

RESEARCH

Open Access



Clinical and genetic characteristics of Chinese patients with congenital cranial dysinnervation disorders

Hongyan Jia^{1,2†}, Qian Ma^{1,2†}, Yi Liang^{1,2}, Dan Wang^{1,2}, Qinglin Chang³, Bo Zhao³, Zongrui Zhang³, Jing Liang^{1,2}, Jing Song^{1,2}, Yidi Wang^{1,2}, Ranran Zhang^{1,2}, Zhanhan Tu⁴ and Yonghong Jiao^{1,2*} 

Abstract

Background: Congenital cranial dysinnervation disorders (CCDDs) are a group of diseases with high clinical and genetic heterogeneity. Clinical examinations combined with Magnetic resonance imaging (MRI) and whole exome sequencing (WES) were performed to reveal the phenotypic and genotypic characteristics in a cohort of Chinese CCDDs patients.

Results: A total of 122 CCDDs patients from 96 families were enrolled. All patients showed restrictive eye movements, and 46 patients from 46 families (47.9%, 46/96) were accompanied by multiple congenital malformations. Multi-positional high-resolution MRI was performed in 94 patients from 88 families, of which, all patients had hypoplasia of the cranial nerves except HGPPS patients and 15 patients from 15 families (17.0%, 15/88) were accompanied by other craniocerebral malformations. WES was performed in 122 CCDDs patients. Ten pathogenic variants were detected in *KIF21A*, *TUBB3*, and *CHN1* genes in 43 families. Three variants were unreported, including *KIF21A* (c.1064T > C, p.F355S), *TUBB3* (c.232T > A, p.S78T) and *CHN1* (c.650A > G, p.H217R). Of the 43 probands harboring pathogenic variants, 42 were diagnosed with Congenital Fibrosis of Extraocular Muscles (CFEOM) and one was Duane Retraction Syndrome (DRS). No definite pathogenic variants in known candidate genes of CCDDs were found in sporadic DRS, Möbius Syndrome (MBS) and Horizontal Gaze Palsy with Progressive Scoliosis (HGPPS) patients. The CFEOM patients harboring R380C, E410K and R262H variants in *TUBB3* gene and F355S variant in *KIF21A* gene exhibited syndromic phenotypes.

Conclusions: This study broadened the phenotypic and genotypic spectrums of CCDDs, and it was the largest clinical and genetic investigation for CCDDs patients from China. *KIF21A* and *TUBB3* were the common pathogenic genes in Chinese CFEOM. MRI coupled with WES can provide a supportive diagnosis in patients with clinically suspected CCDDs.

Keywords: Congenital cranial dysinnervation disorders, Magnetic resonance imaging, Whole exome sequencing, Phenotypic and genotypic characteristics, Genetics

Background

Congenital cranial dysinnervation disorders (CCDDs) are a group of congenital, non-progressive, sporadic or familial abnormalities of innervation of extraocular and cranial musculature with high clinical and genetic heterogeneity. The CCDDs can be resulted from hypoplasia or absence of one or more cranial nerves with primary or

[†]Hongyan Jia and Qian Ma have contributed equally to this work

*Correspondence: yhjiao2001@aliyun.com

¹ Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China
Full list of author information is available at the end of the article



© The Author(s) 2022. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

secondary muscle dysinnervation [1]. The disease spectrum of CCDDs encompasses Congenital Fibrosis of Extraocular Muscles (CFEOM), Duane Retraction Syndrome (DRS), Möbius Syndrome (MBS), Horizontal Gaze Palsy with Progressive Scoliosis (HGPPS) and Hereditary Congenital Facial Paresis (HCFP) etc. [2]. CCDDs can be accompanied by multiple congenital malformations, for instance, craniofacial malformations, musculoskeletal defects of limb and/or trunk, intellectual and behavioral impairments etc. [3, 4].

So far, causative variants have been identified in a dozen genes, including *KIF21A*, *TUBB3*, *TUBA1A*, *TUBB2B*, *TUBB6*, *PHOX2A*, *CHN1*, *MAFB*, *SALL4*, *HOXA1*, *HOXB1*, *ROBO3*, *PLXND1*, *REV3L*, *COL25A1* and *ECEL1* etc. [5–19]. Previous reports have established that at least a subset of these genes is critical to the differentiation and migration of the cranial motor neurons, patterning of brainstem, as well as the axon growth and guidance [2, 5–19].

The phenotypic spectrum of CCDDs is broad, and the phenotypes among different constituent diseases may be overlapping [3]. Hypoplasia of the cranial nerves is the most common characteristic of CCDDs. Magnetic resonance imaging (MRI) can display brain structure, cranial nerves and extraocular muscles in high resolution, so MRI can provide supporting evidence for clinical suspected CCDDs cases [20, 21]. Furthermore, whole exome sequencing (WES) can assist with genetic diagnosis and classification of CCDDs [3]. In this study, clinical examination, MRI and WES were used to reveal the clinical and genetic characteristics in a cohort of Chinese Han CCDDs patients.

Results

Clinical and MRI evaluation results of CCDDs

A total of 122 Chinese self-reported Han patients from 96 not known to be related families with CCDDs were enrolled in the study (24 familial and 72 sporadic; age ranged from 5 months to 60 years); their demographic data and systemic features are provided in Table 1. The family trees of familial CFEOM patients were shown in Figs. 1 and 2. All 122 patients have different degrees of restriction of eye movements. Of them, 46 patients from 46 families (47.9%, 46/96) were accompanied by multiple congenital malformations, including facial weakness, joint constricture and psychomotor retardation etc.

94 patients from 88 families underwent MRI, of which, 90 patients exhibited hypoplasia of cranial nerves except HGPPS patients. Furthermore, fifteen patients from 15 families (17.0%, 15/88) were accompanied by other craniocerebral dysplasia, which affected commissural fiber, basal ganglia, thalamus, cerebral ventricle, brainstem and cerebellum etc. An additional table file shows the clinical data of all CCDDs patients in more detail (see Additional file 1).

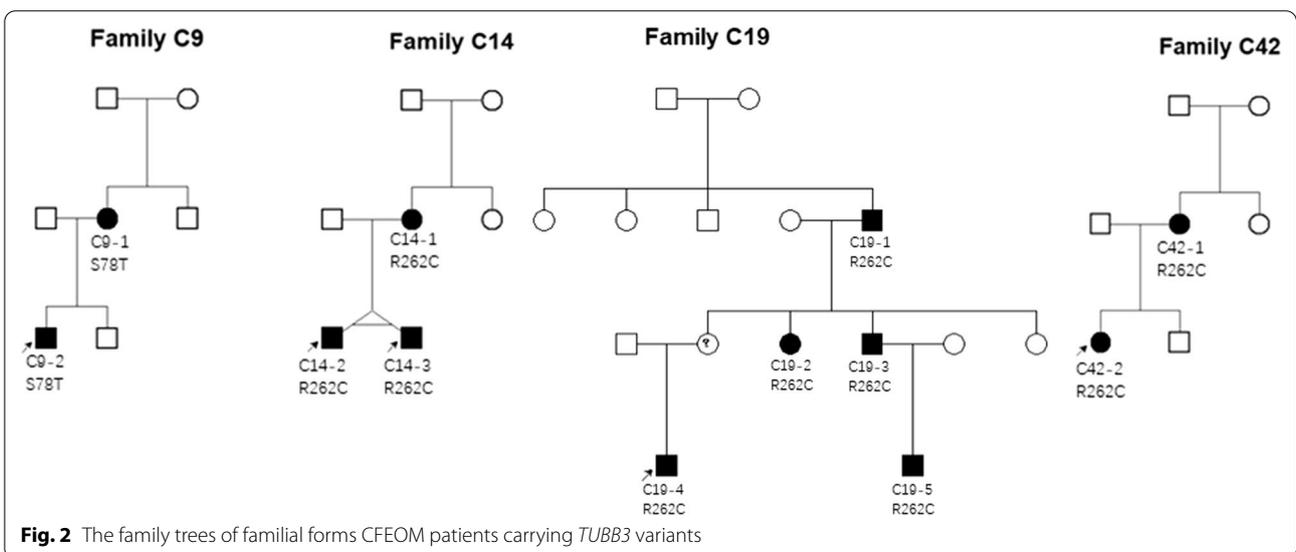
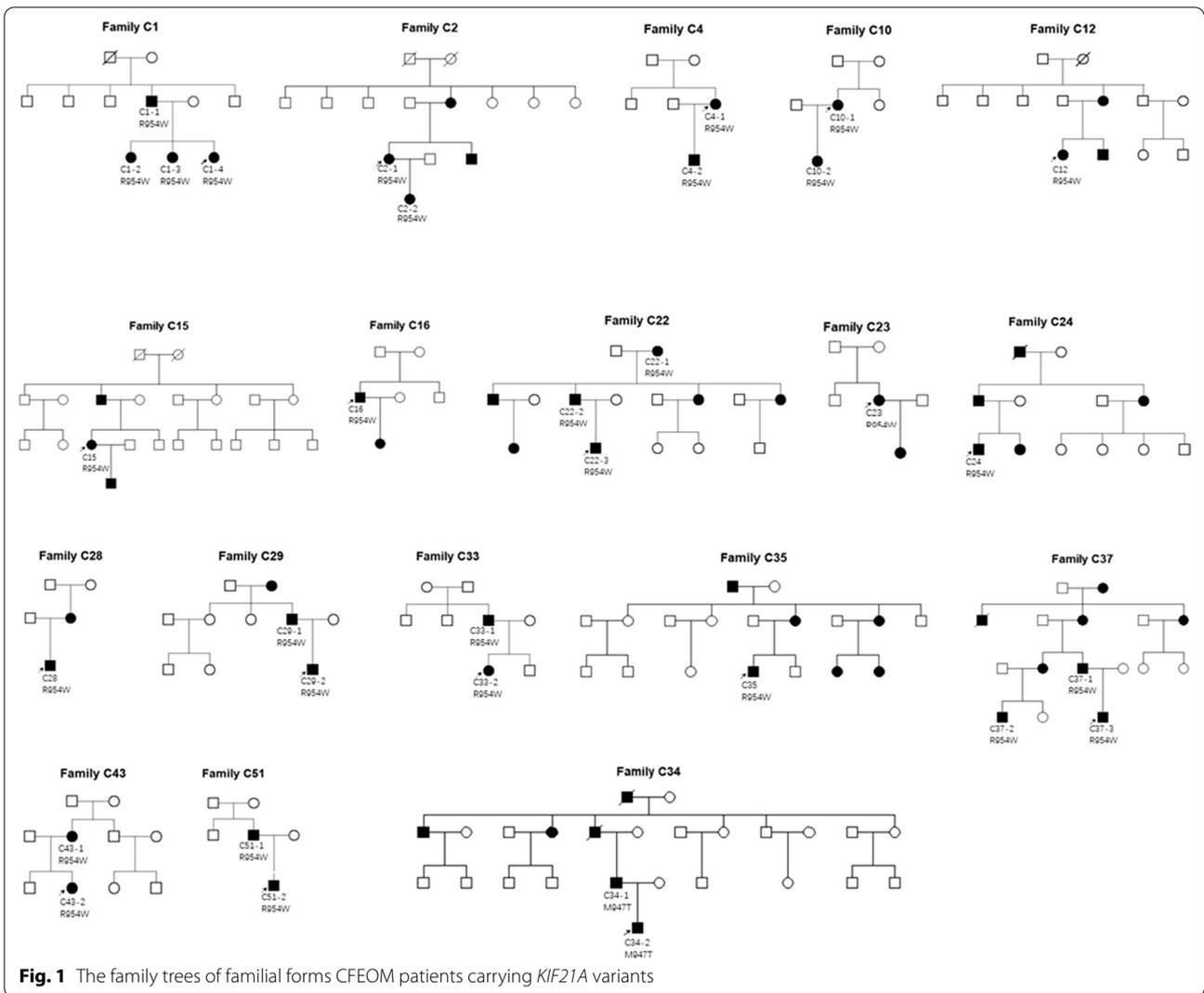
WES evaluation results of CCDDs

