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Congenital heart defects in *CTNNB1* syndrome: Raising clinical awareness

Lorenzo Sinibaldi[1] | Giacomo Garone[2,3] | Alessandra Mandarino[4] | Giancarlo Iarossi[5] | Laura Chioma[6] | Maria Lisa Dentici[1] | Giuseppe Merla[7,8] | Emanuele Agolini[9] | Alessia Micalizzi[9] | Cecilia Mancini[10] | Marcello Niceta[10] | Marina Macchiaiolo[11] | Daria Diodato[12] | Roberta Onesimo[13,14] | Rita Blandino[14] | Angelica Bibiana Delogu[14] | Gabriella De Rosa[14] | Valentina Trevisan[13] | Mariella Iademarco[13] | Giuseppe Zampino[13,14,15] | Marco Tartaglia[10] | Antonio Novelli[9] | Andrea Bartuli[11] | Maria Cristina Digilio[1] | Giulio Calcagni[16]

[1]Medical Genetics Unit, IRCCS Bambino Gesù Children Hospital, Rome, Italy

[2]Clinical and Experimental Neurology, IRCCS Bambino Gesù Children Hospital, Rome, Italy

[3]Department of Neuroscience, Mental Health and Sensory Organs (NESMOS), Faculty of Medicine and Psychology, Sapienza University of Rome, Rome, Italy

[4]Child and Adolescent Neuropsychiatry Unit, IRCCS Bambino Gesù Children's Hospital, Rome, Italy

[5]Department of Ophthalmology, IRCCS Bambino Gesù Children Hospital, Rome, Italy

[6]Endocrinology and Diabetology Unit, IRCCS Bambino Gesù Children's Hospital, Rome, Italy

[7]Laboratory of Regulatory & Functional Genomics, Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Foggia, Italy

[8]Department of Molecular Medicine & Medical Biotechnology, University of Naples Federico II, Naples, Italy

[9]Laboratory of Medical Genetics, Translational Cytogenomics Research Unity, IRCCS Bambino Gesù Children Hospital, Rome, Italy

[10]Molecular Genetics and Functional Genomics, IRCCS Bambino Gesù Children Hospital, Rome, Italy

[11]Rare Diseases and Medical Genetics Unit, IRCCS Bambino Gesù Children Hospital, Rome, Italy

[12]Neuromuscular and Neurodegenerative Disorders Unit, IRCCS Bambino Gesù Children Hospital, Rome, Italy

[13]Rare Diseases Unit, IRCCS Fondazione Policlinico Universitario Agostino Gemelli, Rome, Italy

[14]Pediatric Unit, IRCCS Fondazione Policlinico Universitario Agostino Gemelli, Rome, Italy

[15]Dipartimento Universitario Scienze della Vita e Sanità Pubblica, Università Cattolica Sacro Cuore, Rome, Italy

[16]Department of Cardiac Surgery, Cardiology and Heart and Lung Transplant, IRCCS Bambino Gesù Children's Hospital, Rome, Italy

Correspondence
Lorenzo Sinibaldi, Medical Genetics Unit, IRCCS Bambino Gesù Children Hospital, Rome, Italy.
Email: lorenzo.sinibaldi@opbg.net

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Abstract

CTNNB1 [OMIM *116806] encodes β-catenin, an integral part of the cadherin/catenin complex, which functions as effector of Wnt signaling. *CTNNB1* is highly expressed in brain as well as in other tissues, including heart. Heterozygous *CTNNB1* pathogenic variations are associated with a neurodevelopmental disorder characterized by spastic

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diplegia and visual defects (NEDSDV) [OMIM #615075], featuring psychomotor delay, intellectual disability, behavioral disturbances, movement disorders, visual defects and subtle facial and somatic features. We report on a new series of 19 NEDSDV patients (mean age 10.3 years), nine of whom bearing novel *CTNNB1* variants. Notably, five patients showed congenital heart anomalies including absent pulmonary valve with intact ventricular septum, atrioventricular canal with hypoplastic aortic arch, tetralogy of Fallot, and mitral valve prolapse. We focused on the cardiac phenotype characterizing such cases and reviewed the congenital heart defects in previously reported NEDSDV patients. While congenital heart defects had occasionally been reported so far, the present findings configure a higher rate of cardiac anomalies, suggesting dedicated heart examination to NEDSDV clinical management.

