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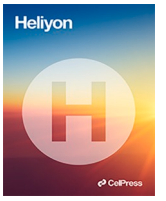

Research article

Whole-exome sequencing in a cohort of Chinese patients with isolated cervical dystonia

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ABSTRACT

Background: Dystonia is a kind of movement disorder but its pathophysiological mechanisms are still largely unknown. Recent evidence reveals that genetical defects may play important roles in the pathogenesis of dystonia.
Objectives and Methods: -To explore possible causative genes in Chinese dystonia patients, DNA samples from 42 sporadic patients with isolated cervical dystonia were subjected to whole-exome sequencing. Rare deleterious variants associated with dystonia phenotype were screened out and then classified according to the American College of Medical Genetics and Genomics (ACMG) criteria. Phenolyzer was used for analyzing the most probable candidates correlated with dystonia phenotype, and SWISS-MODEL server was for predicting the 3D structures of variant proteins.
Results: Among 42 patients (17 male and 25 female) recruited, a total of 36 potentially deleterious variants of dystonia-associated genes were found in 30 patients (30/42, 71.4 %). Four disease-causing variants including a pathogenic variant in *PLA2G6* (c.797G > C) and three likely pathogenic variants in *DCTN1* (c.73C > T), *SPR* (c.1A > C) and *TH* (c.56C > G) were found in four patients separately. Other 32 variants were classified as uncertain significance in 26 patients. Phenolyzer prioritized genes *TH*, *PLA2G6* and *DCTN1* as the most probable candidates correlated with dystonia phenotype. Although 3D prediction of *DCTN1* and *PLA2G6* variant proteins detected no obvious structural alterations, the mutation in *DCTN1* (c.73C > T:p.Arg25Trp) was closely adjacent to its key functional domain.
Conclusion: Our whole-exome sequencing results identified a novel variant in *DCTN1* in sporadic Chinese patients with isolated cervical dystonia, which however, needs our further study on its exact role in dystonia pathogenesis.

1. Introduction

Dystonia is a kind of movement disorder characterized by abnormal involuntary movements and/or postures in one or more parts of the body owing to sustained or intermittent muscle contractions [1]. The clinical manifestations of dystonia are highly heterogeneous, which involve the face (blepharospasm or Meige syndrome), the neck (cervical dystonia or torticollis), the larynx (larynx dystonia or spastic dysphonia), the limb (writer's cramp and other focal hand dystonia) or trunk and lower extremities [2]. Cervical

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dystonia is the most common form of focal dystonia, with an incidence of 1.18/100,000 person-years [3]. It is a highly disabling movement disorder characterized by involuntary, usually painful, head posturing [4].

The pathophysiological mechanisms of dystonia remain largely unclear [5]. At present, it is considered as a kind of "neural network disorder" [6], that may be related to dopaminergic receptor hypersensitivity or dopamine transmitter imbalance, low function of GABAergic neurons, and hyperactivity of cholinergic effect [7]. The brain regions may involve cerebral cortex, basal ganglia (including striatum, subthalamic nucleus, and substantia nigra pars reticula), cerebellum, thalamus, brainstem, etc. [8]. In recent years, more attentions are focusing on the field of genetics about dystonia, and a number of novel genes have been identified, which may be involved in dystonia pathogenesis. With the rapid development of the next-generation sequencing technology, it provides a new perspective for in-depth understanding of the occurrence and pathophysiology of dystonia [9], and also provides an important reference for early diagnosis, treatment guidance, prognosis judgment and genetic counseling of dystonia. At present, the majority of genetic discoveries were from the patients with a family history of dystonia. However, given the clinical practice that most dystonia cases are sporadic, here we performed the whole exome sequencing in 42 sporadic Chinese patients with isolated cervical dystonia and tried to explore the possible causative genes in Asian population.

2. Methods

2.1. Patients

Forty-two Chinese Han patients (17 male and 25 female) in middle adulthood were recruited from April 2020 to April 2022. They were diagnosed with isolated cervical dystonia by an experienced specialist in movement disorder. All the patients underwent head magnetic resonance imaging, revealing no obvious abnormalities (data not shown). Those who were suspected of other acquired etiologies or diagnosed as combined dystonia were excluded in this study. Clinical subtypes of cervical dystonia were classified according to the Col-Cap concept [10]. The severity and functional disability of patients were evaluated by the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) and Tsui Score [11]. Relatively younger ages were chosen here, trying to avoid the data were not affected by either early development, mutation accumulation with age, or environment. All the patients had no family history of different types of dystonia. This study was approved by the local Institutional Ethics Committee. All subjects completed informed consent for participation and publication of potential identifying information before the original sample collection. This study was conducted in accordance with the principles of the Declaration of Helsinki.

2.2. Sample preparation and sequencing

After obtaining patients' informed consents, their peripheral blood samples were collected and genomic DNAs were extracted. Then DNA samples were fragmented to an average size of 180-280bp and subjected to DNA library creation according to established Illumina paired-end protocols. An Agilent SureSelect Human All Exon V6 Kit (Agilent Technologies, Santa Clara, CA, USA) was used for exome capture according to the manufacture's introduction. The Illumina Novaseq 6000 platform (Illumina Inc., San Diego, CA, USA) was utilized for genomic DNA sequencing. The average sequencing depth was 133.7 ± 15.0 with a depth-of-coverage $\geq$10x for at least 99 % of the targeted regions. After removing low quality reads, valid FastQ data were aligned to the reference human genome (GRCH37) by using the Burrows-Wheeler Aligner (Ver.0.7.8-r455), and duplicate reads were marked by Sambamba tools (Ver. 1.6). The single nucleotide variants (SNVs) and INDELs were called with Samtools (Ver. 1.6), and the copy number variants were detected by CoNIFER software (Ver. 0.2.2). Then the deleteriousness and conservation of variant data were annotated by using ANNOVAR (2017June8).