Using WES, we identified 10 pathogenic variants in the *KIF21A*, *TUBB3* and *CHN1* genes in 43 families (24 familial and 19 sporadic). Genomic sequence chromatograms of CFEOM individuals with *KIF21A* and *TUBB3* variants were shown in Fig. 3. Three variants were novel: *KIF21A* (c.1064T>C, p.F355S) (NM_001173464), *TUBB3* (c.232T>A, p.S78T) (NM_006086) and *CHN1* (c.650A>G, p.H217R) (NM_001371513), and they were not found in Chinese ethnically-matched databases, gnomAD, etc. (see Additional file 2). CNVs were not identified in known pathogenic genes of CCDDs. For three unreported variants, the 3D structure prediction and

Table 1 Demographics and systemic features of CCDDs patients

	CFEOM	DRS	MBS	HGPPS	Total
No. of patients/families	89/66	11/8	17/17	5/5	122/96
Sex					
Males	48/89 (53.9%)	6/11 (54.5%)	8/17 (47.1%)	3/5 (60%)	65/122 (53.3%)
Female	41/89 (46.1%)	5/11 (45.5%)	9/17 (52.9%)	2/5 (40%)	57/122 (46.7%)
No. of pedigrees	22	2	0	0	24
Sporadic cases	44	6	17	5	72
MRI (performed)					
No. of patients	64	10	16	4	94
Multiple malformations*	17	7	17	5	46
Craniocerebral malformation	9	1	1	4	15

*Multiple malformations contain craniocerebral malformation and other systemic malformations



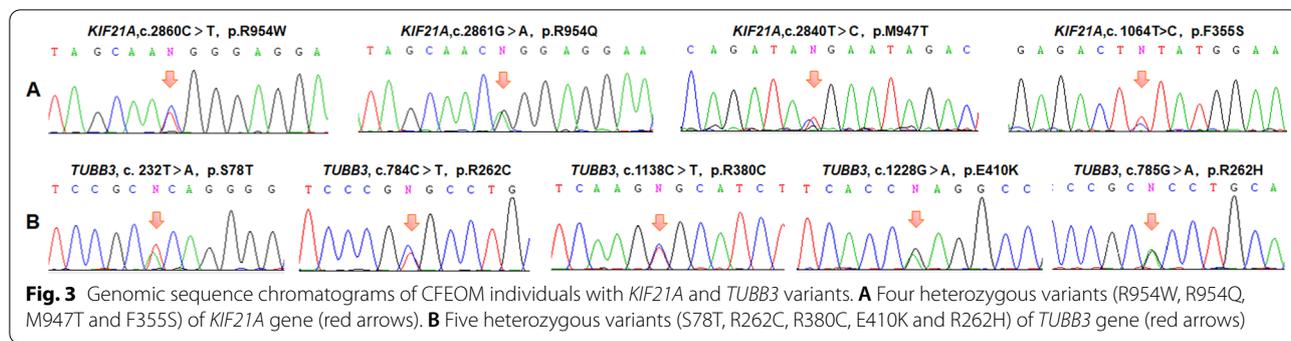


Table 2 The stability analysis of mutations by different servers and software

Mutation (kcal/mol)	$\Delta\Delta G$ DynaMut	$\Delta\Delta G$ ENCoM	$\Delta\Delta G$ mCSM	$\Delta\Delta G$ SDM	$\Delta\Delta G$ DUET	$\Delta\Delta G$ FoldX
KIF21A F355S	-1.974 Destabilizing	-0.998 Destabilizing	-1.709 Destabilizing	-1.400 Destabilizing	-1.717 Destabilizing	3.006 Destabilizing
CHN1 H217R	-0.395 Destabilizing	0.021 Destabilizing	-1.270 Destabilizing	-0.390 Destabilizing	-1.108 Destabilizing	0.204 Stabilizing
TUBB3 S78T	-0.066 Destabilizing	-0.063 Destabilizing	-0.206 Destabilizing	0.090 Stabilizing	0.227 Stabilizing	-0.4751 Stabilizing

stability analysis of mutated protein were performed (Table 2 and Fig. 4).

The protein spatial structure of KIF21A was predicted by SWISS-MODEL, and F355 is located in the possible protein binding pocket (Fig. 4A, B). Force analysis showed a weakened interaction force between the amino acid residues at F355 and the surrounding amino acid residues after the mutation (Fig. 4C, D). The free energy analysis indicated that the free energy of the whole structure changed greatly, and the stability of the protein structure decreased after the mutation (Table 2).

The CHN1 protein structure came from PDBID:3CXL, and H217 is located in the binding pocket of the spatial structure (Fig. 4E, G). Force analysis showed a weakened interaction force after the mutation (Fig. 4H, I). The free energy analysis indicated that the free energy changed greatly, and the stability of the protein structure decreased after the mutation, suggested that H217 is a key site to maintain the structural stability of CHN1 (Table 2).

The TUBB3 protein structure came from PDBID:7PJE, and S78 is located in the surface of spatial structure (Fig. 4K, L). No obvious change in interaction force was found after the mutation (Fig. 4M, N). No obvious change in the free energy was found after the mutation, indicated that the structural stability remains unchanged after mutation (Table 2).