KEYWORDS

clinical management, congenital heart defects, *CTNNB1*, neurodevelopmental disorder, Wnt-β-catenin signaling

1 | INTRODUCTION

Neurodevelopmental Disorder with Spastic Diplegia and Visual Defects (NEDSDV) [OMIM #615075] is caused by de novo heterozygous loss-of-function (LoF) pathogenic variants in *CTNNB1* [*116806], mapping to chromosome 3p22.1.[1] This rare autosomal dominant disorder, often referred to as *CTNNB1*-syndrome, is characterized by developmental delay (DD), intellectual disability (ID), speech delay, ranging from limited to absent speech, learning and memory disturbances. Affected individuals can also present with axial hypotonia, spasticity, dystonia, microcephaly, exudative vitreoretinopathy, feeding difficulties, musculoskeletal defects, susceptibility to pilomatrixomas, and minor somatic features.[1] NEDSDV was originally recognized in 2012 when *CTNNB1* pathogenic variants were identified in three patients showing DD, ID, limited/absent speech, microcephaly, and spasticity.[2] Since then *CTNNB1*-syndrome appeared to occur more frequently than previously estimated. To date, NEDSDV has been reported in more than 400 patients worldwide,[3] including cases clinically diagnosed as cerebral palsies in which a pathogenic *CTNNB1* variant was eventually recognized as the cause of the disorder.[4,5] Notably, gain-of-function (GoF) somatic variants have been linked to a variety of phenotypes, including colorectal cancer [OMIM #114500], hepatocellular carcinoma [OMIM #114550], medulloblastoma [OMIM #155255], and pilomatrixoma [OMIM #13260], while LoF constitutional variants have been also associated with dominant exudative vitreoretinopathy-7 (EVR7) [OMIM #61757]. Such observations testify to a polyhedric action of *CTNNB1* both in development and cell physiology. *CTNNB1* comprises 16 exons, with exons 2–15 (2346 bp) encoding the central, mainly active part of the protein consisting of 781 amino acids related to the armadillo structural proteins.[6] *CTNNB1* encodes β-catenin, an integral part of the cadherin/catenin complex connected to the activation of the Wnt signaling pathway. B-catenin acts both as a transcriptional effector of Wnt signaling, and as structural protein mediating intracellular cell connections and adhesion.[6]

Wnt/β-catenin signaling plays a key role in human development.[7] *CTNNB1* is highly expressed in brain[8]; consistently, *WNT1* and *CTNNB1* contribute in coordinating brain development, maturation, and function.[9,10] Indeed, β-catenin involvement in different phases of central nervous system development and differentiation has been explored.[9–11] Recently, *CTNNB1* role in other organs physiology and pathology has started being clarified. The gene is highly expressed in heart,[12] and an association between *CTNNB1* LoF variants and congenital heart defects (CHDs) has been recently reported.[3,13–17] However, CHDs manifestations in *CTNNB1*-NEDSDV have not been specifically and thoroughly studied to date. Hitherto the prevalence of CHDs in *CTNNB1*-NEDSDV is not precisely known, since only anecdotal patients affected by cardiac anomalies have been reported.

In the present study, we analyzed the CHDs frequency and anatomic characteristics in 19 patients with *CTNNB1*-NEDSDV and report on five cases presenting with different types of CHDs, including absent pulmonary valve (APV) with intact ventricular septum (IVS), atrioventricular canal defect (AVCD), tetralogy of Fallot (ToF), and mitral valve prolapse (MPV). We also reviewed previous NEDSDV cases showing CHDs aiming to provide an overview of *CTNNB1* related cardiac anomalies to date.

2 | MATERIALS AND METHODS

2.1 | Patient data

Between January 2019 and February 2023, 19 new patients with *CTNNB1*-NEDSDV (8 females, 11 males) were recruited at the Bambino Gesù Children Hospital and Fondazione Policlinico Agostino

Gemelli University Hospital IRCCS. The clinical characteristics of the present cohort were retrospectively assessed. Mean age at the last evaluation was 10.3 ± 6 years (range 3.6–23 years). All patients were evaluated by clinical geneticists, pediatric cardiologists, pediatricians, neurologists, neuropsychiatrists, endocrinologists and ophthalmologists. In particular, the cardiac evaluation included chest x-ray, electrocardiogram, and two-dimensional and color Doppler echocardiography. Cardiac catheterization and/or cardiac surgery were executed when necessary. No data specifically dealing with the assessment of cardiac dysfunctions were reported in the clinical records, particularly in those cases without morphological defects.