2.3. Determination for rare deleterious variants

To determine rare deleterious variants, above annotated variants were further filtered as follows: (1) Variants with a minor allele frequency less than 1 % in population databases, including 1000g_all (2015), esp6500siv2_all (2014), gnomAD_ALL (2017) and gnomAD_EAS (2017) were reserved; (2) Only SNVs occurring in exons or splice sites (splicing junction 10 bp) were selected, and synonymous SNVs which are not relevant to the amino acid alternation predicted by dbscSNV database were discarded; (3) Small fragment non-frameshift (<10bp) INDELs in the repeat region defined by Repeat Masker were discarded; (4) Potentially deleterious variations were scored by following 4 professional tools or databases, the SIFT (https://sift.bii.a-star.edu.sg/), the Polyphon-2 (http://genetics.bwh.harvard.edu/pph2/), the Mutation Taster (http://www.mutationtaster.org/) and the CADD (http://cadd.gs.washington.edu/). CADD score $\geq$15 [12] were used as the cut-off. Those reaching to the harmful criteria scored by $\geq$ 2 tools or databases were reserved. SNP mutations predicted by dbscSNV database to affect splicing were reserved, and dbscSNV score more than 0.6 was used as a cut-off [13]. Sites (>2bp) did not affect alternative splicing were removed.

After rare deleterious variants were determined, the corresponding genes associated with dystonia phenotype were screened out from the ClinVar database, the Online Mendelian Inheritance in Man (OMIM) database and the Human Gene Mutation database (HGMD), in which the genes are documented to involve the diseases accompanied with dystonic symptoms, such as Parkinsonism, dyskinesia, ataxia, chorea et al. Then dystonia phenotype-associated deleterious variants were classified into pathogenic, likely pathogenic, uncertain significance (VUS), likely benign or benign by ACMG criteria of the standards and guidelines for the interpretation of sequence variants [14]. In addition, above mutation genes and dystonia phenotype were input on the Phenolyzer website (https://phenolyzer.wglab.org/) [15] and ranked the correlation by the final scoring results. All the deleterious variants were

Table 1
Phenotypic profile of 42 index patients with cervical dystonia.

Patient No.	Sex	age	Age at onset	Course of disease (m)	Forms of CD[a]	Combined symptoms	TWSTRS score	Tsui score	Related Gene
S2	F	57	57	2	Torticaput with retrocaput	Tremor	32	12	*DCTN1*
S3	F	50	48	22	Torticaput	No	20	9	*KMT2B*
S4	M	40	38	26	Torticaput with laterocaput	Tremor and pain	42	13	*CACNA1B*
S5	F	51	51	3	Torticaput with retrocaput	Tremor and pain	44	15	*ADCY5*
S6	M	52	50	23	Torticollis with antecollis	Pain	38	11	*ATP1A2*
S7	M	30	27	25	Torticollis	No	28	10	*CACNA1B DCTN1*
S8	F	38	38	6	Torticaput	Tremor	36	14	*ADCY5 COL6A3 SGCE PANK2*
S9	M	27	26	8	Torticaput with retrocaput	Tremor and pain	40	16	*CACNA1B PANK2*
S10	F	47	47	2	Torticaput	Pain	30	6	*COL6A3*
S11	M	17	17	2	Torticaput with torticollis	Tremor and pain	42	8	
S12	M	43	43	6	Torticaput	Tremor and pain	42	17	*COL6A3*
S13	M	44	43	6	Torticollis	Tremor	36	12	*ATM*
S14	M	40	38	18	Torticaput	No	27	6	*PLA2G6*
S15	F	47	46	12	Torticaput with laterocaput	Tremor and pain	43	16	
S16	F	23	23	1	Torticaput	Tremor	29	7	*PINK1*
S17	F	26	23	36	Torticaput	Tremor	34	6	*PANK2*
S18	F	47	47	3	Torticaput	Tremor and pain	45	11	
S19	M	52	51	9	Laterocaput	No	20	5	
S20	M	30	30	2	Lateral shift	Tremor and pain	42	13	
S21	F	51	48	36	Torticaput with laterocaput	Tremor	32	14	*COL6A3*
S22	F	39	39	4	Torticaput with retrocaput	Tremor	39	13	*KMT2B THAP1*
S23	F	29	27	24	Torticaput	Pain	27	8	*COL6A3 NKX2-1*
S24	F	47	39	85	Torticaput with retrocaput	No	20	7	*COL6A3*
S25	F	35	33	15	Torticaput with laterocaput	Pain	37	13	*PANK2 RAB12 WDR45*
S26	M	43	43	2	Laterocaput	Tremor	29	6	*PLA2G6*
S27	M	31	31	3	Torticollis	Pain	32	9	
S28	M	42	42	6	Torticollis with retrocollis	Tremor and pain	46	15	
S29	F	36	35	12	Torticaput with laterocaput	Pain	36	14	
S30	F	42	40	24	Torticaput with retrocaput	Pain	30	8	*COL6A3 KMT2B TUBB4A*
S31	F	43	40	13	Torticaput	Tremor	25	6	
S32	F	39	38	8	Torticaput	Tremor	22	6	
S33	M	24	21	36	Torticaput with laterocaput	Tremor and pain	32	7	*TH*
S34	F	43	39	40	Torticaput	Tremor and pain	36	12	*COL6A3*
S35	F	36	36	6	Torticaput with laterocaput	pain	32	10	
S36	F	37	36	13	Torticaput	Tremor	25	8	*COL6A3 KMT2B*
S37	M	47	47	1	Torticaput	No	20	5	
S38	F	45	45	1	Laterocaput	Tremor	21	6	*KCNA4*
S39	M	41	40	8	Torticaput with laterocaput	Tremor and pain	32	7	*SPR*
S40	F	39	39	5	Torticaput	Tremor	20	5	*KMT2B*
S41	F	46	45	6	Torticollis with Retrocollis	Pain	28	8	*TBC1D24*
S42	M	45	43	22	Torticaput with laterocaput	Pain	22	6	*SGCE*
S43	F	51	48	35	Lateral shift	Tremor	18	5	*SPR*

Abbreviations: F, female; M, male; m, months; TWSTRS, Toronto Western Spasmodic Torticollis Rating Scale.
[a] , Forms of cervical dystonia according to the Col-Cap concept [10].

Table 2
Pathogenic or likely-pathogenic variants identified by whole-exome sequencing.