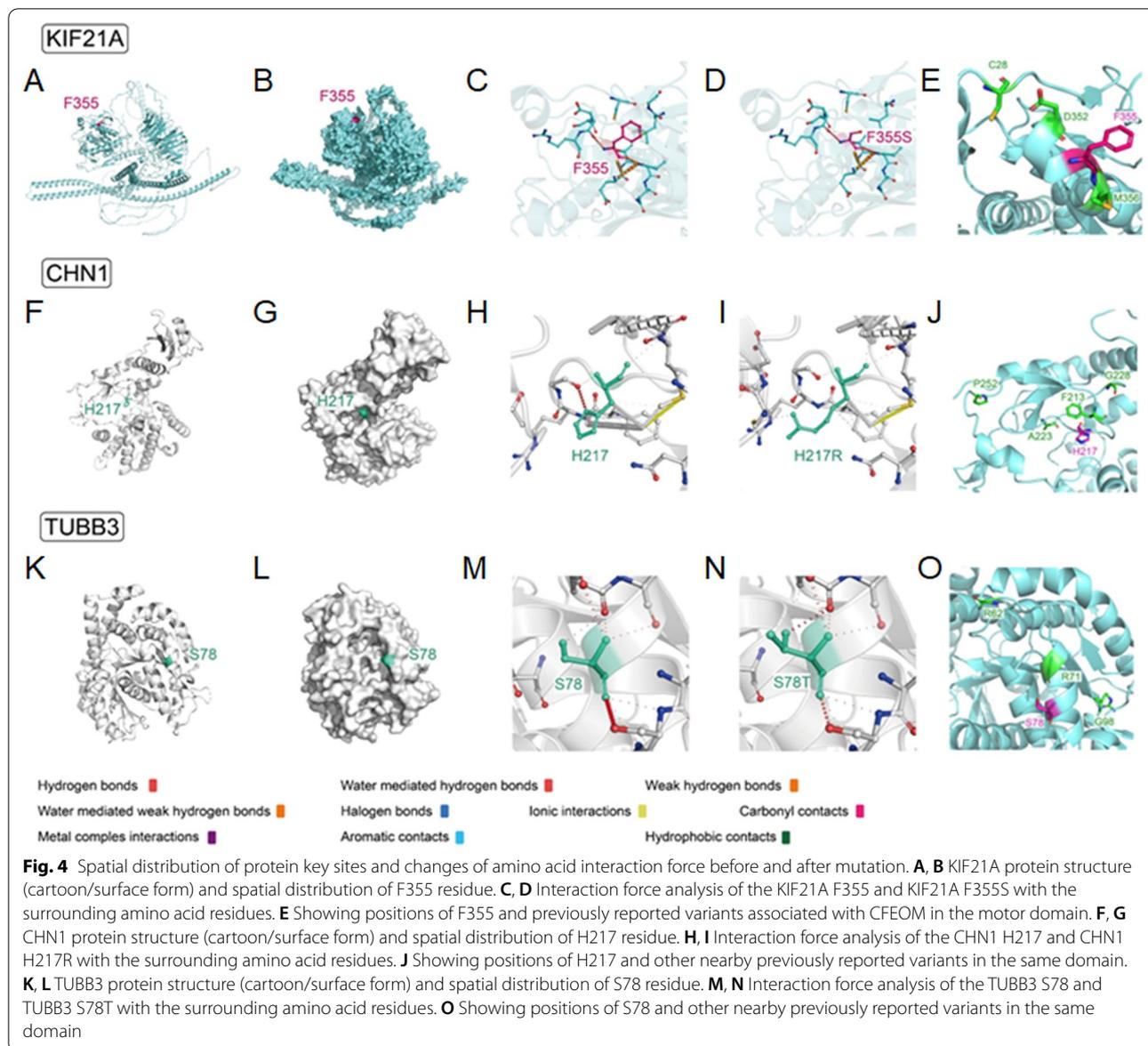
Among the 43 patients harboring variants, 42 were diagnosed with CFEOM and one was familial DRS. No potential pathogenic variants in known candidate genes of CCDDs variant were found in sporadic DRS and MBS. For HGPPS, more than ten heterozygous variants were found in *ROBO3* gene, according to American College of Medical Genetics and Genomics (ACMG) standards, no variants were classified as pathogenic or likely pathogenic.

Genotype–phenotype results in CCDDs
CFEOM

The study enrolled 89 CFEOM cases from 66 families (22 familial and 44 sporadic), all patients showed ptosis, variable limited eye movements and restrictive strabismus. The overall mutation detection rate of CFEOM was 63.6% (42/66), and mutations were detected in 100% familial CFEOM cases. Nine mutations (7 previously reported and 2 novel) in the *KIF21A* (73.8%, 31/42) and *TUBB3* (26.2%, 11/42) genes were identified in 42 probands (22 familial and 20 sporadic).

KIF21A

Four variants (R954W, R954Q, M947T and F355S) in *KIF21A* gene were identified in 31 CFEOM families. The two most common variants were R954W (77.4%, 24/31) and R954Q (16.1%, 5/31), and the other two less common variants were M947T (3.2%, 1/31) and F355S

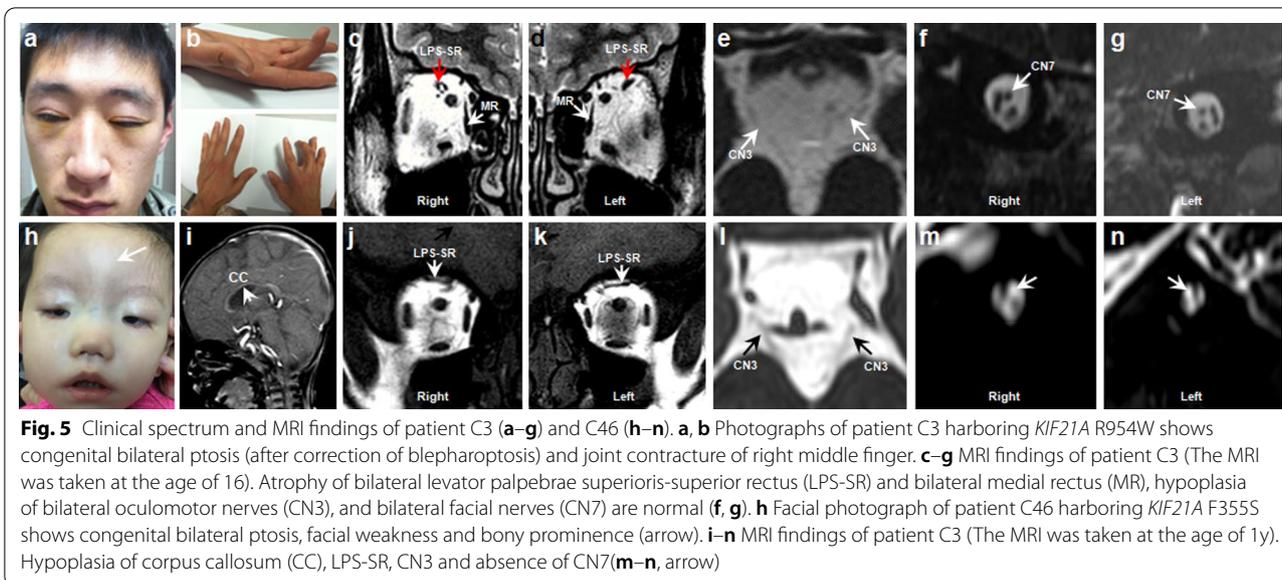


(3.2%, 1/31). The c.1064T>C, p.F355S was an unreported de novo variant. We observed that almost all CFEOM patients harboring *KIF21A* variants showed similar phenotypes, that is isolated CFEOM, except for patients C3 (R954W) and C46 (F355S). The patient C3 was also accompanied by congenital distal joint contracture, and the patient C46 exhibited facial weakness, frontal bony prominence, delayed developmental milestones and thinning of corpus callosum in MRI (Fig. 5). Furthermore, we also observed that patients harboring the same *KIF21A* variants may also have different severity of CFEOM, even if they come from the same family.

TUBB3

Five variants (R262C, R262H, E410K, R380C and S78T) were identified in *TUBB3* gene in 11 CFEOM families. The two most common variants were R262C (54.5%, 6/11) and E410K (18.2%, 2/11), less commonly, R380C (9.1%, 1/11), R262H (9.1%, 1/11) and a novel variant S78T (9.1%, 1/11). Clinical and genetic findings of the 11 CFEOM probands with *TUBB3* variants were listed in Table 3.

The patients harboring R262C and S78T showed milder phenotypes compared to the patients with R380C, E410K and R262H variants. The majority of R262C patients and one S78T patient showed isolated CFEOM, except patient C65 (R262C) who showed the absence of



anterior commissure and the hypoplasia of callosum corpus and internal capsule (Fig. 6). All the CFEOM patients with R380C, E410K and R262H variants were accompanied by multiple congenital craniocerebral and systemic malformations (Figs. 6, 7 and Table 3). Facial weakness and hypogonadotropic hypogonadism occurred in the patients harboring E410K and R262H variants. Psychomotor retardation occurred in patients with R380C, E410K and R262H variants. The patient carrying the R380C variant had delayed developmental milestones, and he was given rehabilitation treatment for 3 months at the age of 2. At the age of 3, he could walk with the help of others. When walking, his legs were not straight, and he had impaired gait.

Common features of MRI from patients with R380C, R262H and E410K variants were malformations of the corpus callosum, anterior commissure and lateral ventricle. Malformations of basal ganglia, thalamus, brainstem and cerebellar were correlated with specific variants—R380C and R262H variants. Absence of facial nerves (CN7) and dysplasia of olfactory bulbs and sulci (OBS) were correlated with R262H and E410K variants (Figs. 6, 7).

DRS

A total number of 11 DRS patients from 8 families (2 familial and 6 sporadic) were enrolled, and they all exhibited congenital esotropia with limited horizontal eye movement accompanied by globe retraction which results in narrowing of the palpebral fissure. MRI revealed hypoplasia of bilateral abducens nerves (CN6) and dysinnervation of CN3.

No pathogenic variants were identified in sporadic DRS cases. Only one pedigree (D2) found a novel variant (c. 650A > G, p. H217R) in *CHN1* gene. The patient D2-2 had congenital esotropia, nystagmus, limited horizontal eye movements and globe retraction with narrowing of the palpebral fissure on attempted adduction. The patient had classic DRS and additional vertical movement anomalies, with the right adducted eye slightly depressed and the left adducted eye slightly elevated. MRI revealed hypoplasia of CN6 and aberrant innervation of the lateral rectus (LR) by the inferior branches of CN3 (Fig. 8).

MBS and HGPPS

The study enrolled 17 MBS and 5 HGPPS patients. All MBS patients were sporadic and showed congenital, non-progressive abduction deficits and facial weakness. MRI revealed hypoplasia of CN6 and CN7 in all MBS patients (Fig. 9). Notably, nine MBS patients (52.9%, 9/17) had intrauterine exposure to adverse factors.

All HGPPS patients were from families of non-consanguineous marriage and showed absence of conjugate horizontal eye movements with intact vertical eye movements, accompanied by variable degrees of scoliosis (Fig. 10). Among four patients who underwent MRI, all had the typical radiographic features of HGPPS, including a deep posterior midsagittal cleft of the pons and butterfly-like morphology in the medulla.

No definite pathogenic variants in known candidate genes were identified in MBS and HGPPS patients.

