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Genetic and clinical characteristics of 24 mainland Chinese patients with *CTNNB1* loss-of-function variants

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Abstract

Background: Neurodevelopmental disorder with spastic diplegia and visual defects (NEDSDV) is a rare autosomal dominant syndrome, which is caused by the heterozygous germline loss-of-function variants in *CTNNB1*.

Methods: We evaluated the clinical and genetic findings of 24 previously undescribed Chinese patients affected by *CTNNB1*-related disorders and explored the possible ethnicity-related phenotypic variations.

Results: Twenty-one loss-of-function variants were identified within these 24 NEDSDV patients, including 14 novel *CTNNB1* variants and 7 recurrent ones. The prominent clinical manifestations in our cohort are developmental delay/intellectual disability (100%), motor delay (100%), speech impairment (100%), dystonia (87.5%) and microcephaly (69.6%). The common facial dysmorphisms were consistent with previous reports, including wide nasal bridge (58.3%), bulbous nose (45.8%), long philtrum (45.8%) and thin upper lip (45.8%). In addition, 19 patients (79.2%) in our cohort had mild visual defects, while one affected individual (4.2%) had familial exudative vitreoretinopathy. Notably, we discovered that 20 patients (83.3%) exhibited various behavioral abnormalities, which is described in Chinese patients for the first time.

Conclusion: We provided the largest known Chinese cohort with pathogenic *CTNNB1* variants, which not only helps to expand the variant spectrum of *CTNNB1* gene, but further delineates the typical phenotype of this disorder in Chinese population.

Yongguo Yu and Yongkun Zhan equal contribution as co-corresponding author.

1 | INTRODUCTION

CTNNB1 (MIM *116806) was first described to be related with intellectual disability by de Ligt et al. (2012). Since then, many individuals with CTNNB1 loss-of-function variants have been reported to suffer from a rare multisystem neurodevelopmental disorder, which was defined as neurodevelopmental disorder with spastic diplegia and visual defects (NEDSDV, MIM #615075). NEDSDV is an autosomal dominant disorder and its characteristic features include developmental delay/intellectual delay (DD/ID), speech impairment, microcephaly, motor delay, autistic spectrum disorder (ASD), hypotonia, progressive peripheral spasticity, dysmorphic craniofacial, and various degrees of visual abnormalities (Ho et al., 2021; Kharbanda et al., 2017; Kuechler et al., 2014; Rossetti et al., 2021; Tucci et al., 2014).

The CTNNB1 gene is located on chromosome 3p22.1 and encodes the ubiquitously expressed and evolutionarily conserved protein beta-catenin, a 781-amino acid protein with an N-terminal domain (NTD), 12 central armadillo (ARM) repeat domains, and the C-terminal domain (CTD) (Xu & Kimelman, 2007). As a multitasking protein, beta-catenin is not only a core component of the cadherin complex but also a key factor in the canonical Wnt signaling, which plays an important role in stem cell renewal, as well as cell proliferation and differentiation during embryogenesis (Steinhart & Angers, 2018; Valenta et al., 2012). Many studies have identified that aberrant activation of beta-catenin may promote various tumorigenesis, such as colorectal cancer and hepatocellular carcinoma (Bian et al., 2020; Zhang & Wang, 2020), while ablation of beta-catenin would affect the nervous system development (Zechner et al., 2003).

To date, less than 100 different loss-of-function variants in CTNNB1 gene have been reported in the literatures, mostly from non-Chinese populations. In this study, we reported the clinical characteristics and genetic findings of 24 mainland Chinese patients with pathogenic CTNNB1 variants. So far, this is the largest case series for NEDSDV patients of Chinese ethnicity. We also attempted to review the clinical features of our CTNNB1 variant patients together with 13 previously reported Chinese patients and 37 non-Chinese patients with CTNNB1 variants (Ho et al., 2021; Ke & Chen, 2020; Li et al., 2017), and

explore the possible ethnicity related phenotypic and genetic diversity in CTNNB1-related NEDSDV.

2 | MATERIALS AND METHODS

2.1 | Editorial policies and ethical considerations

Written informed consent for publishing clinical information with photographs was obtained from the parents of patients. The study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Xinhua Hospital, School of Medicine, Shanghai Jiao Tong University (XHEC-D-2022-099).