Gene.[a]	PatientNo.	cDNA	Protein	SNPID	Het/Hom	Allelefrequency.[b]	dbscSNVscore.[c]	SIFT	Polyphen2HVARHDIV		MutationTaster	CADD.[d]	OMIMID.[e]	HGMDID.[f]	ACMG	ACMGclassification(evidence)
PLA2G6	S26	c.797G > C	p.Gly266Ala	–	Het	−/−	1.0000, 0.908	D	–	–	DC	13.45	256600, 610217, 612953	–	Pathogenic	1PVS+1PM+1 PP
DCTN1	S2	c.73C > T	p.Arg25Trp	rs756611519	Het	−/−	−/−	–	PSD	PBD	DC	33	105400, 607641, 168605	–	Likely Pathogenic	2PM+2 PP
SPR	S39	c.1A > C	p.Met1Leu	–	Het	−/−	−/−	D	B	B	DC	15.74	612716	CM171543	Likely Pathogenic	1PVS+1PM
TH	S33	c.56C > G	p.Ser19Cys	rs766704202	Het	−/−	−/−	D	PBD	PBD	DC	26.2	605407	CM135180	Likely Pathogenic	2PM+2 PP

Abbreviations: B, benign; D, deleterious; DC, disease causing; Het, heterozygous; Hom, homozygous; PBD, probably damage; PolyPhen2, polymorphism phenotyping v2; PSD, possible damage; SIFT, sorting intolerant from tolerant; PVS, very strong evidence of pathogenicity; PM, moderate evidence of pathogenicity; PP, supporting evidence of pathogenicity.

−, not available.

[a] Reference sequences used are: NM_001004426 (PLA2G6); NM_001135040 (DCTN1); NM_003124 (SPR); NM_000360 (TH).

[b] Allele frequency: Allele frequency in public population databases: 1000g_Chinese and 1000 Genomes, respectively.

[c] dbscSNV Score: The score more than 0.6 is considered for predicting their potential for alternative splicing.

[d] CADD: The score more than 15 is considered as deleteriousness for SNP.

[e] OMIM disease ID: 256600, Infantile neuroaxonal dystrophy 1; 610217, Neurodegeneration with brain iron accumulation 2B; 612953, Parkinson disease 14; 105400, Amyotrophic lateral sclerosis; 607641, Distal hereditary motor neuronopathy VIIB; 168605, Perry syndrome; 612716, Dopa-responsive dystonia (sepiapterin reductase deficiency); 605407, Segawa syndrome.

[f] HGMD disease ID: CM171543, Dopa-responsive dystonia (sepiapterin reductase deficiency); CM135180, Dopa-responsive dystonia.

Table 3
Uncertain significance variants in reportedly confirmed dystonia-causing genes identified by whole-exome sequencing.

Gene[a]	Patient No.	cDNA	Protein	SNP ID	Het/ Hom	Allele frequency[b]	SIFT	Polyphen2 HVAR	Polyphen2 HDIV	Mutation Taster	CADD[c]	OMIM ID.[d]	HGMD ID.[e]	ACMG classification (evidence)
COL6A3	S34	c.958G > A	p.Ala320Thr	rs115819851	Het	0.004983/ 0.000799	T	B	PSD	DC	22.8	158810, 616411, 254090	–	1BP+1PM
COL6A3	S10; S30	c.1264G > A	p.Val422Met	rs114511558	Het	0.013289/ 0.001997	D	PSD	PBD	DC	22.6	158810, 616411, 254090	–	1BP+1 PP
COL6A3	S12	c.1478T > C	p.Val493Ala	rs116794756	Het	0.013289/ 0.001997	T	PBD	PBD	DC	21.0	158810, 616411, 254090	_	1BP+1 PP
COL6A3	S21	c.1597C > T	p.Arg533Cys	rs751952844	Het	−/−	D	PBD	PBD	N	20.5	158810, 616411, 254090	–	1PM
COL6A3	S24	c.1762G > A	p.Asp588Asn	rs886043408	Het	−/−	D	PBD	PBD	N	22.2	158810, 616411, 254090	CM1822109	1PM
COL6A3	S8; S23: S36	c.4912G > A	p.Ala1638Thr	rs114322958	Het	0.006645/ 0.000799	D	PBD	PBD	DC	25.5	158810, 616411, 254090	CM1310912	1BP+2 PP
COL6A3	S10	c.8965+9G > A	–	–	Het	−/−	–	–	–	–	12.3	158810, 616411, 254090	–	1PM
KMT2B	S3; S36; S40	c.1760C > G	p.Pro587Arg	rs2242519	Het	0.009967/ 0.003395	D	PBD	PBD	DC	23.8	617284	–	1 PP
KMT2B	S22	c.2421_2422 insCAG	p.Asp807delins AspGln	rs750525059	Het	−/−	–	–	–	–	–	617284	–	2PM
KMT2B	S30	c.2702G > A	p.Arg901Gln	rs755335733	Het	−/−	D	PBD	PBD	DC	31	617284	–	1PM+1 PP
SGCE	S8	c.1027C > T	p.Arg343Trp	rs757796461	Het	−/−	D	PBD	PBD	DC	32	159900	–	1BP+1PM+1 PP
SGCE	S42	c.1282G > A	p.Gly428Arg	rs368892695	Het	0.001661/ 0.0002	D	PBD	PBD	N	15.72	159900		1BP+1PM
SPR	S43	c.112G > A	p.Val38Ile	rs146099322	Het	0/0.000998	D	B	B	DC	16.33	612716	CM171543	1BP+2PM
THAP1	S22	c.449A > C	p.His150Pro	–	Het	−/−	T	B	B	DC	15.03	602629	CM111185	1PM+2 PP
TUBB4A	S30	c.766G > A	p.Glu256Lys	–	Het	−/−	D	B	PSD	DC	23.4	128101, 612438	–	2PM+1 PP

Abbreviations: B, benign; BP, supporting evidence of benign. D, deleterious; DC, disease causing; Het, heterozygous; Hom, homozygous; N, polymorphism; PBD, probably damage; PM, moderate evidence of pathogenicity; PolyPhen2, polymorphism phenotyping v2; PP, supporting evidence of pathogenicity; PSD, possible damage; SIFT, sorting intolerant from tolerant; T, tolerate.
−, not available.