Table 3 Clinical and genetic findings of the 11 CFEOM probands with *TUBB3* variants

Subject	C9-2	C5	C14-2	C19-4	C31	C42-2	C65	C6	C21	C32	C52
Gender/Age	M/3y	F/3y	M/5y	M/3y	F/14y	F/6y	F/3y	M/10y	M/11y	F/19y	M/3y
Family history	+	-	+	+	-	+	-	-	-	-	-
Genetic findings											
cDNA variation	c.232T>A	c.784C>T	c.784C>T	c.784C>T	c.784C>T	c.784C>T	c.784C>T	c.1138C>T	c.1228C>T	c.1228C>T	c.785G>A
AA change	p. S78T	p. R262C	p. R380C	p. E410K	p. E410K	p. R262H					
Ophthalmological characteristics											
Prosis	R	B	L	B	B	B	B	B	B	B	R
Ocular alignment	ET, HoT	XT, HoT	XT, HoT	XT, HoT	ET, HoT	HoT	XT, HoT	XT, HoT	XT, HoT	XT	XT, HoT
Limited horizontal eye movements	-	B	L	B	-	-	B	-	B	B	B
Limited vertical eye movements	B	B	B	B	B	B	B	B	B	B	B
Nystagmus	-	-	-	-	+	-	-	-	-	-	-
Corneal exposure	-	-	-	-	-	-	-	-	+	+	+
brain malformations (MRI)											
OBS	-	-	-	-	-	-	-	-	+	+	+
CN3	+	+	+	+	+	+	+	+	+	+	+
CN7: Facial weakness	-	-	-	-	-	-	-	-	+	+	+
EOMs	+	+	+	+	+	+	+	+	+	+	+
Corpus callosum	/	-	-	-	-	-	-	-	+	+	+
Cerebellum	/	-	-	-	-	-	-	-	-	-	-
Anterior commissure	/	-	-	-	-	-	+	+	+	+	+
Basal ganglia	/	-	-	-	-	-	-	+	-	-	+
Internal capsule	/	-	-	-	-	-	+	+	-	-	+
External capsule	/	-	-	-	-	-	-	+	-	-	-
Thalamus	/	-	-	-	-	-	-	+	-	-	+
Lateral ventricle	/	-	-	-	-	-	-	+	+	+	+
Sylvian fissure	/	-	-	-	-	-	-	-	-	-	+
Hippocampus	/	-	-	-	-	-	-	-	-	-	+
Others											
Intellectual impairment	-	-	-	-	-	-	-	+	+	+	+
Congenital contractures	-	-	-	-	-	-	-	-	-	-	+
Leg length discrepancy	-	-	-	-	-	-	-	-	-	-	+
Impaired gait	-	-	-	-	-	-	-	+	+	+	+
Cryptorchidism	-	+	-	-	+	+	+	-	+	+	+
Infertility	-	-	-	-	-	-	-	-	+	+	+
Cyclic vomiting	-	-	-	-	-	-	-	-	-	+	+

M, Male; F, Female; y, year; AA, Amino acid; R, Right; B, Bilateral; L, Left; XT, Exotropia; ET, Esotropia; HoT, Hypotropia; OBS, Olfactory bulbs and sulci; EOMs, Extraocular muscles; /, Not available; + Not applicable; +*, Infertility was found in subsequent clinical follow-up

Discussion

In this study, we combined MRI and WES to reveal and broaden the phenotypic and genotypic spectrums in 122 Chinese CCDDs patients from 96 families.

KIF21A and *TUBB3* were the common pathogenic genes of Chinese CFEOM

The mutation detection rate of CFEOM was the highest (63.6%, 42/66), which was much higher than other disease groups (DRS, MBS and HGPPS). And the mutation detection rate of familial CFEOM was 100%. The common pathogenic genes of CFEOM were *KIF21A* (73.8%, 31/42) and *TUBB3* (26.2%, 11/42) genes. Whitman et al. summarized the results of previous researches and found that the variants of *KIF21A*, *TUBB3*, *PHOX2A*, *TUBA1A*, *COL25A1* and *TUBB2B* were identified in 55%, 35%, 10%, <1%, <1% and <1% of the CFEOM patients, respectively [22]. These results are consistent with our current research findings.

KIF21A belongs to a family of kinesin motor protein, which involved in cargo transport along microtubules [5]. Pathogenic variants in *KIF21A* gene may cause CFEOM by modifying the autoinhibitory interaction between the motor domain and a regulatory region in the stalk [23]. The majority of previously reported variants located in coiled coil domains of *KIF21A*, meanwhile, the CFEOM patients are almost all present with isolated phenotype [5]. However, the novel variant F355S identified in our study was located in conserved N-terminal motor domain, and the patient showed rare syndromic phenotype. To our knowledge, only three variants located in the motor region of *KIF21A* have been reported, they were C28W, D352E, M356T [5, 24, 25]. The patients harboring C28W and M356T variants showed isolated CFEOM, while the patients harboring D352E variant present with syndromic phenotype. Notably, patient harboring F355S in this study and the patient harboring D352E had the similar phenotypes, including CFEOM, facial weakness, frontal bony prominence, exposure keratitis and delayed developmental milestones. The difference is that the F355S patient also had the hypoplasia of corpus callosum in MRI. We speculate that the F355S may attenuate auto-inhibition to a greater degree, or induce disease via different pathways [26].

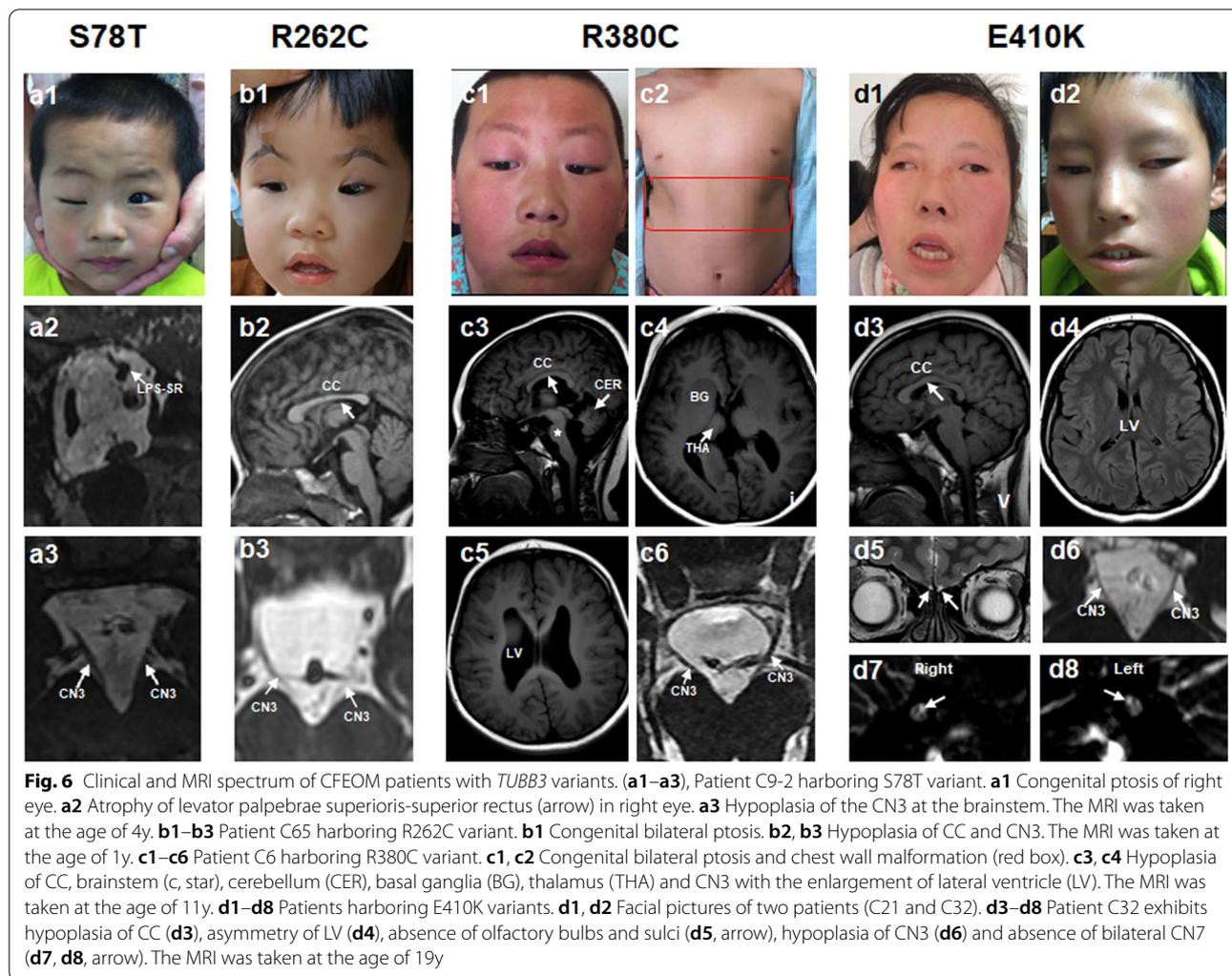
TUBB3 gene encodes the neuron-specific beta-tubulin isotype 3 which plays a critical role in axon guidance and neuronal migration. Pathogenic variants in *TUBB3* may cause CFEOM by altering dynamic instability of microtubules or disrupting the interaction of microtubules with kinesin motors [27].