2.2 | Cohort

We gathered 24 patients with CTNNB1-related neurodevelopmental disorder from 23 families in Mainland China: 14 males (58%) and 10 females (42%) were included in our cohort, with ages ranging from 0.6 to 11 years old. We abstracted the information about the developmental delay, autism diagnosis, and MRI or CT scans from the medical record. Besides, detailed information of patients was collected through the questionnaire completed by the parents, including the growth parameters, head circumference, development/neurological features, behavioral abnormalities, and other clinical features. Additionally, the pictures of the frontal and bilateral views of the face of the patients were provided by their parents. Two clinicians from Xinhua Hospital, School of Medicine, Shanghai Jiao Tong University estimated the pictures of the patients to analyze their craniofacial features.

2.3 | Genetic analysis

In our cohort, different sequencing methods and platforms were applied: exome sequencing (ES) were performed in most patients (23/24), while multigene panel testing were performed in 1 patient. The disease-causing CTNNB1 variants identified from the genetic sequencing reports provided by the parents were rechecked and unified based

on the human genome reference assembly GRCh37/hg19 and *CTNNB1* transcript NM_001904.4.

2.4 | Statistical analysis

We designed Chinese (our 24 patients and 13 Chinese patients previously reported) and non-Chinese groups (37 non-Chinese patients previously reported) with the same patients' number ($n = 37$) artificially to explore the similarities and differences between ethnicities. Relevant literatures were searched through PubMed database using the keywords "*CTNNB1*" and "Intellectual disability" or "Neurodevelopmental disorder". We preferred to include the reports with series cases rather than single case reports, for more consistent evaluation criteria and description method. As reports about Chinese patients are still few, we included two single-case reports in our comparison. Meanwhile, these cases should cover most of the phenotypes we concerned. Reports only focusing on ocular disease were excluded in our study. The statistical significance in phenotype and genotype differences of these two groups have been assessed by Fisher's exact test.

3 | RESULTS

3.1 | Cohort characteristics

A total of 24 molecularly diagnosed Chinese patients from 23 unrelated families were recruited into this study. All patients had no positive family history of *CTNNB1*-related diseases except for patients 23 and 24, who are siblings. There were 10 females (42%) and 14 males (58%) with an average age of 3.07 years (range: 0.6 to 11y). The age of initial assessment was from 3 months to 2 years and the mean age of diagnosis was 1.94 years. None of these patients have been published in the literature. Their clinical findings were summarized in Tables 1 and 2 (details in Supplementary Table S1) and presented in Figure 1. For this study, eight previous publications reporting 13 Chinese patients and 37 patients from other countries were included for assessing the similarities and differences between Chinese NEDSDV patients and those of other ethnicities (Table 2) (Ho et al., 2021; Ke & Chen, 2020; Kharbanda et al., 2017; Kuechler et al., 2014; Li et al., 2017; Rossetti et al., 2021; Tucci et al., 2014; Wang, Zhao, et al., 2019).

3.2 | Dysmorphic features

Twenty-two patients in our cohort presented with similar but not prominent facial features to what had

TABLE 1 Main clinical features of patients with *CTNNB1* pathogenic variants in our cohort

| Feature	N	Total	%
Dysmorphisms			
Primary microcephaly (<3rd percentile)	3	20	15.0
Microcephaly (<3rd percentile)	16	23	69.6
Narrow forehead	1	24	4.2
Fair skin	2	24	8.3
Sparse hair	3	24	12.5
Sparse eyebrows	9	24	37.5
Long eyelashes	10	24	41.7
Wide nasal bridge	14	24	58.3
Bulbous nose	11	24	45.8
Long philtrum	11	24	45.8
Thin upper lip	11	24	45.8
Ear anomalies	11	24	45.8
Short stature/delayed growth (<3rd percentile)	3	24	12.5
Visual defects			
Astigmatism	3	24	12.5
Shortsighted	1	24	4.2
Hyperopia	11	24	45.8
Strabismus	15	24	62.5
Amblyopia	1	24	4.2
Retinopathy/Vitreous opacities/FEVR	2	24	8.3
Behavioral features			
Autism spectrum disorder	3	24	12.5
Impulsiveness	7	24	29.2
Anxiety	8	24	33.3
Hyperactivity	5	24	20.8
Self-injury	2	24	8.3
Repetitive behaviors	8	24	33.3
Sleep disturbance	17	24	70.8
Other clinical features			
Gastroesophageal reflux	1	24	4.2
Diarrhea/constipation	6	24	25.0
Recurring upper respiratory tract infections	1	24	4.2
Congenital heart defect	1	24	4.2
Allergies	1	24	4.2