[a] Reference sequences used are: NM_004369 (COL6A3); NM_014727 (KMT2B); NM_001099401 (SGCE); NM_003124 (SPR); NM_018105 (THAP1); NM_001289130 (TUBB4A).

[b] Allele frequency: Allele frequency in public population databases: 1000g_Chinese and 1000 Genomes, respectively.

[c] CADD: The score more than 15 is considered as deleteriousness for SNP.

[d] OMIM disease ID: 158810, Bethlem myopathy 1; 616411, Dystonia 27; 254090, Ullrich congenital muscular dystrophy 1; 617284, Childhood-onset dystonia 28; 159900, Myoclonic dystonia-11; 612716, Dopa-responsive dystonia (sepiapterin reductase deficiency); 602629, Torsion dystonia 6; 128101, Torsion dystonia 4; 612438, Hypomyelinating leukodystrophy 6.

[e] HGMD disease ID: CM1822109, Limb-girdle muscular dystrophy 1A; CM1310912, Intermediate ullrich congenital muscular dystrophy; CM111185, Dystonia 6; CM171543, Dopa-responsive dystonia (sepiapterin reductase deficiency).

Table 4
Uncertain significance variants in dystonia phenotype-associated genes identified by whole-exome sequencing.

Gene[a]	Patient No.	cDNA	Protein	SNP ID	Het/ Hom	Allele frequency[b]	SIFT	Polyphen2 HVAR	Polyphen2 HDIV	Mutation Taster	CADD[c]	OMIM ID.[d]	HGMD ID.[e]	ACMG classification (evidence)
ADCY5	S5; S8	c.242C > T	p.Pro81Leu	rs77439349	Het	0.003322/ 0.000998	D	B	B	DC	11.24	606703	–	2BS
ATM	S13	c.6503C > T	p. Ser2168Leu	rs200431631	Het	0.001661/ 0.0002	D	PSD	PBD	DC	29.5	208900	–	1BP+1PM+1 PP
ATP1A2	S6	c.194G > T	p.Arg65Leu	rs187733403	Het	0.003322/ 0.000998	T	B	B	DC	20.3	104290, 619605	–	1BS+1BP+1PM
CACNA1B	S9	c.95C > T	p.Pro32Leu	rs777004745	Het	−/−	D	B	B	DC	17.3	618497	–	1PM+1 PP
CACNA1B	S7	c.2869C > T	p.Arg957Trp	–	Het	−/−	D	B	B	N	23.5	618497	–	1PM+1 PP
CACNA1B	S4	c.5455G > A	p. Ala1819Thr	rs200122209	Het	0/0.000399	D	PSD	PBD	DC	24.6	618497	–	1BS2+2 PP
CACNA1B	S7	c.6340C > T	p. Arg2114Trp	rs374411386	Het	−/−	D	PBD	PBD	DC	35	618497	–	1PM+2 PP
DCTN1	S7	c.3724G > C	p. Glu1242Gln	rs146083590	Het	−/−	T	B	PSD	N	15.29	105400, 607641, 168605	–	1PM+1 PP
KCNA4	S38	c.146C > T	p.Ala49Val	–	Het	−/−	D	B	PSD	N	.	618284	–	2PM
NKX2−1	S23	c.964G > A	p.Gly322Ser	rs200560568	Het	0.003322/ 0.000599	T	B	B	DC	13.89	118700, 610978	–	1BS+1 PP
PANK2	S8; S17; S25	c.280C > G	p.Arg94Gly	rs199680057	Het	0.003322/ 0.000799	D	B	B	DC	24.3	607236, 234200	–	–
PANK2	S9	c.383G > A	p.Arg128Gln	rs546381069	Het	0.004983/ 0.001198	D	B	PSD	DC	23.4	607236, 234200	CM1717209	–
PINK1	S16	c.158G > A	p.Gly53Asp	–	Het	−/−	D	PSD	PBD	N	12.3	605909	–	1PM
PLA2G6	S14	c.2255C > G	p.Pro752Arg	rs140758033	Het	0.01495/ 0.002995	D	PBD	PBD	DC	23.8	256600, 610217, 612953	–	1BP + PP
RAB12	S25	c.64C > T	p.Pro22Ser	rs752093779	Het	−/−	D	B	B	DC	21.5	616448	–	–
TBC1D24	S41	c.1552C > T	p.Arg518Trp	rs78644690	Het	−/−	T	B	B	DC	23.4	615338, 608105, 605021	–	1BP + PM
WDR45	S25	c.331C > T	p.Arg111Cys	rs781985024	Het	−/−	D	B	B	DC	25.2	300894	–	1BP+2PM

Abbreviations: B, benign; BS: strong evidence of benign; BP, supporting evidence of benign; D, deleterious; DC, disease causing; Het, heterozygous; Hom, homozygous; N, polymorphism; PBD, probably damage; PM, moderate evidence of pathogenicity; PolyPhen2, polymorphism phenotyping v2; PP, supporting evidence of pathogenicity; PSD, possible damage; SIFT, sorting intolerant from tolerant; T, tolerate.

−, not available.

[a] Reference sequences used are: NM_183357 (ADCY5); NM_000051 (ATM); NM_000702 (ATP1A2); NM_000718 (CACNA1B); NM_001135040 (DCTN1); NM_002233 (KCNA4); NM_003317 (NKX2-1); NM_001324192 (PANK2); NM_032409 (PINK1); NM_001004426 (PLA2G6); NM_001025300 (RAB12); NM_020705 (TBC1D24); NM_001029896 (WDR45).

[b] Allele frequency: Allele frequency in public population databases: 1000g_Chinese and 1000 Genomes, respectively.

[c] CADD: The score more than 15 is considered as deleteriousness for SNP.