We found a novel variant *TUBB3*-S78T in a CFEOM pedigree, the proband has normal developmental history (C9-2). The S78T is located in the N-terminal domain of

TUBB3, so far, several variants have been reported in the region of S78. Whitman et al. [28] reported the patients harboring G71R and G98S variations presented with syndromic phenotypes, manifested as CFEOM, nystagmus, torticollis, developmental delay, intellectual and social disabilities. MRI showed the hypoplasia of the corpus callosum and anterior commissure, malformations of hippocampi, thalami, basal ganglia and cerebella, and hypoplasia of brainstem and cranial nerve. The patients harboring the S78T variant in this study and the patient harboring R62Q variant reported by Max A. Tischfield in 2010 presented with isolated CFEOM [6]. Although the S78T patient (C9-2) underwent MRI exam of the ocular motor nerves, and had the normal corpus callosum configuration, however the high resolution brain MRI data was absent, so we cannot rule out the possibility that he has subtle cortical dysplasia. In addition, we noted that G82R have been reported to cause MCD without CFEOM [29].

Compared to patients harboring *KIF21A* variants, the CFEOM patients with *TUBB3* variants tend to show syndromic phenotypes. In the past, researchers named the disease caused by the *TUBB3* variants as the “*TUBB3* Syndromes” [6]. In recent years, researchers found that different variants of *TUBB3* usually produce different syndromic phenotypes [6]. In our cohort, the patients harboring E410K and R262H variants of *TUBB3* gene exhibited CFEOM and accompanied by different multiple congenital malformations, and their clinical and MRI findings were highly similar to previous reports [4, 30], which confirmed that the correlations between specific variants and phenotypes were strong. Therefore, researchers preferred to name these syndromic phenotypes after specific variants. Up to date, “*TUBB3* E410K syndrome”, “*TUBB3* R262H syndrome” and “*TUBB3* M323V syndrome” were used to name the diseases caused by E410K, R262H and M323V variants, respectively [4, 31, 32]. Furthermore, the patients with “*TUBB3* E410K syndrome” and “*TUBB3* R262H syndrome” were easily misdiagnosed as MBS owing to similar clinical manifestations (facial weakness and limitation of eye movements etc.) [33]. MRI, WES and specific general examinations based on the good phenotype-genotype correlations can contribute to accurate diagnosis and follow-up.

TUBB3 R380C variant was identified in one CFEOM patient (C6) in our cohort, and this variant has been previously reported only once in a CFEOM patient [6]. The clinical and MRI findings of these two patients were highly similar, both of them manifested CFEOM with intellectual impairment, multiple congenital craniocerebral malformations which involved in commissural fiber, brainstem, basal ganglia, thalamus, cerebral ventricle and



cerebellum etc. However, “bilateral chest wall malformation” and impaired gait were observed only in our R380C patient. Previous research indicated sensorimotor polyneuropathy occurred in individuals harboring R262H, E410K and D417H/N [6]. We speculated that the R380C patient in this study may have similar sensorimotor polyneuropathy, and electromyography results are needed to confirm the suspected diagnosis.

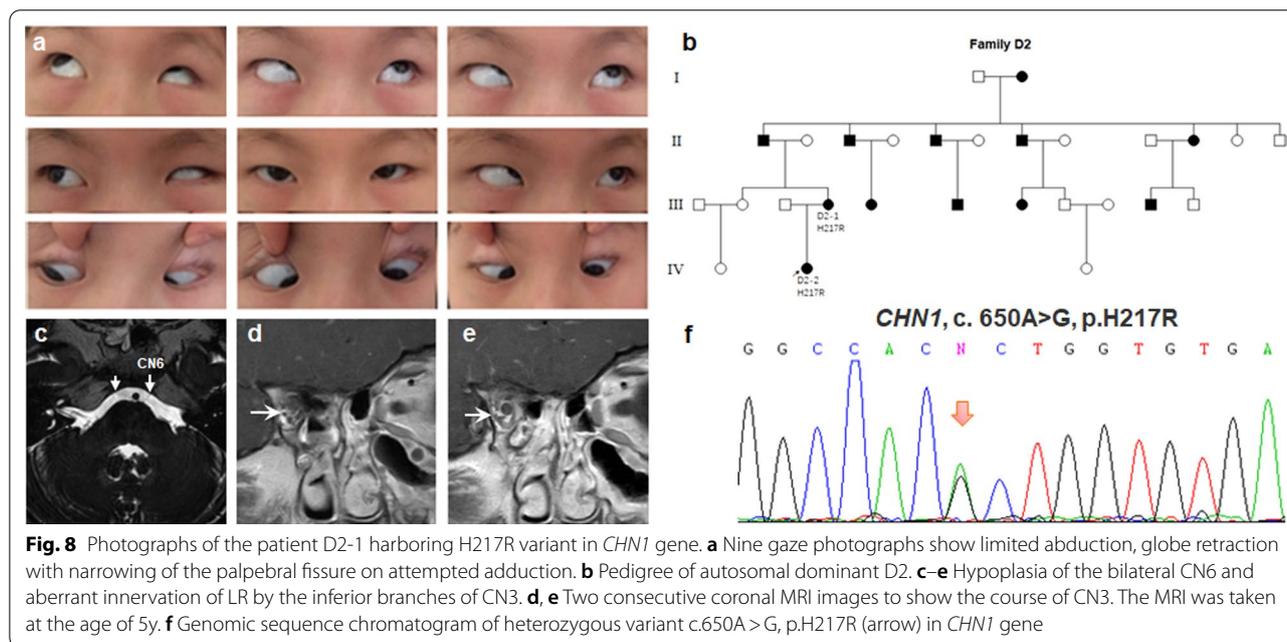
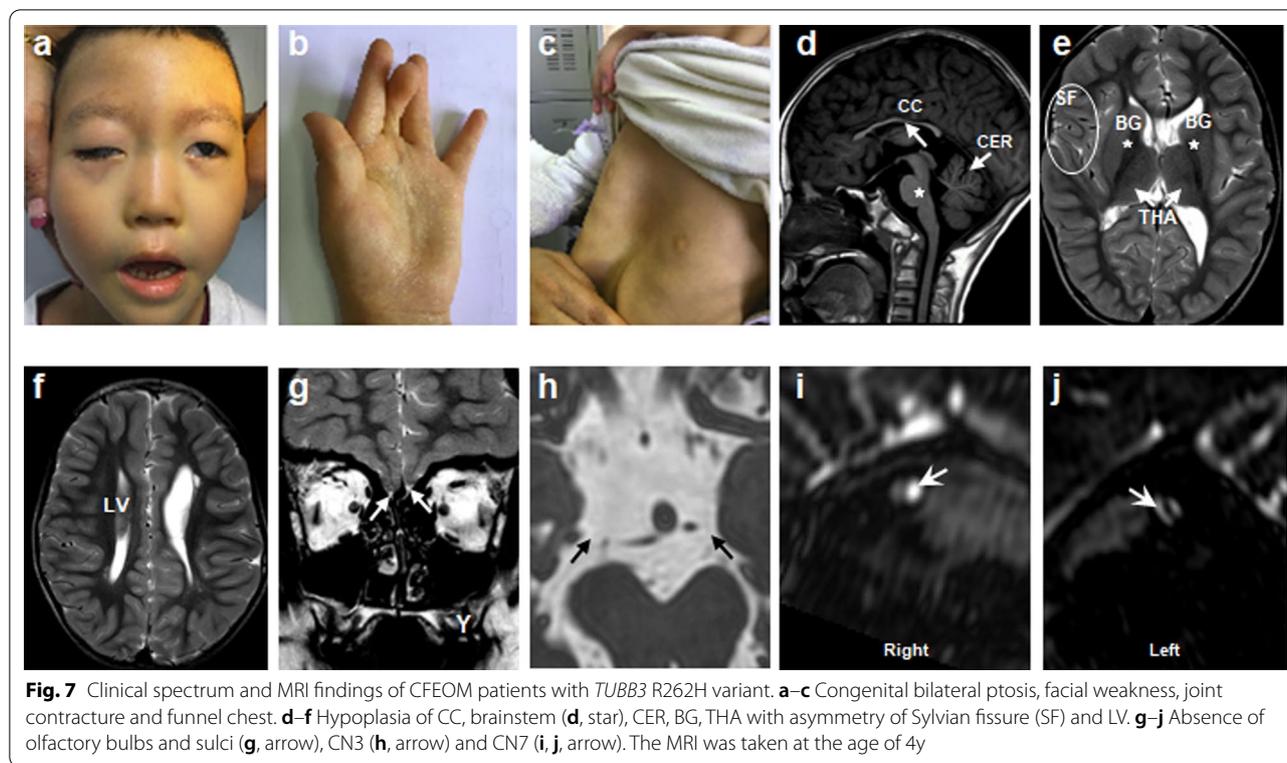
Furthermore, we also noted that *TUBB3* R380C variant had been reported in patients diagnosed with “neurodevelopmental disabilities” and “cerebellar dysplasia (CD)” [34, 35], however the ocular manifestations of the patients were not described clearly by authors.

We noted that Chew et al. and Whitman et al. reported CFEOM patients harboring E410K and R262H variants were accompanied by Kallmann syndrome (anosmia with hypogonadal hypogonadism) [4, 31]. Furthermore, Yasuko Nakamura et al. also reported E410K patients presented with Kallmann syndrome [33].

In present study, three patients with *TUBB3* E410K and R262H variants (C21, C32 and C52) fitted the diagnostic criteria of Kallmann syndrome, confirmed the previous report results and suggested that *TUBB3* gene may involve in the pathogenesis of Kallmann syndrome [4].