Patients harbored heterozygous pathogenic/likely pathogenic *CTNNB1* variants. Nine novel previously unreported variants were detected.

The molecular and clinical characterization of the individual patients featuring in the present cohort were tabulated (Tables 1 and 2).

Informed consent for the genomic analyses and clinical data collection was obtained from the patients' parents.

TABLE 1 Cardiac and molecular details of patients with *CTNNB1* pathogenic variants and CHDs from the present series and previous literature reports.

Reference/present cases	Sex	Congenital heart defect	*CTNNB1* pathogenic variant Transcript variant Reference sequence NM_001904.4 Protein variant Reference sequence NP_001895.1	Variant type	Segregation
Kayumi et al.[3]	M	Ventricular septal defect	c.268C>T; p.Arg90*	Stop	De novo
Present series—Case 5	M	Absent pulmonary valve with intact ventricular septum Patent ductus arteriosus	c.976_979delAATA; p.Asn326Ter	Stop	De novo
Monies et al.[15]	F	Atrial septal defect Tricuspid valve regurgitation	c.999C>G; p.Tyr333*	Stop	Unknown
Present series—Case 7	F	Mitral valve prolapse with insufficiency	c.999delC; p.Tyr333*	Stop	De novo
Rossetti et al.[16]	M	Pulmonary valve stenosis	c.1005delA; p.Lys335Asnfs*10	Frameshift	De novo
Present series—Case 9	M	Atrioventricular canal defect, partial Hypoplastic aortic arch	c.1081+1_1082−1_(2346+?)del p.?	Stop	De novo
Kayumi et al.[3]	M	Atrial septal defect	c.1139A>T; p.Asn380Ile	Missense	De novo
Kayumi et al.[3]	Unknown	Tetralogy of Fallot	c.1543C>T; p.Arg515*	Stop	Unknown
Ke et al.[14]	F	Atrial septal defect, right heart enlargement, pulmonary hypertension, tricuspid regurgitation	c.1603C>T; p.Arg535*	Stop	De novo
Kayumi et al.[3]	M	Patent ductus arteriosus	c.1683G>C; p.Val561=	Splice region variant	Unknown
Homsy et al.[13]	Unknown	Partial anomalous pulmonary venous return Atrial septal defect (sinus venosus)	c.1690delG; p.Val564Serfs*6	Frameshift	De novo
Morton et al.[17]	Unknown	Atrial septal defect	c.1690delG; p.Val564Serfs*6	Frameshift	Unknown
Morton et al.[17]	Unknown	Dilated tricuspid valve	c.1711dup; p.Glu571Glyfs*38	Frameshift	Unknown
Kayumi et al.[3]	M	Bicuspid aortic valve Patent foramen ovalis	c.1723G>A; p.Gly575Arg	Missense	De novo
Kayumi et al.[3]	F	Pulmonary valve stenosis	c.1804-2A>G; intron 11 of 15 position 504 of 505 (splicing, intronic)	Splice acceptor	De novo
Present series—Case 16	F	Mitral valve insufficiency Patent foramen ovalis	c.1852del; p.Leu618SerfsTer23	Stop	De novo
Present series—Case 19	M	Tetralogy of Fallot	c.2089G>T; p.Gly697Ter	Stop	Unknown
Morton et al.[17]	Unknown	Tetralogy of Fallot	c.2172T>G; p.Tyr724Ter	Stop	Unknown

Abbreviations: F, female; M, male.