[d] OMIM disease ID: 606703, Familial dyskinesia with facial myokymia; 208900, Ataxia-telangiectasia; 104290, Alternating hemiplegia of childhood 1; 619605, Developmental and epileptic encephalopathy 98; 618497, Neurodevelopmental disorder with seizures and nonepileptic hyperkinetic movements; 105400, Amyotrophic lateral sclerosis; 607641, distal Hereditary motor neuronopathy VIIB; 168605, Perry syndrome; 618284, Microcephaly, cataracts, impaired intellectual development, and dystonia with abnormal striatum; 118700, Hereditary benign chorea; 610978, Choreoathetosis, hypothyroidism, and neonatal respiratory distress; 607236, HARP syndrome; 234200, Neurodegeneration with brain iron accumulation 1; 605909, Parkinson disease type 6; 256600, Infantile neuroaxonal dystrophy 1; 610217, Neurodegeneration with brain iron accumulation 2B; 612953, Parkinson disease 14; 616448, RAS-associated protein RAB12; 615338, Developmental and epileptic encephalopathy 16; 608105, Epilepsy, rolandic, with proxysmal exercise-induce dystonia and writer's cramp; 605021, Familial infantile myoclonic epilepsy; 300894, Neurodegeneration with brain iron accumulation.

[e] HGMD disease ID: CM1717209, Pantothenate kinase-associated neurodegeneration.

validated by Sanger sequencing on ABI 3730xl Genetic Analyzer (Applied Biosystems, USA), and the forward and reverse primers used for amplifying the mutation genes were showed in Supplemental Table 1.

Amino acid conservation analysis using multiple sequence alignment of *PLA2G6* and *DCTN1* protein sequences from different species was performed by MEGA8.0 software [16]. The 3D protein structures of the wild-type and variant proteins were predicted using SWISS-MODEL server [17] (https://swissmodel.expasy.org/). and visualized by Visual Molecular Dynamics software [18].

3. Results

3.1. Clinical characteristics of patients

General information, clinical manifestations and relevant examination of all recruited patients were shown in Table 1. A total of 42 patients (17 male and 25 female) were recruited in this study. The mean age at onset was 40.3 ± 9.0 years ranging from 17 to 57 years. The course of disease ranged from 1 to 85 months. The forms of cervical dystonia were described by the Col-Cap concept [10]. In detail, the most common primary form was torticaput (31/42, 73.8 %), and the second form was laterocaput (12/42, 28.6 %). Other forms

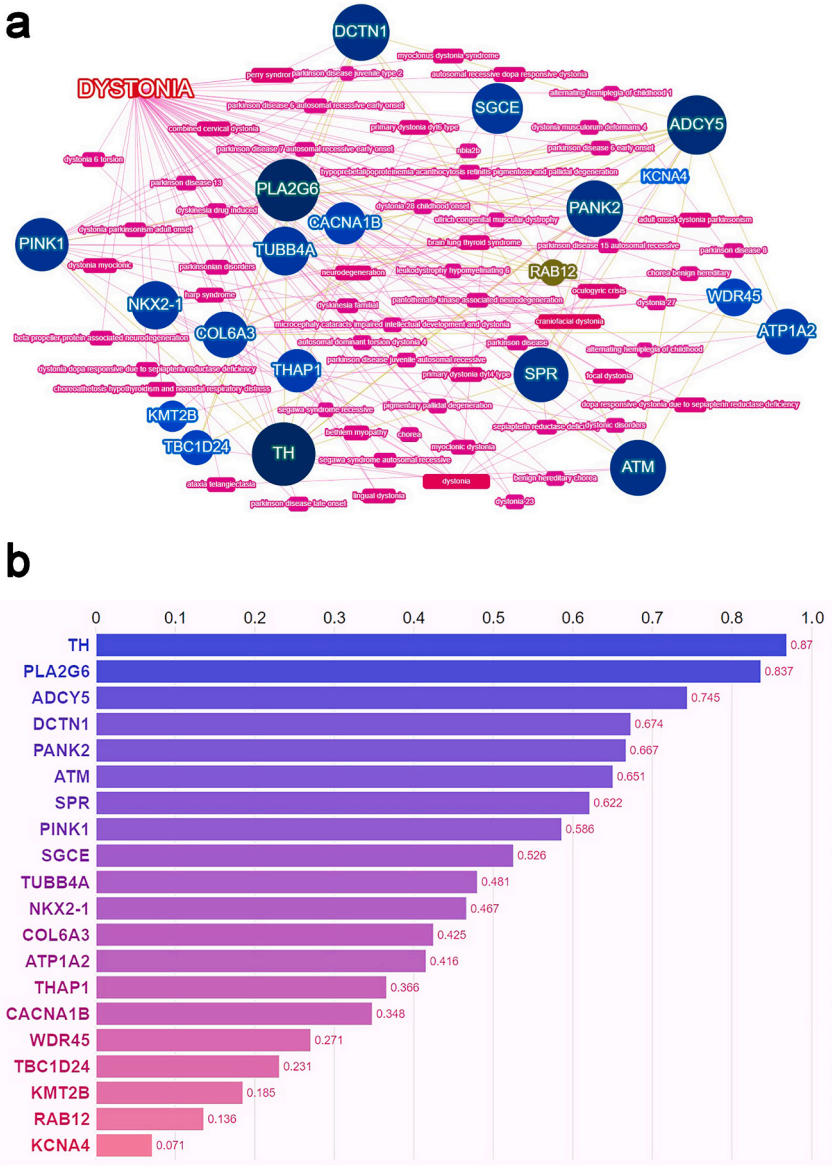

Fig. 1. Gene-dystonia phenotypic association analysis. **(a)** The correlation between the mutation genes and dystonia phenotype was analyzed on the Phenolyzer website. Word in red indicates dystonia phenotype; blue and yellow-green dots indicate genes related to dystonia; pink rounded rectangles indicate related diseases searched by dystonia. **(b)** Candidate genes correlated with dystonia phenotype were scored by Phenolyzer.

included torticollis (7/42, 16.7 %), retrocaput (6/42, 14.3 %), retrocollis (2/42, 4.8 %), antecollis (1/42, 2.4 %) and lateral shift (2/42, 4.8 %). Pure forms were observed in 23 patients (23/42, 54.8 %). Torticaput was combined with laterocaput in 9 patients (9/42, 21.4 %), and with retrocaput in 6 patients (6/42, 14.3 %). Other combined forms included torticaput with torticollis, torticollis with antecollis, and torticollis with retrocollis. Additionally, 36 patients were accompanied by other symptoms including tremor (14/36, 38.9 %), pain (10/36, 27.8 %) and the both (12/36, 33.3 %). Tsui score and TWSTRS scale were used for evaluating the severity of cervical dystonia. The mean score of TWSTRS was 31.7 ± 8.1 ranging from 18 to 46 and that of Tsui was 9.6 ± 3.7 ranging from 5 to 17.