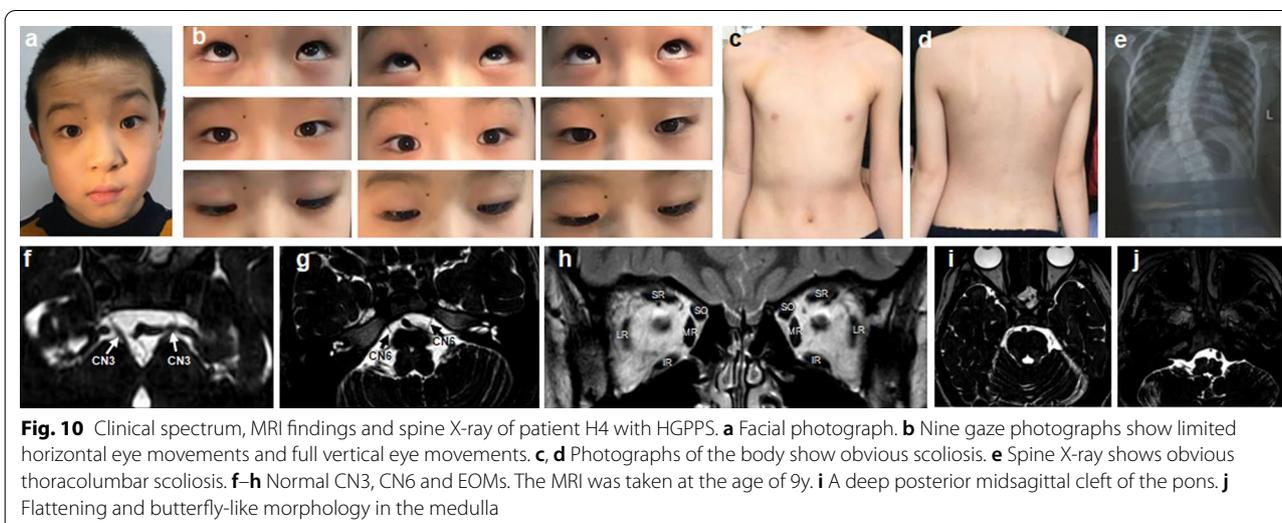
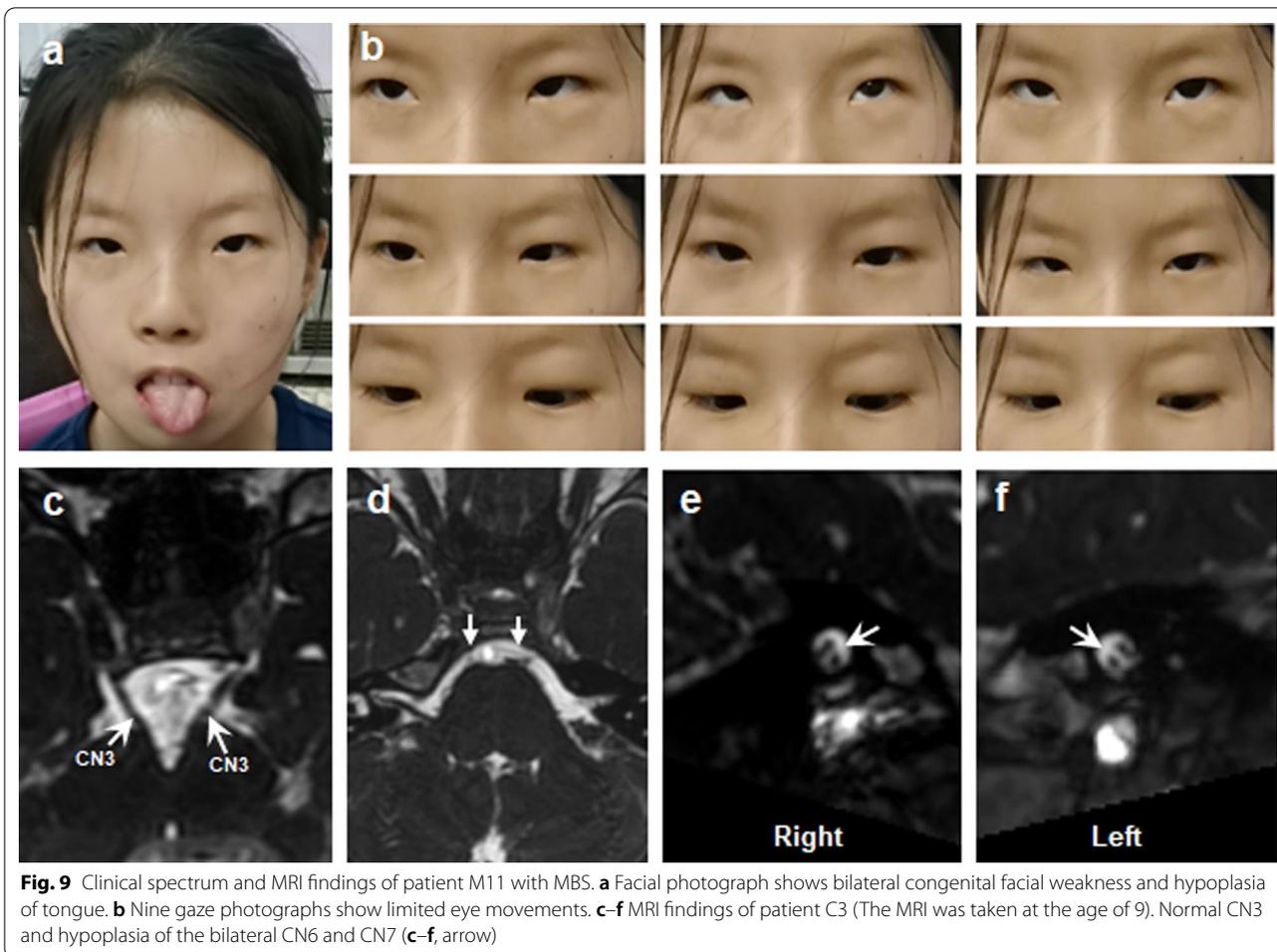
Genetic screening results of sporadic DRS, MBS and HGPPS cases were poor

DRS is a congenital motility disorder which characterized by restricted abduction or adduction and accompanied by globe retraction and narrowing of the palpebral fissure [36]. *CHN1* gene was a common causative gene for familial non-syndromic DRS, which was identified in approximately 35% of familial DRS cases [11]. Functional analysis revealed that *CHN1* variants caused CN6 growth stalled, leading to secondary dysinnervation of the LR by the CN3 [37]. To date, variant detection rate was poor in sporadic isolated DRS patients, so genetic screening is suggestive for those individuals with positive family history



[38]. In our DRS cohort (2 familial and 6 sporadic), we identified one novel variant H217R in *CHN1* gene in a large DRS family, no causative genes were identified in all sporadic DRS cases and one DRS family. These results were similar to the previous study [38].

MBS is a rare disease with minimum diagnostic criteria of congenital limitation of ocular abduction and facial weakness [3]. In 2015, Laura Tomas-Roca et al. reported that *PLXND1* and *REV3L* may be pathogenic genes for MBS, and animal researches indicated *PLXND1* and



REV3L may cause a defect in the facial branchiomotor neuron migration [17]. This was the first report of pathogenic gene for MBS, however, these two genes have not been reported again in other MBS cases to date. In addition to genetic factors, intrauterine environmental factors were also reported to be involved in MBS [39]. In our study, no causative gene was identified in all MBS patients, and more than half of the patients had intrauterine exposure to adverse factors. These results suggested that multifactorial pathogenic mechanism may exist in MBS.

HGPPS is a rare autosomal recessive disorder characterized by severe restriction of conjugate horizontal eye movements and progressive scoliosis [16]. Reviewed the published reports about HGPPS, it could be found that *ROBO3* was the pathogenic gene and the HGPPS patients harboring *ROBO3* variants often existed parental consanguinity [40, 41]. *ROBO3* plays an important role in axonal guidance which mediate midline crossing of neurons during embryogenesis of the spinal cord [16]. Our study enrolled five sporadic HGPPS patients who were from non-consanguineous family. No homozygous variants of *ROBO3* gene were found, and the possibility of compound heterozygous mutation could not be ruled out. More than a dozen heterozygous variants of *ROBO3* gene were identified in five HGPPS patients, however, no variants were classified as pathogenic or likely pathogenic according to ACMG standards, so the pathogenicity of these variants cannot be determined.

The mutation detection rates in sporadic isolated DRS, MBS and non-consanguineous families with HGPPS were poor in this study. The reasons could be multiple, such as epigenetic factors, environmental or other non-genetic factors may be involved in the pathogenesis of diseases.

The phenotypes of CCDDs are diverse and complex, often involving multiple tissues and organs, many new MRI and clinical phenotypes have been reported and discovered. Face to face communication with radiologist is necessary. At the same time, the participation of clinical multidisciplinary doctors is required to carry out professional examinations, diagnosis and treatment. Considering these facts, future efforts need to focus on mining, refining and quantifying extraocular manifestations to improve our understanding of clinical and genetic basis of CCDDs. Meanwhile, the novel variants need future functional studies to determine its pathogenicity.

Conclusions

This study was the largest clinical and genetic research for CCDDs patients from China, the results broadened the phenotypic and genotypic spectrums of CCDDs. *KIF21A* and *TUBB3* were the common pathogenic genes of

Chinese CFEOM. Compared with patients with *KIF21A* variants, patients with *TUBB3* variants have better genotype–phenotype associations. The patients harboring R380C, E410K and R262H variants in *TUBB3* gene and F355S amino acid variant in *KIF21A* gene exhibited syndromic phenotypes. Genetic screening results of sporadic DRS, MBS and HGPPS cases were poor. These findings were highly consistent with previous results. The combination of MRI and WES was effective for accurate and supportive diagnosis of clinical suspected CCDDs, and the research results would be valuable for future gene therapy and facilitate further understanding of pathogenic mechanism of CCDDs.