TABLE 2 *CTNNB1*-NEDSDV clinical features of the present cohor.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
Transcript variant—Reference sequence NM_001904.4	c.675_676insGA	c.680dup	c.808delA	c.975delA	c.976_979delAATA	c.998dupA	c.999delC	c.999C>A	c.(1081+1_1082−1)_(2346+?)del	c.1420C>T
Mutation site	Exon 5	Exon 5	Exon 6	Exon 7	Exon 7	Exon 7	Exon 7	Exon 7	8–15 exons deletion	Exon 9
Protein variant—Reference sequence NP_001895.1	p.Gly227Argfs*16	p.Leu229Thrfs*5	p.Met271Trpfs*5	p.Asn326Ilefs*2	p.Asn326*	p.Tyr333*	p.Tyr333*	p.Tyr333*	p.?	p.Arg474*
Variant type	Frameshift	Frameshift	Frameshift	Nonsense	Nonsense	Nonsense	Nonsense	Nonsense	Nonsense	Nonsense
Previously reported/novel	Novel	Novel	Novel	Novel	Novel	Reference 47	Reference 20	Reference 47	Novel	Reference 1
ACMG classification	Class 4	Class 4	Class 4	Class 4	Class 4	Class 5	Class 5	Class 5	Class 5	Class 5
Inheritance pattern	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo
Sex	M	F	F	M	M	M	F	F	M	F
Auxological features										
Age at observation (years + months)	15.8	5	11	10	7.6	4.8	19.8	4.9	14.8	9.2
Height SDS (cm/SDS)	158 cm (−1.98 SD)	104 cm (0.7 SD)	143.5 cm (−0.07 SD)	138.7 cm (0.6 SD)	123.8 (−0.28 SD)	100.3 cm (−1.59 SD)	164 cm (0.12 SD)	105 cm (−0.43 SD)	171.5 cm (0.11 SD)	138.8 cm (0.75 SD)
Weight SDS (kg/SD)	55 kg (−0.95 SD)	16.3 kg (0.2 SD)	53.4 kg (1.44 SD)	35.7 kg (0.1 SD)	29 kg (0.76 SD)	17.2 kg (−0.4 SD)	53.9 kg (−0.51 SD)	20 kg (6.4 SD)	45.7 kg (−1.51 SD)	45.6 kg (1.88 SD)
BMI SDS	0.76	15.1 (−0.2 SD)	1.77	18.6 (0.8 SD)	1.18	0.94	−0.61	−1.75	−2.27	1.96
Occipito-frontal circumference (cm/SDS)	51.5 cm (−2.4 SD)	46 cm (−3.1 SD)	51 cm (−1.31 SD)	49.5 cm (−2.6 SD)	50.15 cm (−1.49 SD)	49 cm (−1.34 SD)	51.5 cm (−2.64 SD)	46.4 (−2.74 SD)	49 cm (−4.03 SD)	49 cm (−2.42 SD)
Congenital heart disease	–	–	–	–	Absent pulmonary valve with intact ventricular septum Patent ductus arteriosus	–	Mitral valve prolapse with insufficiency	–	Partial atrioventricular canal, aortic coarctation, patent ductus arteriosus and mitral valve insufficiency	–
Neurodevelopmental issues										
Developmental delay (mild, moderate, severe)	Severe	Moderate	Severe	Mild	Moderate	Mild	Moderate	Moderate	Severe	Severe
Intellectual disability (mild, moderate, severe)	Severe	Moderate	Severe	Mild	Moderate	Moderate	Moderate	Moderate	Moderate	Severe
Speech delay (+/−)	+	+	+	+	+	+	+	+	+	+
Motor delay (+/−)	+	+	+	+	+	+	+	+	+	+
Behavioral issues										
Autistic features (+/−)	–	+	+	+	–	–	–	–	–	+
ADHD (+/−)	–	–	+	–	+	+	–	+	–	+
Aggression/self-mutilation (+/−)	–	+	+	–	–	–	+	–	–	–
Sleep disturbances (+/−)	–	–	+	–	–	–	–	+	–	–

(Continues)

TABLE 2 (Continued)