Abbreviation: FEVR, familial exudative vitreoretinopathy.

been previously reported in the literature (Table 1 and Supplementary Table S2). Characteristic facial phenotype included long eyelashes (10/24), wide nasal bridge (14/24), bulbous nasal tip (11/24), long philtrum (11/24), and thin upper lip (11/24). One affected individual was found to

TABLE 2 Incidences and percentages of phenotypes in Chinese *CTNNB1* variant patients and comparison with the literatures

Clinical features (Not every characteristic is recorded for all patients)	24 Novel Chinese patients	13 Previously published Chinese patients (Ho et al., Ke et al., Li et al., Wang et al.)	Total 37 Chinese patients	37 Previously published non-Chinese patients (Kharbanda et al., Kuechler et al., Rossetti et al., Tucci et al)	*p*-value (by Fisher exact test)
Gender (M:F)	14:10	6:7	20:17	17:20	.6423
Developmental/neurological					
Microcephaly	16/23 (69.6%)	8/11 (72.7%)	24/34 (70.6%)	32/37 (86.5%)	.1464
Dystonia	21/24 (87.5%)	11/12 (91.7%)	32/36 (88.9%)	36/37 (97.3%)	.1992
Motor delay	24/24 (100%)	12/12 (100%)	36/36 (100%)	37/37 (100%)	1.0000
Speech impairment (age ≥3)	5/5 (100%)	3/4 (75.0%)	8/9 (88.9%)	35/36 (97.2%)	.3636
DD/ID	18/18 (100%)	13/13 (100%)	31/31 (100%)	35/36 (97.2%)	1.0000
Brain imaging anomalies	7/13 (53.8%)	0/10 (0%)	7/23 (30.4%)	5/32 (15.6%)	.2078
Craniofacial dysmorphism	22/24 (91.7%)	12/12 (100%)	34/36 (94.4%)	35/36 (97.2%)	1.0000
Behavioral anomalies	20/24 (83.3%)	NA	20/24 (83.3%)	25/37 (67.6%)	.2373
Visual defects	20/24 (83.3%)	9/13 (69.2%)	29/37 (78.4%)	25/36 (69.4%)	.4328
Variant type					
Nonsense	10/24 (41.7%)	5/13 (38.5%)	15/37 (40.5%)	18/37 (48.6%)	.6403
Frameshift	11/24 (45.8%)	5/13 (38.5%)	16/37 (43.2%)	13/37 (35.1%)	.6343
Splice site	3/24 (12.5%)	3/13 (38.5%)	6/37 (16.2%)	2/37 (5.4%)	.2611
Missense	0/24 (0%)	0/13 (0%)	0/37 (0%)	2/37 (5.4%)	.4932
Inframe mutation	0/24 (0%)	0/13 (0%)	0/37 (0%)	1/37 (2.7%)	1.0000
Whole gene	0/24 (0%)	0/13 (0%)	0/37 (0%)	1/37 (2.7%)	1.0000