Methods

Patients

This was a prospective cohort study with retrospective data analysis. A total of 122 self-reported Han patients from 96 not known to be related families with CCDDs were enrolled in the outpatient department of Beijing Tongren Hospital affiliated to Capital Medical University from January 2013 to December 2021.

The inclusion criterion was (1) congenital, non-progressive restrictive eye movements and/or dysplasia of the cranial nerves in MRI, or (2) congenital, non-progressive limitation of horizontal eye movements and progressive scoliosis, with or without dysplasia of the cranial nerves [1].

The study complied with the tenets of the Declaration of Helsinki and was approved by the Institution Review Board of Beijing Tongren Hospital, Beijing, China (TRECKY2018-035). All participants we enrolled gave written informed consent.

Clinical evaluation and MRI

All participants underwent a detailed ophthalmic and physical examinations. The ophthalmic examinations included cycloplegic refraction, best-corrected visual acuity (BCVA), binocular alignment, eye movements, measurement of the height of palpebral fissures and the function of levator palpebrae muscles, slit lamp examination and fundus examination.

Multi-positional high-resolution MRI was performed in 96 patients from 88 families with a General Discovery MR750 3.0-T Twinspeed scanner. Children under 10 years old were given oral chloral hydrate (10–15 mg/kg) 30 min before examination. Imaging of cranial nerves at the brainstem was conducted with an 8HR brain coil and adopted the three-dimensional fast imaging employing steady–state acquisition (3D-FIESTA) sequence. Parameters were as follows: axial scanning; repetition time (TR), 4.28 ms; echo time (TE), 2.04 ms; flip angle

(FA), 60°; the field of view (FOV), 16 × 16 cm; Matrix, 256 × 320; section thickness, 0.8 mm; number of excitation (NEX), 4. Orbital imaging was performed with an 8HR brain coil in oblique coronal planes and applied the following parameters: TR, 325 ms; TE, 9.38 ms; FOV, 17 × 17 cm; matrix, 264 × 256; section thickness, 3.0 mm; layer spacing, 0.3 mm; NEX, 2. Scanning of the brain structure was conducted with a phased array coil in head-cross section and the parameters were as follows: Fast spin-echo/T2-weighted images (FSE/T2WI); section thickness, 6.0 mm; layer spacing, 1.0 mm.

Whole exome sequencing and bioinformatics analysis

Whole blood samples were collected from all participants and their nuclear family members, genomic DNA was extracted using TianGen Biochemical DNA isolation kit (Beijing Tiangen Biochemical Co., Ltd, Beijing, China).

Whole exome sequencing was performed at Beijing Novogene Bioinformatics Technology Co., Ltd (Beijing, China). Agilent SureSelect Human All ExonV6 kit was used to capture whole exome sequence. Sequencing was performed on illumina HiSeq4000 platform using PE150 sequencing strategy.

Valid sequencing data was mapped to the reference genome (GRCh37/hg19) by BurrowsWheeler Aligner (BWA) software. Samtools and Sambamba were used to sort bam files. Samtools and bcftools were used to do variants calling and identified SNPs and indels. ANNOVAR was used to do annotation for VCF (Variant Call Format) file with a variety of databases, such as dbSNP, 1000 Genome, GnomAD, CADD and HGMD. Gene transcript annotation databases, such as Consensus CDS, RefSeq, Ensemble and UCSC, were also applied for annotation to determine amino acid alternation. Variants were filtered if MAF (minor allele frequency) > 1% in more than one of the four frequency databases (1000 genomic data, esp6500, gnomAD_ALL and gnomAD_EAS). Nonsynonymous variants were screened according to scores of SIFT, Polyphen, VariantTaster and CADD. CNVs analyses were also performed. ACMG guidelines were used to evaluate variants. After the candidate pathogenic variants (*KIF21A*, *TUBB3*, *TUBA1A*, *TUBB2B*, *TUBB6*, *PHOX2A*, *CHN1*, *MAFB*, *SALL4*, *HOXA1*, *HOXB1*, *ROBO3*, *PLXND1*, *REV3L*, *COL25A1* and *ECEL1*) were found, Sanger sequencing was used for validation. Trios/pedigree analysis were performed to establish co-segregation or de novo status.

Abbreviations

BCVA: Best-corrected visual acuity; CCDDs: Congenital cranial dysinnervation disorders; CD: Cerebellar dysplasia; CFEOM: Congenital Fibrosis of Extraocular Muscles; CN3: Oculomotor nerves; CN6: Abducens nerves; CN7: Facial nerves;

CNVs: Copy number variations; DRS: Duane Retraction Syndrome; EOMs: Extraocular muscles; FA: Flip angle; FOV: The field of view; FSE/T2WI: Fast spin-echo/T2-weighted images; HCFP: Hereditary Congenital Facial Paresis; HGPPS: Horizontal Gaze Palsy with Progressive Scoliosis; LR: Lateral rectus; MBS: Möbius Syndrome; MRI: Magnetic resonance imaging; NEX: Number of excitation; OBS: Olfactory bulbs and sulci; TE: Echo time; TR: Repetition time; WES: Whole exome sequencing; 3D-FIESTA: Three-dimensional fast imaging employing steady-state acquisition.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13023-022-02582-5>.

Additional file 1. Clinical and genetic data of 122 CCDDs patients. Description of data: The table presents detailed data of 122 patients with CCDDs, including the results of ophthalmic and physical examinations, neuroimaging findings and genetic test results.

Additional file 2. Allele frequency of three novel variants. Description of data: Three unreported variants were not found in Chinese ethnically-matched databases, gnomAD, etc.

Acknowledgements

The authors are grateful to the families for their participation.

Author contributions

YJ and HJ conceived and designed the experiments. HJ and QM performed experiments. HJ, QM, YL, DW, JL, JS, YW and RZ provided the clinical data. BZ and ZZ performed the MRI. HJ, YJ and QC analyzed the clinical and genetic data. HJ, QM and YJ wrote the paper. ZT provided critical review of the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (82070999), and the Beijing Municipal Natural Science Foundation (7212017). The funders had no role in the design of the study nor in the collection, analysis and interpretation of data or in writing the manuscript.

Availability of data and materials

All data in this study are available upon reasonable request.

Declarations

Ethical approval and consent to participate

All participants we enrolled gave written informed consent. The study complied with the tenets of the Declaration of Helsinki and was approved by the Institution Review Board of Beijing Tongren Hospital, Beijing, China.

Consent for publication

Written consent was obtained for all involved patients.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China. ²Beijing Ophthalmology and Visual Science Key Lab, Beijing 100730, China. ³Department of Radiology, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China. ⁴Department of Neuroscience, Psychology and Behaviour, Ulverscroft Eye Unit, University of Leicester, Robert Kilpatrick Clinical Sciences Building, Leicester Royal Infirmary, Leicester LE2 7LX, UK.