3.2. Genetic analysis

By whole-exome sequencing, about 120,000 deleterious and conserved variants per sample were first obtained (Supplemental Tables 2 and 3). After filtration, around 350 rare deleterious variants were then determined (Supplemental Table 4). By screening from the ClinVar, the OMIM and the HGMD databases, total 20 mutated genes associated with dystonia phenotype were identified and contained 36 variants, distributing in 30 patients (30/42, 71.4 %). According to ACMG criterion classification, four disease-causing variants were identified, including a pathogenic variant in *PLA2G6* (c.797G > C) in S26 patient, and three likely pathogenic variants in *DCTN1* (c.73C > T), *SPR* (c.1A > C) and *TH* (c.56C > G) in S2, S39 and S33 patients, respectively (Table 2). Other 32 VUS variants were found in *ADCY5*, *ATM*, *ATP1A2*, *CACNA1B*, *COL6A3*, *DCTN1*, *KCNA4*, *KMT2B*, *NKX2-1*, *PANK2*, *PINK1*, *PLA2G6*, *RAB12*, *SGCE*, *SPR*, *TBC1D24*, *THAP1*, *TUBB4A* and *WDR45* in 26 patients (Tables 3 and 4).

Among 32 VUS variants, 15 variants (15/32, 46.9 %) in 14 patients were identified in *COL6A3* (DYT27), *KMT2B* (DYT28), *SGCE* (DYT11), *SPR* (dopa-responsive dystonia), *THAP1* (DYT6) and *TUBB4A* (DYT4), which have been reported to be involved in the pathogenesis of dystonia. Intriguingly, 9 patients (9/42, 21.4 %) had variations in *COL6A3* (OMIM 616411) with 7 variants (c.958G >

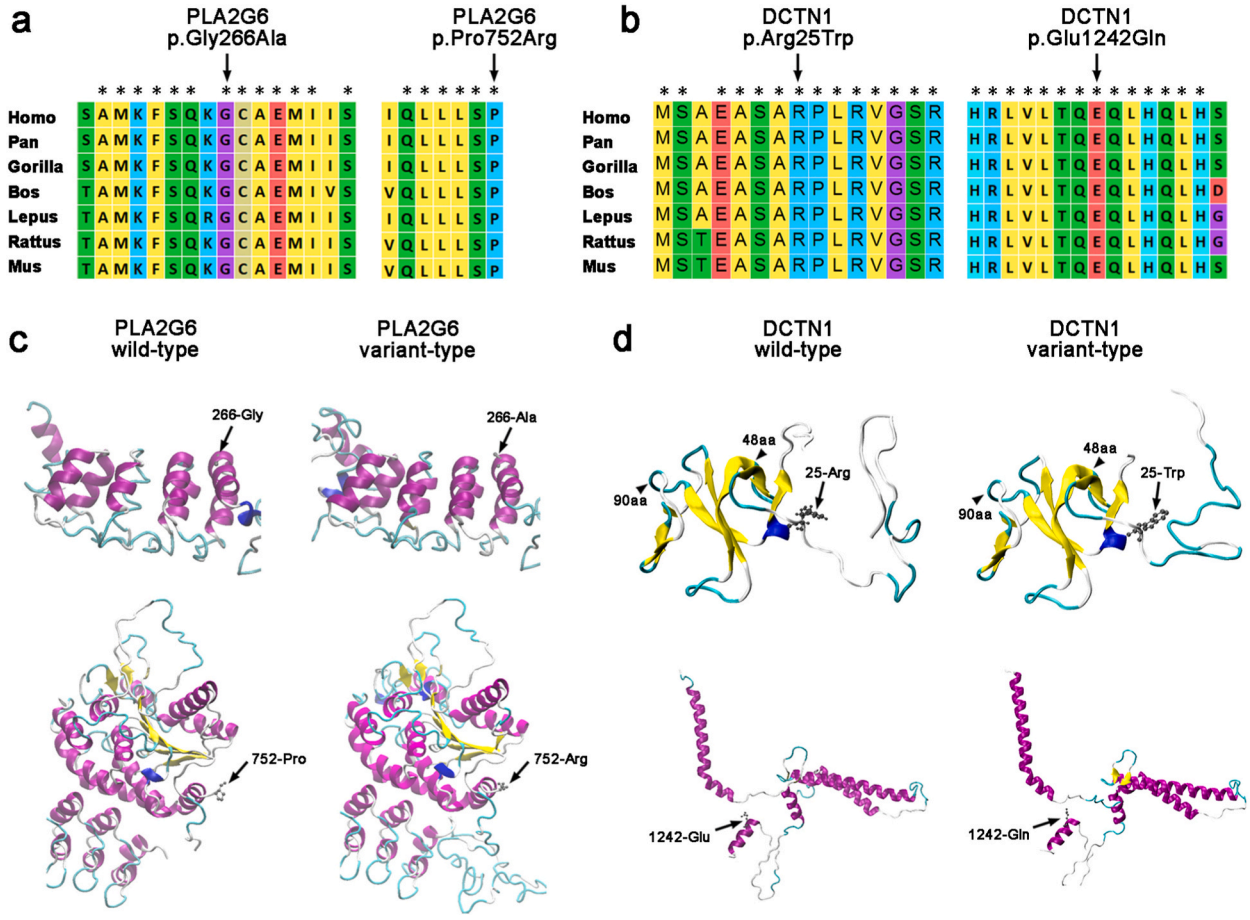

Fig. 2. Protein structure prediction of variant proteins of *PLA2G6* and *DCTN1*. **(a–b)** Conservation of amino acid (aa) residues across different species in *PLA2G6* **(a)** and *DCTN1* **(b)** were aligned using MEGA 7.0 software. The aa substitutions identified in this study are marked by arrows. Asterisk (*) indicates the positions that have a single, fully conserved residue. **(c–d)** The 3D structures of wild-type and variant-type proteins of *PLA2G6* **(c)** and *DCTN1* **(d)** were predicted by SWISS-MODEL server. Protein models were shown in secondary structures. Variant sites were shown in aa structures (black arrows). The CAP-Gly domain of *DCTN1* that ranges from 48 to 90 aa are indicated by arrowheads. aa amino acid.