Abbreviations: DD, developmental delay; F, female; ID, intellectual disability; M, male; NA, not applicable.
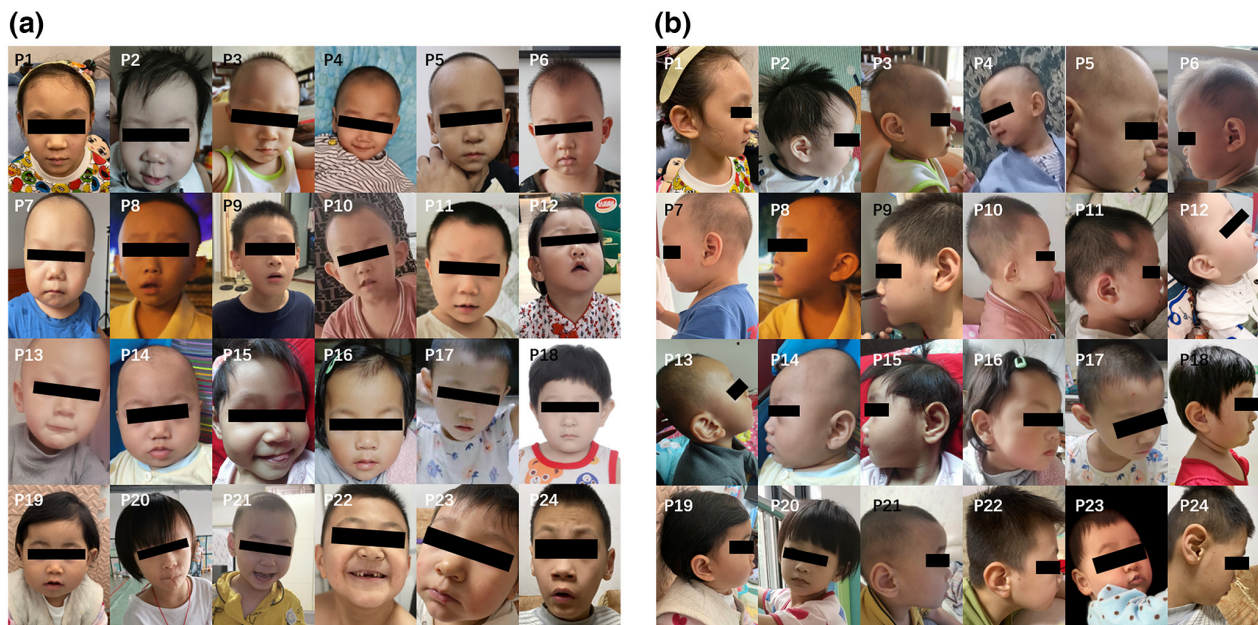
FIGURE 1 Frontal (a) and lateral (b) facial photos of 24 Chinese individuals with *CTNNB1* loss-of-function variants

have narrowed forehead and nine with sparse eyebrows. Intriguingly, we also noticed 11 of these patients showed large or protruding ears, which had been first reported in Ho et al's case (Ho et al., 2021), hence we proposed this may be a notable feature of Chinese. Besides, one patient had aural deformities and received auricular repairment. The detailed dysmorphic features of individuals were summarized in Supplementary Table S2, and clinical photos of affected individuals were shown in Figure 1.

3.3 | Development and growth

The growth details for our cohort were summarized in Supplementary Tables S1 and S3. All 24 patients in our cohort were described with motor delay involving fine and gross motor areas, while 5 patients showed speech impairment, including four could only speak single word or phases over the age of 3 years, and one only speaks short sentences at 6 years. Sixteen patients were diagnosed with microcephaly (occipitofrontal head circumference [OFC] below 3rd percentile for sex, age, and ethnicity), including two had primary microcephaly (at birth). Meanwhile, most patients had age-appropriate weight and height except for seven of them presenting short stature (height below 3rd percentile) or low weight (body weight below 3rd percentile).

3.4 | Neurological features

DD/ID was described in the medical records of 18 patients. Among them, 12 cases were professionally assessed and

diagnosed with mild-to-severe DD/ID. However, due to the differences in evaluation institutions and reporting forms, only seven of them had a full-scale score, while the remaining five did not. As a common syndrome of *CTNNB1*-related disorder, dystonia was also presented in 21 patients of our cohort. Meanwhile, abnormal gait was noted in all 21 patients who were able to walk with or without help. Thirteen patients had received neurological examination, and seven of them showed abnormal brain magnetic resonance imaging (MRI) or computed tomography (CT) findings, including widened extracerebral spaces ($n = 2$), white matter abnormalities ($n = 2$), enlargement of ventricles ($n = 2$), and delayed myelination ($n = 1$). Besides, 2/6 patients had abnormal electroencephalography (EGG), with one of them developing seizures. Their neurological features were summarized in Table 2 and Supplementary Table S1.