Received: 18 June 2022 Accepted: 20 November 2022

Published online: 09 December 2022

References

- Gutowski NJ, Bosley TM, Engle EC. 110th ENMC International Workshop: the congenital cranial dysinnervation disorders (CCDDs). Naarden, The Netherlands, 25–27 October, 2002. *Neuromuscul Disord* 2003;13(7–8):573–8.
- Chilton JK, Guthrie S. Axons get ahead: Insights into axon guidance and congenital cranial dysinnervation disorders. *Dev Neurobiol*. 2017;77(7):861–75.
- MacKinnon S, Oystreck DT, Andrews C, Chan WM, Hunter DG, Engle EC. Diagnostic distinctions and genetic analysis of patients diagnosed with moebius syndrome. *Ophthalmology*. 2014;121(7):1461–8.
- Whitman MC, Barry BJ, Robson CD, Facio FM, Van Ryzin C, Chan WM, et al. TUBB3 R262H causes a recognizable syndrome including CFEOM3, facial palsy, joint contractures, and early-onset peripheral neuropathy. *Hum Genet*. 2021;140(12):1709–31.
- Yamada K, Andrews C, Chan WM, McKeown CA, Magli A, et al. Heterozygous mutations of the kinesin KIF21A in congenital fibrosis of the extraocular muscles type 1 (CFEOM1). *Nat Genet*. 2003;35(4):318–21. <https://doi.org/10.1038/ng1261>.
- Tischfield MA, Baris HN, Wu C, Rudolph G, Van Maldergem L, He W, et al. Human TUBB3 mutations perturb microtubule dynamics, kinesin interactions, and axon guidance. *Cell*. 2010;140(1):74–87. <https://doi.org/10.1016/j.cell.2009.12.011>.
- Jurgens JA, Barry BJ, Lemire G, Chan WM, Whitman MC, Shaaban S, et al. Novel variants in TUBA1A cause congenital fibrosis of the extraocular muscles with or without malformations of cortical brain development. *Eur J Hum Genet*. 2021;29(5):816–26.
- Cederquist GY, Luchniak A, Tischfield MA, Peeva M, Song Y, Menezes MP, et al. An inherited TUBB2B mutation alters a kinesin-binding site and causes polymicrogyria, CFEOM and axon dysinnervation. *Hum Mol Genet*. 2012;21(26):5484–99. <https://doi.org/10.1093/hmg/ddc393>.
- Fazeli W, Herkenrath P, Stiller B, Neugebauer A, Fricke J, Lang-Roth R, et al. A TUBB6 mutation is associated with autosomal dominant non-progressive congenital facial palsy, bilateral ptosis and velopharyngeal dysfunction. *Hum Mol Genet*. 2017;26(20):4055–66.
- Nakano M, Yamada K, Fain J, Sener EC, Selleck CJ, Awad AH, et al. Homozygous mutations in ARIX(PHOX2A) result in congenital fibrosis of the extraocular muscles type 2. *Nat Genet*. 2001;29(3):315–20. <https://doi.org/10.1038/ng744>.
- Miyake N, Chilton J, Psatha M, Cheng L, Andrews C, Chan WM, et al. Human CHN1 mutations hyperactivate alpha2-chimaerin and cause Duane's retraction syndrome. *Science*. 2008;321(5890):839–43. <https://doi.org/10.1126/science.1156121>.
- Park JG, Tischfield MA, Nugent AA, Cheng L, Di Gioia SA, Chan WM, et al. Loss of MAFB function in humans and mice causes Duane syndrome, aberrant extraocular muscle innervation, and inner-ear defects. *Am J Hum Genet*. 2016;98(6):1220–7. <https://doi.org/10.1016/j.ajhg.2016.03.023>.
- Al-Baradie R, Yamada K, St Hilaire C, Chan WM, Andrews C, McIntosh N, et al. Duane radial ray syndrome (Okiihiro syndrome) maps to 20q13 and results from mutations in SALL4, a new member of the SAL family. *Am J Hum Genet*. 2002;71(5):1195–9. <https://doi.org/10.1086/343821>.
- Tischfield MA, Bosley TM, Salih MA, Alorainy IA, Sener EC, Nester MJ, et al. Homozygous HOXA1 mutations disrupt human brainstem, inner ear, cardiovascular and cognitive development. *Nat Genet*. 2005;37(10):1035–7. <https://doi.org/10.1038/ng1636>.
- Webb BD, Shaaban S, Gaspar H, Cunha LF, Schubert CR, Hao K, et al. HOXB1 founder mutation in humans recapitulates the phenotype of Hoxb1^{-/-} mice. *Am J Hum Genet*. 2012;91(1):171–9. <https://doi.org/10.1016/j.ajhg.2012.05.018>.
- Jen JC, Chan WM, Bosley TM, Wan J, Carr JR, Rüb U, et al. Mutations in a human ROBO gene disrupt hindbrain axon pathway crossing and morphogenesis. *Science*. 2004;304(5676):1509–13. <https://doi.org/10.1126/science.1096437>.
- Tomas-Roca L, Tsalibsi-Shtylik A, Jansen JG, Singh MK, Epstein JA, Altunoglu U, et al. De novo mutations in PLXND1 and REV3L cause Möbius syndrome. *Nat Commun*. 2015;6:7199. <https://doi.org/10.1038/ncomms8199>.
- Shinwari JM, Khan A, Awad S, Shinwari Z, Alaiya A, Alanazi M, et al. Recessive mutations in COL25A1 are a cause of congenital cranial dysinnervation disorder. *Am J Hum Genet*. 2015;96(1):147–52.
- Khan AO, Shaheen R, Alkuraya FS. The ECEL1-related strabismus phenotype is consistent with congenital cranial dysinnervation disorder. *J AAPOS*. 2014;18(4):362–7.
- Demer JL, Ortube MC, Engle EC, Thacker N. High-resolution magnetic resonance imaging demonstrates abnormalities of motor nerves and extraocular muscles in patients with neuropathic strabismus. *J AAPOS*. 2006;10(2):135–42.
- Jiao YH, Zhao KX, Wang ZC, Qian XH, Wu X, Man FY, et al. Magnetic resonance imaging of the extraocular muscles and corresponding cranial nerves in patients with special forms of strabismus. *Chin Med J (Engl)*. 2009;122(24):2998–3002.
- Whitman MC, Jurgens JA, Hunter DG, Engle EC. Congenital fibrosis of the extraocular muscles overview. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Mirzaa GM, Amemiya A, editors. *GeneReviews*[®]. Seattle: University of Washington; 2004.
- Cheng L, Desai J, Miranda CJ, Duncan JS, Qiu W, Nugent AA, et al. Human CFEOM1 mutations attenuate KIF21A autoinhibition and cause oculomotor axon stalling. *Neuron*. 2014;82(2):334–49. <https://doi.org/10.1016/j.neuron.2014.02.038>.
- Lu S, Zhao C, Zhao K, Li N, Larsson C. Novel and recurrent KIF21A mutations in congenital fibrosis of the extraocular muscles type 1 and 3. *Arch Ophthalmol*. 2008;126(3):388–94. <https://doi.org/10.1001/archophth.126.3.388>.
- Ali Z, Xing C, Anwar D, Itani K, Weakley D, Gong X, Pascual JM, Mootha VV. A novel de novo KIF21A mutation in a patient with congenital fibrosis of the extraocular muscles and Möbius syndrome. *Mol Vis*. 2014;20:368–75.
- Bianchi S, van Riel WE, Kraatz SH, Olieric N, Frey D, Katrukha EA, Jaussi R, Missimer J, Grigoriev I, Olieric V, Benoit RM, Steinmetz MO, Akhmanova A, Kammerer RA. Structural basis for misregulation of kinesin KIF21A autoinhibition by CFEOM1 disease mutations. *Sci Rep*. 2016;6:30668. <https://doi.org/10.1038/srep30668>.
- Minoura I, Takazaki H, Ayukawa R, Saruta C, Hachikubo Y, Uchimura S, et al. Reversal of axonal growth defects in an extraocular fibrosis model by engineering the kinesin-microtubule interface. *Nat Commun*. 2016;7:10058. <https://doi.org/10.1038/ncomms10058>.
- Whitman MC, Andrews C, Chan WM, Tischfield MA, Stasheff SF, Brancati F, et al. Two unique TUBB3 mutations cause both CFEOM3 and malformations of cortical development. *Am J Med Genet A*. 2016;170A(2):297–305. <https://doi.org/10.1002/ajmg.a.37362>.
- Poirier K, Saillour Y, Bahi-Buisson N, Jaglin XH, Fallet-Bianco C, Nabbout R, et al. Mutations in the neuronal β -tubulin subunit TUBB3 result in malformation of cortical development and neuronal migration defects. *Hum Mol Genet*. 2010;19(22):4462–73. <https://doi.org/10.1093/hmg/ddq377>.
- Balasubramanian R, Chew S, MacKinnon SE, Kang PB, Andrews C, Chan WM, et al. Expanding the phenotypic spectrum and variability of endocrine abnormalities associated with TUBB3 E410K syndrome. *J Clin Endocrinol Metab*. 2015;100(3):E473–7. <https://doi.org/10.1210/jc.2014-4107>.
- Chew S, Balasubramanian R, Chan WM, Kang PB, Andrews C, Webb BD, et al. A novel syndrome caused by the E410K amino acid substitution in the neuronal β -tubulin isotype 3. *Brain*. 2013;136(Pt 2):522–35. <https://doi.org/10.1093/brain/aws345>.
- Jin S, Park SE, Won D, Lee ST, Han SH, Han J. TUBB3 M323V syndrome presents with infantile nystagmus. *Genes (Basel)*. 2021;12(4):575. <https://doi.org/10.3390/genes12040575>.
- Nakamura Y, Matsumoto H, Zaha K, Uematsu K, Nonoyama S. TUBB3 E410K syndrome with osteoporosis and cough syncope in a patient previously diagnosed with atypical Möbius syndrome. *Brain Dev* 2018;40(3):233–237.
- Srivastava S, Cohen JS, Vernon H, Barañano K, McClellan R, Jamal L, et al. Clinical whole exome sequencing in child neurology practice. *Ann Neurol*. 2014;76(4):473–83. <https://doi.org/10.1002/ana.24251>.
- Romaniello R, Arrigoni F, Panzeri E, Poretti A, Micalizzi A, Citterio A, et al. Tubulin-related cerebellar dysplasia: definition of a distinct pattern of cerebellar malformation. *Eur Radiol*. 2017;27(12):5080–92. <https://doi.org/10.1007/s00330-017-4945-2>.
- Barry BJ, Whitman MC, Hunter DG, Engle EC. Duane Syndrome. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Mirzaa GM, Amemiya A, editors. *GeneReviews*[®]. Seattle: University of Washington; 2007.
- Nugent AA, Park JG, Wei Y, Tenney AP, Gilette NM, DeLisle MM, et al. Mutant α 2-chimaerin signals via bidirectional ephrin pathways in Duane

retraction syndrome. *J Clin Invest.* 2017;127(5):1664–82. <https://doi.org/10.1172/JCI88502>.

38. Miyake N, Andrews C, Fan W, He W, Chan WM, Engle EC. CHN1 mutations are not a common cause of sporadic Duane's retraction syndrome. *Am J Med Genet A.* 2010;152A(1):215–7. <https://doi.org/10.1002/ajmg.a.33168>.
39. Verzijl HT, van der Zwaag B, Cruysberg JR, Padberg GW. Möbius syndrome redefined: a syndrome of rhombencephalic maldevelopment. *Neurology.* 2003;61(3):327–33.
40. Butcher J. Mutations in ROBO3 cause HGPPS. *Lancet Neurol.* 2004;3(6):328. [https://doi.org/10.1016/s1474-4422\(04\)00786-0](https://doi.org/10.1016/s1474-4422(04)00786-0).
41. Xiu Y, Lv Z, Wang D, Chen X, Huang S, Pan M. Introducing and reviewing a novel mutation of ROBO3 in horizontal gaze palsy with progressive scoliosis from a Chinese family. *J Mol Neurosci.* 2021;71(2):293–301. <https://doi.org/10.1007/s12031-020-01650-4>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