A, c.1264G > A, c.1478T > C, c.1597C > T, c.1762G > A, c.4912G > A, c.8965+9G > A), among which c.4912G > A appeared spontaneously in patients S8, S23 and S36, and c.1264G > A appeared in patients S10 and S30. Five patients (5/42, 11.9 %) had variations in *KMT2B* (OMIM 617284) with 3 variants (c.1760C > G, c.2421_2422insCAG, c.2702G > A), among which c.1760C > G appeared spontaneously in patients S3, S36 and S40. Additionally, other variants, e.g., c.1027C > T and c.1282G > A in *SGCE* (OMIM 159900), c.112G > A in *SPR* (OMIM 612716), c.449A > C in *THAP1* (OMIM 602629) and c.766G > A in *TUBB4A* (OMIM 128101), were found in S8, S42, S43, S22 and S30, respectively (Table 3).

In addition to above confirmed dystonia-causing genes, 17 VUS variants (17/32, 53.1 %) involving *ADCY5*, *ATM*, *ATP1A2*, *CACNA1B*, *DCTN1*, *KCNA4*, *NKX2-1*, *PANK2*, *PINK1*, *PLA2G6*, *RAB12*, *TBC1D24* and *WDR45* were found in 14 patients as well (Table 4). Among these dystonia phenotype-associated genes, *PANK2* mutations appeared in 4 patients (S8, S9, S17 and S25), *CACNA1B* mutations in 3 patients (S4, S7 and S9), and *ADCY5* mutations in 2 patients (S5 and S8). Other mutations in *ATM*, *ATP1A2*, *DCTN1, KCNA4, NKX2-1*, *PINK1*, *PLA2G6*, *RAB12*, *TBC1D24* and *WDR45* appeared in one patient respectively (Table 4).

Next, we analyzed the correlation between all the mutation genes above and dystonia phenotype on the Phenolyzer website (Fig. 1a). Phenolyzer scored the correlation according to the information of dystonic symptoms and genes in the databases, and ranked the correlation based on the final scoring results. Through the analysis of the results, it can be seen that among the pathogenic and likely-pathogenic genes, except the reported dystonia-causing genes (namely *TH and SPR*), *PLA2G6* and *DCTN1* were prioritized as the most probable candidates correlated with dystonia phenotype (Fig. 1b).

To further explore possible effects of causative variants on gene function, mutated *PLA2G6* and *DCTN1* protein sequences were analyzed. There were two variants in both *PLA2G6* (c.797G > C:p.Gly266Ala in patient S26 and c.2255C > G:p.Pro752Arg in patient S14) and *DCTN1* (c.73C > T:p.Arg25Trp in patient S2 and c.3724G > C:p.Glu1242Gln in patient S7). Through multiple sequence alignment by MEGA8.0, p.Gly266Ala and p.Pro752Arg in the *PLA2G6* and p.Arg25Trp and p.Glu1242Gln in the *DCTN1* were found highly conserved across known mammalian species (Fig. 2a and b), indicating variants in these loci may have an effect on protein function. Although 3D structure prediction of these variants showed no obvious structural alterations (Fig. 2c and d), it was noted that variant p.Arg25Trp in *DCTN1* was closely adjacent to its N-terminal microtubule-binding cytoskeleton-associated protein glycine-rich (CAP-Gly) domain (Fig. 2d, arrowheads), a highly conserved region of *DCTN1*.

4. Discussion

The etiology and pathophysiological mechanisms of dystonia remain largely unknown. With the development of gene detection technology, a number of mutation genes have been reported to be involved in the pathogenesis of dystonia [9]. In this study, we analyzed the genetic information of 42 sporadic Chinese patients with isolated cervical dystonia by using whole exome sequencing, and found several potentially deleterious variants in dystonia-related genes such as *COL6A3* (DYT27), *KMT2B* (DYT28), *SGCE* (DYT11), *SPR* (dopa-responsive dystonia), *TH* (DYT5b), *THAP1* (DYT6) and *TUBB4A* (DYT4), which have been reported in the pathogenesis of dystonia. Importantly, some novel mutations were also identified in other genes, such as *PLA2G6* and *DCTN1*, which are associated with dystonia phenotype but not reported to be involved in dystonia at present.

Among reportedly dystonia-causing genes, *TH* and *SPR* had likely pathogenic variants (Table 2), which were involved in dopa-responsive dystonia [19,20]. However, both patients (S33 and S39) who had respective variants in *TH* and *SPR* did not have fluctuating movement symptoms and had no response to levodopa (data not shown). Alternatively, they mainly presented torticaput combined with obvious head tremor. Other confirmed dystonia-causing genes, such as *COL6A3*, *KMT2B*, *SGCE*, *SPR*, *THAP1* and *TUBB4A*, were identified to have VUS variants (Table 3). Previous studies reported that the patients with *COL6A3* mutations had initial symptoms with the neck or hand, and the distribution was segmental that pronounced in the cranio-cervical region [21,22]. *KMT2B*-related dystonia was characterized phenotypically by intellectual disability and limb-onset childhood dystonia that tended to spread progressively, resulting in generalized dystonia with cranio-cervical involvement [23]. *SGCE* mutations were found to cause myoclonus-dystonia syndrome, *SPR* mutations were related to dopa-responsive dystonia, *THAP1*-related dystonia involved craniofacial muscles and secondary generalization, and *TUBB4A* mutation-causing dystonia was manifested with laryngeal dysphonia [9]. Overall, the phenotypes caused by above dystonia-causing genes seemed to be inconsistent with our patients' manifestations mainly featured by cervical dystonia. The discrepancy of clinical phenotypes might be partly explained by the different mode of transmission; for instance, *TH* and *SPR* mutations have an autosomal recessive mode of inheritance. In addition, the discovered VUS mutations in *COL6A3*, *KMT2B*, *SGCE*, *SPR*, *THAP1* and *TUBB4A* were different from previously reported variants, but the causality of these VUS and cervical dystonia in the affected individuals still needs to be clarified.