3.5 | Behavioral features

As reported, the majority of our patients exhibited different behavioral abnormalities similar to those observed in Western patients (Table 1 and Supplementary Table S1). Among them, 3 patients were noticed to suffer from ASD, and two of them were finally diagnosed by formal autism assessment. Furthermore, 11 patients were more anxious or hyperactive in daily life, 8 patients exhibited repetitive behaviors, 7 patients were reported as impulsiveness, and 2 patients showed overly sensitivity to touch or voice. Even more, two patients had once self-injury behaviors. Sleep disturbances were also found in 18 out of

24 patients, mainly including difficulties to fall asleep and night terror.

3.6 | Ocular findings

Consistent with previous reports, our patients also presented a series of visual defects (Table 1 and Supplementary Table S2), including astigmatism, shortsighted, hyperopia, strabismus, amblyopia, and even familial exudative vitreoretinopathy (FEVR). Among these, strabismus is the most common one, with 15 patients presenting this symptom. One patient was noticed unable to chase objects with her eyes after birth, and then was diagnosed with binocular cataract and bilateral FEVR. At the age of 9 months, she was treated with vitrectomy combined with a lens and retinal resection.

3.7 | Bone and joint deformity

Nine patients showed flatfoot, three cases exhibited strephexopodia, while four cases showed limited finger/wrist movement, and four individuals had spinal deformity. In addition, one patient had polydactyly, and had been treated by surgery.

3.8 | Other clinical features

Six patients were found to have diarrhea or constipation, and the gastroesophageal reflux was also noticed in one patient. One patient had atrial septal defect and recurring upper respiratory tract infections.

3.9 | Genotype

We identified 21 different variants in 24 patients, all are loss-of-function variants, including 11 frameshift (45.8%), 10 nonsense (41.7%), and 3 splice site variants (12.5%). Fourteen out of these variants were novel, which were not described in the literature, ClinVar, Genome aggregation database (gnomAD) and Human Gene Mutation Database (HGMD), including c.495 + 2dup, c.1857_1858del, c.1154dup, c.735-1G > T, c.299_300del, c.987dup, c.1843dup, c.2010del, c.931del, c.1760_1761del, c.1853dup, c.950del, c.1689_1690dup, and c.2098dup. All variants were de novo, except for one variant that had not been verified in father and another variant that had not ruled out low-grade parental mosaicism. All of the variants were classified as pathogenic or likely pathogenic variants based on the American College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al., 2015). These variants were predicted to result in various amino acid changes, while amino acids 90 and 587 were the most common sites of variation, each accounting for 12.5% (3/24). The genotypic information in our cohort and the pathogenic variants previously reported in the literature are showed in Figure 2 and summarized in Table 3.

4 | DISCUSSION

Few Chinese NEDSDV patients had been reported up till now. Herein, we described the clinical and molecular genetic characterization of 24 mainland Chinese NEDSDV individuals to expand the genotype and phenotype spectrum of NEDSDV. We also compared the major clinical presentations of our 24 patients and 13 previous reported Chinese NEDSDV individuals with 37 patients of other ethnic backgrounds, attempting to elucidate the ethnicity-related features of this disorder (Ho et al., 2021; Ke & Chen, 2020; Kharbanda et al., 2017; Kuechler et al., 2014; Li et al., 2017; Rossetti et al., 2021; Tucci et al., 2014; Wang, Zhao, et al., 2019).

