Hills, H. Houlden, J.A. Hurst, Z. Israel, M. Kaminska, P. Limousin, D. Lumsden, S. McKee, S. Misra, S.S. Mohammed, V. Nakou, J. Nicolai, M. Nilsson, H. Pall, K.J. Peall, G.B. Peters, P. Prabhakar, M.S. Reuter, P. Rump, R. Segel, M. Sinnema, M. Smith, P. Turnpenny, S.M. White, D. Wieczorek, S. Wiethoff, B.T. Wilson, G. Winter, C. Wragg, S. Pope, S.J. Heales, D. Morrogh, U.K. Consortium, S. Deciphering Developmental Disorders, N.B.R.D. Consortium, A. Pittman, L.J. Carr, B. Perez-Duenas, J.P. Lin, A. Reis, W.A. Gahl, C. Toro, K.P. Bhatia, N.W. Wood, E.J. Kamsteeg, W.K. Chong, P. Gissen, M. Topf, R.C. Dale, J.R. Chubb, F.L. Raymond, M.A. Kurian, Mutations in the histone methyltransferase gene KMT2B cause complex early-onset dystonia, Nat. Genet. 49 (2) (2017) 223–237.

[28] A. Schmidt, K.R. Kumar, K. Redyk, A. Grunewald, M. Leben, A. Munchau, C.M. Sue, J. Hagenah, H. Hartmann, K. Lohmann, H.J. Christen, C. Klein, Two faces of the same coin: benign familial infantile seizures and paroxysmal kinesigenic dyskinesia caused by PRRT2 mutations, Arch. Neurol. 69 (5) (2012) 668–670.

[29] C. Ganos, B. Crowe, M. Stamelou, N. Kresojevic, M.J. Lukic, J. Bras, R. Guerreiro, F. Taiwo, B. Balint, A. Batla, S.A. Schneider, R. Erro, M. Svetel, V. Kostic, M.A. Kurian, K.P. Bhatia, The clinical syndrome of dystonia with anarthria/ aphonia, Park. Relat. Disord. 24 (2016) 20–27.

[30] L. Dai, C. Ding, F. Fang, An inherited KMT2B duplication variant in a Chinese family with dystonia and/or development delay, Park. Relat. Disord. 63 (2019) 227–228.