Additionally, in the reported hereditary dystonia, *ANO3* (DYT24) and *GNAL* (DYT25) mutations were reported to most commonly involve the neck [9]. However, of all 42 patients with cervical dystonia in this study, no variants were identified in these two genes. Different race (only Han people in northwestern China) and absence of family history might account for this discrepancy. Interestingly, in our study *COL6A3* (DYT27) variants were occurred with the highest frequency. Its mutation was found in 9 patients, 3 of whom had c.4912G > A, and 2 had c.1264G > A. *KMT2B* (DYT28) variants ranked second and appeared in 5 patients, 3 of whom had c.1760C > G (Table 3). Therefore, we assumed that these newly-identified mutations (c.4912G > A in *COL6A3* and c.1760C > G in *KMT2B*) might be worth further research for their roles in cervical dystonia in Chinese Han people.

Apart from reported dystonia-causing genes, other genes, such as *ADCY5*, *ATM*, *ATP1A2*, *CACNA1B*, *DCTN1*, *KCNA4*, *NKX2-1*, *PANK2*, *PINK1*, *PLA2G6*, *RAB12*, *TBC1D24* and *WDR45*, were found to bear mutations as well. These genes were so far not reported to be involved in dystonia pathogenesis but associated with dystonia phenotypes. Among these genes, a pathogenic variant was identified in *PLA2G6* and a likely-pathogenic variant was in *DCTN1*. With Phenolyzer analysis, *PLA2G6* and *DCTN1* were prioritized as the most probable candidates correlated with dystonia phenotype, except confirmed dystonia-causing genes (Fig. 1). *PLA2G6* encodes the group

VI calcium-independent phospholipase A2, a member of the A2 phospholipase family, functioning in inflammation, immune responses, cell proliferation, apoptosis and remodeling of membrane phospholipids [24]. *PLA2G6* mutations may result in so-called *PLA2-G6*-associated neurodegeneration (PLAN), including a series of neurodegeneration diseases. The patients with PLAN present with gait disturbance or neuropsychiatric changes in their early adulthood, and consistently developed dystonia and Parkinsonism in their late teens to early twenties [25]. *DCTN1* encodes the p150Glued, the largest subunit of the dynactin complex, which binds directly to microtubules and the intermediate chain of dynein, engaging retrograde axonal transport [26]. *DCTN1* mutations cause several neurodegeneration diseases, such as Perry syndrome (characterized by Parkinsonism, hypoventilation, depression, and weight loss), distal hereditary motor neuronopathy type 7B, frontotemporal dementia, and amyotrophic lateral sclerosis [27]. In our study, a pathogenic variant c.797G > C (p.Gly266Ala) in *PLA2G6* was found in patient S26 who was, however, only manifested with isolated cervical dystonia. 3D protein structure prediction of p.Gly266Ala showed no structural alteration (Fig. 2c), indicating *PLA2G6* may be not a likely cause or risk factor for dystonia pathogenesis. For the *DCTN1*, a likely-pathogenic variant c.73C > T (p.Arg25Trp) in patient S2 was present. Although protein structure prediction also detected no structural alteration, p.Arg25Trp was found to be closely adjacent to the CAP-Gly domain that ranges from 48 to 90 amino acids. The CAP-Gly domain is essential for its preferential binding to tyrosinated microtubules and for promoting the sustained interaction of the dynein motor with microtubules [28]. p.Arg25Trp converts 25 Arginine (an alkaline amino acid with positive charge) to Tryptophan (a nonpolar amino acid), which may affect the dynein-mediated retrograde axonal transport. In addition, considering that *DCTN1* mutation is autosomal dominant inheritance (OMIM 601143) and patient S2 has no other mutant genes related to the phenotype of dystonia except for *DCTN1*, we supposed that *DCTN1* variant c.73C > T (p.Arg25Trp) might be related with the pathogenesis of cervical dystonia. However, its exact role in the development of dystonia needs our further study.

At present, nearly 100 gene mutations have been found to be related to hereditary dystonia [9,29]. In addition to the inheritance from parents to offspring, gene mutation also occurred spontaneously or was caused by environmental factors. For instance, Demy DJS et al. found that *EIF2AK2* mutation may account for the early-onset isolated generalized dystonia in 5 patients of a Taiwanese family, then they sequenced *EIF2AK2* in 191 unrelated patients with unexplained dystonia and found 3 patients with identical heterozygous or de novo variants [30]. In our study, 42 unrelated patients with isolated cervical dystonia had no family history of dystonia, 30 of whom had various novel variants in either reported dystonia-causing genes (e.g., *COL6A3*, *KMT2B*, etc.) or dystonia phenotype-associated genes (e.g., *DCTN1*, *PLA2G6*, etc.). Given high clinical and genetic heterogeneity of dystonia, these results suggest that apart from hereditary factors, these variants might be one of important causes or risk factors for dystonia pathogenesis as well.

There are still some limitations of our study. First, a relatively small sample size was included in the study, which may omit the patients with a family history of dystonia, therefore weakening the robustness of our findings. Second, among various types of sporadic dystonia, we selectively recruited the patients with cervical dystonia under middle adulthood. Although relatively younger age might minimize the impact of early development, mutation accumulation with age and environment [31], this may also omit some other genes involved in dystonia. Third, genetic information of patients' family members should be examined to determine the mode of inheritance and support the validity of our results. Therefore, in the next study we will recruit more cases including different types of dystonia with all ages, and perform a comprehensive family-based segregation analysis.

5. Conclusion

In the present study, we analyzed gene mutation characteristics of 42 sporadic Chinese patients with isolated cervical dystonia by whole-exome sequencing, and revealed a novel variant (c.73C > T:p.Arg25Trp) of *DCTN1*. These findings might expand our understanding of the pathogenic gene spectrum for dystonia and also provide help for the diagnosis and treatment of disease.

Data availability statement

The datasets presented in this study can be found in online repositories. The name of the repository and accession number can be found below: National Center for Biotechnology Information (NCBI) ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/, SCV002553215 - SCV002553248 and SCV002555548.

CRediT authorship contribution statement

Rui Wu: Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Wen-Tian Chen:** Visualization, Software, Formal analysis. **Wei-Kang Dou:** Resources, Methodology. **Hui-Min Zhou:** Data curation. **Ming Shi:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31885.

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