This study discovered 21 different *CTNNB1* variants, including 14 novel pathogenic variants, which broadened *CTNNB1* variant spectrum. Most of the variants reported in our cohort are localized in ARM repeat domains and no particular mutational hotspots identified. So far, all of the reported NEDSDV-related *CTNNB1* variants are heterozygous variants, except that recently Taylor et al reported a patient with bi-allelic variant which was inherited from her unaffected parents (Taylor et al., 2022). The majority of reported pathogenic variants in *CTNNB1* are loss-of-function variants such as frameshift, nonsense, or splicing variants, except for a few cases with missense variants (Kuechler et al., 2014; Rossetti et al., 2021). Similar to previous reports, all of our patients had heterozygous loss-of-function variants. As for the type of variants, there is no significant difference between the Chinese group and the non-Chinese group, with the nonsense (40.5% vs 48.6%, $p = .6403$) and frameshift (43.2% vs 35.1%, $p = .6343$) variants being the most frequent. In addition, most of the reported variants arise de novo, although some familial cases have been described (Ho et al., 2021; Kuechler et al., 2014; Wang, Zhao, et al., 2019). Consistent with these results, the great majority of our variants are de novo. However, we noticed that both siblings in our cohort carried a heterozygous *CTNNB1* nonsense variant c.268C > T, p.(Arg90*). Neither parent was confirmed to carry this variant by means of Sanger sequencing of whole blood DNA, indicating the possibility of parental germline mosaicism.
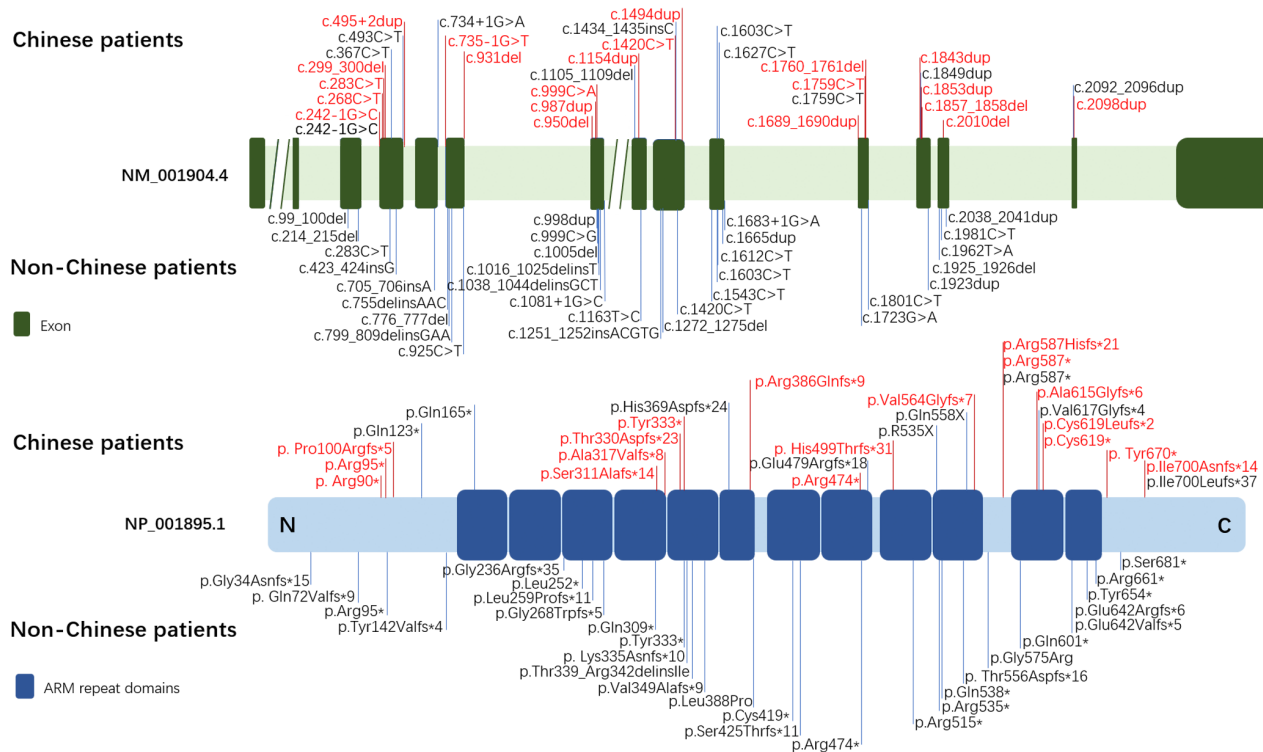
FIGURE 2 Summary of *CTNNB1* pathogenic variants in 37 Chinese patients and 37 non-Chinese patients. The variants in our study are marked in red

DD/ID is the cardinal clinical feature of *CTNNB1*-related disorders. In vivo experiments with beta-catenin knockout mice also demonstrated that haploinsufficiency of this gene leads to dysregulation of synaptic plasticity, neuronal network connectivity, and synaptic adhesion, providing the underlying pathogenic mechanism for neurodevelopmental disorder (Tucci et al., 2014; Wickham et al., 2019). Consistent with previous reports, 18 patients in our cohort were professionally assessed and all diagnosed as DD/ID. We further compared the frequencies of DD/ID in Chinese patients (31/31) and non-Chinese patients (35/36), and there was no statistically significant difference (100% vs 97.2%, $p = 1.0000$). We also revealed that there was no statistically significant difference between two groups in other neurological features including microcephaly (70.6% vs 86.5%, $p = .1464$), motor delay (100% vs 100%, $p = 1.0000$), dystonia (88.9% vs 97.3%, $p = .1992$), speech impairment (88.9% vs 97.2%, $p = .3636$), and brain imaging anomalies (30.4% vs 15.6%, $p = .2078$).

There was no significant difference concerning the craniofacial dysmorphism between Chinese cohort (34/36) and non-Chinese cohort (35/36). The main common facial features in our cohort are bulbous nasal tip, wide nasal bridge, long philtrum, and thin upper lip. Noteworthy, one of the most common dysmorphism features in our patients is relatively large ears, which has only been reported

in Chinese up to now. Nearly half of the individuals in our study presented it, and in Ho's cohort, its incidence was higher (8/9). It indicates the large ears may be an ethnic-specific phenotype, but we still cannot draw a definitive conclusion as this diagnosis is very subjective to clinicians.

Meanwhile, the visual defect is also a common symptom with an incidence of 78.4% in Chinese and 69.4% in non-Chinese. And among the specific manifestations of visual defect, the strabismus is the most frequent one. A previous study had suggested that the Wnt/beta-catenin signaling pathway plays a pivotal role in the proper formation of both the anterior segment of the eye and neurovascular retina (Wang, Liu, et al., 2019), which may help to explain why many reported cases with *CTNNB1* variants have mild-to-severe visual symptoms. Most notably, patient 15, who had a truncating variant (p.Ser311Alafs*14) in exon 6, was diagnosed with binocular cataract, FEVR, and retinal detachment, showing the most serious ocular phenotype. Since the relationship between *CTNNB1* haploinsufficiency and FEVR was first reported in 2016 (Dixon et al., 2016), a few cases had been reported and most of the patients were of Asian ethnicity (Coussa et al., 2020; Ke & Chen, 2020; Li et al., 2017; Panagiotou et al., 2017; Sun et al., 2019; Wang, Zhao, et al., 2019). However, our results showed that there was no statistically significant difference between Chinese patients (7/37) and non-Chinese patients (2/36) in the frequency of FEVR. Since reports

TABLE 3 Overview of all *CTNNB1* pathogenic variants identified in our cohort

Patient	Genomic position	cDNA position	Protein position	Exon	Variant type	Inheritance	ACMG classification	Reported variant
P1	chr3:41266700	c.495+2dup	splice variant	Intron 4	Splicing	De novo	Likely pathogenic	Novel
P2	chr3:41277893–41,277,894	c.1857_1858del	p.(Cys619*)	12	Nonsense	De novo	Pathogenic	Novel
P3	chr3:41274904	c.1154dup	p.(Arg386Glnfs*9)	8	Frameshift	De novo	Pathogenic	Novel
P4	chr3:41277290	c.1759C>T	p.(Arg587*)	11	Nonsense	De novo	Pathogenic	Reported
P5	chr3:41277290	c.1759C>T	p.(Arg587*)	11	Nonsense	De novo	Pathogenic	Reported
P6	chr3:41275328	c.1494dup	p.(His499Thrfs*31)	9	Frameshift	De novo	Pathogenic	Reported
P7	chr3:41267150	c.735-1G>T	Splice variant	Intron 5	Splicing	De novo	Pathogenic	Novel
P8	chr3:41266471	c.268C>T	p.(Arg90*)	4	Nonsense	De novo	Pathogenic	Reported
P9	chr3:41266502–41,266,503	c.299_300del	p.(Pro100Argfs*5)	4	Frameshift	De novo	Pathogenic	Novel
P10	chr3:41268761	c.999C>A	p.(Tyr333*)	7	Nonsense	De novo	Pathogenic	Reported
P11	chr3:41268749	c.987dup	p.(Thr330Aspfs*23)	7	Frameshift	De novo	Pathogenic	Novel
P12	chr3:41266486	c.283C>T	p.(Arg95*)	4	Nonsense	De novo	Pathogenic	Reported
P13	chr3:41277879	c.1843dup	p.(Ala615Glyfs*6)	12	Frameshift	De novo	Pathogenic	Novel
P14	chr3:41278134	c.2010del	p.(Tyr670*)	13	Nonsense	De novo	Pathogenic	Novel
P15	chr3:41267347	c.931del	p.(Ser311Alafs*14)	6	Frameshift	De novo	Pathogenic	Novel
P16	chr3:41277291–41,277,292	c.1760_1761del	p.(Arg587Hisfs*21)	11	Frameshift	De novo	Pathogenic	Novel
P17	chr3:41277889	c.1853dup	p.(Cys619Leufs*2)	12	Frameshift	De novo	Pathogenic	Novel
P18	chr3:41268712	c.950del	p.(Ala317Valfs*8)	7	Frameshift	De novo	Pathogenic	Novel
P19	chr3:41277220–41,277,221	c.1689_1690dup	p.(Val564Glyfs*7)	11	Frameshift	De novo	Pathogenic	Novel
P20	chr3:41266444	c.242-1G>C	Splice variant	Intron 3	Splicing	NK	Likely pathogenic	Reported
P21	chr3:41279528	c.2098dup	p.(Ile700Asnfs*14)	14	Frameshift	De novo	Pathogenic	Novel
P22	chr3:41275254	c.1420C>T	p.(Arg474*)	9	Nonsense	De novo	Pathogenic	Reported
P23	chr3:41266471	c.268C>T	p.(Arg90*)	4	Nonsense	NK	Pathogenic	Reported
P24	chr3:41266471	c.268C>T	p.(Arg90*)	4	Nonsense	NK	Pathogenic	Reported

Note: Genomic, cDNA, and protein position are given as well as affected exons. All genomic positions are based on CRCh37/hg19; *CTNNB1* reference sequence is NM_001904.4; NK, not known; *, the stop codon; ACMG, American College of Medical Genetics and Genomic.

focusing on eye diseases were not included in our study, further research with larger sample size is needed to confirm whether ethnicity plays a role in phenotypic variations of NEDSDV. In addition, patient 15 also had an atrial septal defect. Congenital heart disease was also observed in previously published patients, but it was not specifically mentioned (Ke & Chen, 2020; Kuechler et al., 2014; Rossetti et al., 2021). Considering that canonical Wnt signaling and beta-catenin play a critically important role in proper cardiogenesis (Piven & Winata, 2017), the occurrence of this phenotype may be related to the disruption of beta-catenin transcriptional activity.

In this study, we described the behavioral features in Chinese patients for the first time. Twenty out of our 24 patients (83.3%) showed behavioral abnormalities, with anxious (33.3%), hyperactivity (20.8%), repetitive (33.3%) or impulsive behaviors (29.2%), and sleep disturbances (70.8%) were the most frequent findings. There was no significant difference in behavioral abnormalities between Chinese cohort (20/24) and non-Chinese cohort (25/37). As reported in Western patients, our study also recorded three patients with ASD phenotype. Previous study had identified that *CTNNB1* directly interacts with multiple top ASD risk genes (O'Roak et al., 2012). Likewise, autism-associated behavioral defects had been noticed in the *Ctnnb1* conditional knockout mice (Dong et al., 2016). Notably, we found that one of these three patients presented polydactyly, a phenotype that was first reported by Ke et al. in a Chinese patient (Ke & Chen, 2020). To our knowledge, this is the second Chinese case. Since the same symptom was not detected in patients from outside China, we considered polydactyly may be a unique phenotype in Chinese patients. However, further studies are needed to confirm our observation.

This study had several limitations. First of all, clinical information obtained from medical records and questionnaires may be limited due to varying medical criteria between hospitals and recall bias. Secondly, we focused only on studies with multiple non-Chinese patients, single-case studies, and reports focusing on eye disease were not included in this study. Given the limitations of our study, further studies with larger sample size are needed to draw more definitive conclusions.

5 | CONCLUSION

In summary, we delineated the genotypes and phenotypes of 24 Chinese NEDSDV patients, which broaden the *CTNNB1* variant spectrum and clinical phenotype spectrum of NEDSDV. We further explored the possible ethnicity-related phenotypic variations in *CTNNB1*-related NEDSDV. However, no ethnic-specific feature had been noticed except relatively large ears.

CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

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