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
Other	−	−	Picacism	−	−	−	−	−	−	−
Movement disorders										
Truncal hypotonia (+/−)	+	+	−	−	−	−	−	−	−	−
Lower limbs spasticity (+/−)	−	+	−	−	+	+	+	−	+	+
Dystonia (+/−)	−	−	−	−	−	−	+	−	+	−
Ataxic gait	+	+	+	−	−	−	−	−	+	−
Other	−	−	−	−	−	−	−	−	−	−
Visual defects										
Strabismus (+/−)	+	+	+	−	−	−	+	+	+	+
Nystagmus (+/−)	+	−	−	−	−	−	−	+	−	−
Astigmatism (+/−)	+	+	+	−	+	+	+	−	+	−
Myopia (+/−)	+	−	−	−	−	−	−	−	−	−
Bilateral FEVR (+/−)	−	−	−	−	−	−	−	−	−	−
Hypermetropia (+/−)	−	+	+	−	+	+	−	−	−	−
Cataract (+/−)	−	−	−	−	−	−	−	+	−	−
Other	−	−	−	−	−	−	−	−	−	−
Facial features (brief description)	Microcephaly, elongated face, almond shaped palpebral fissures, long and prominent nose, dental malocclusion, prominent upper incisors, thin upper lip, large auricles	Microcephaly, anteverted nostrils, long labial philtrum, thin lips	Small OFC, long eyelashes, elongated-almond shaped palpebral fissures, elongated nose, thin and arched upper lip, large and extroverted auricles	Microcephaly, not other features reported	Small OFC, long face with high forehead, horizontal eyebrows, enophthalmos, hypotelorism, narrow palpebral fissures, mild eyelid ptosis, long and prominent nose, retrognathia	Small OFC, almand-shepd eys, bulbous nose tip, thin upper lip	Microcephaly, broad forehead, long face, turned down mouth corners, squared chin, retro-rotated pinnae with simple helix, mild nasal alar hypoplasia, arched palate, hypopigmented skin	Microcephaly, flat occiput, elongated facies, sparse eyebrows, long eyelashes, narrow palpebral fissures, long nose, slight retrognathia, slightly cupped ears and anteverted lobe, crumpled helix, dental diastasis	Microcephaly, elongated facies, narrow palpebral fissures, long nose, open lips, spaced out teeth, retrognathia, large ears	Microcephaly, almond-shaped eyes, prominent nose, thin upper lip, retrognathia
Other (e.g.)										
IUGR	−	+	+	−	−	+	−	−	−	+
Short stature	+	−	+	−	+	+	−	−	−	−
Scoliosis	+ (severe)	+	+	−	+	−	+	−	+	−
Brain MRI anomalies	−	+ (rounded splenium of corpus callosum; oblique orientation of sulci in the superior part of cerebellar verms; millimetric cyst of the pineal gland)	−	−	+ (prevalence of the splanchnocranium over the neurocranium)	−	−	−	−	−
Truncal obesity	+	−	+	−	−	−	−	−	−	+

TABLE 2 (Continued)

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
Other somatic features	Hypotonia, axial hypotonia, tapering fingers, flat feet, outwardly rotated feet	–	Hyperlordosis, knee valgus, outwardly rotated legs, hollow feet	–	Axial hypotonia, lower limbs hypertone, 2nd–3rd toes brachydactyly	Flatfeet, spastic gait with poor hip and knee flexion	Fingers brachydactyly and cutaneous syndactyly	Overpronated feet	Lower limbs hypertonicity	Walking wide based, equinus feet

	Case 11	Case 12	Case 13	Case 14	Case 15	Case 16	Case 17	Case 18	Case 19	Total and relative percentages
Transcript variant—Reference sequence NM_001904.4	c.1420 C>T	c.1759C>T	c.1759C>T	c.1759C>T	c.1759C>T	c.1852del	c.1874del	c.1925_1926del	c.2089G>T	
Mutation site	Exon 9	Exon 11	Exon 11	Exon 11	Exon 11	Exon 12	Exon 12	Exon 12	Exon 14	
Protein variant—Reference sequence NP_001895.1	p.Arg474*	p.Arg587Ter	p.Arg587Ter	p.Arg587Ter	p.Arg587Ter	p.Leu618SerfsTer23	p.Lys625fs	p.Glu642Valfs*5	p.Gly697Ter	
Variant type	Nonsense	Nonsense	Nonsense	Nonsense	Nonsense	Frameshift	Frameshift	Frameshift	Nonsense	6 frameshift and 13 nonsense
Previously reported/novel	Reference 1	Reference 48	Reference 48	Reference 48	Reference 48	Novel	Novel	Reference 1	Novel	9 novel/19 (47%)
ACMG classification	Class 5	Class 5	Class 5	Class 5	Class 5	Class 4	Class 4	Class 5	Class 4	
Inheritance pattern	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo	Unknown	
Sex	M	F	F	M	M	F	M	M	M	8 females, 11 males
Auxological features										
Age at observation (years + months)	4	7.9	10.1	6	23	14.2	10	7.8	12	Mean age 10.3 years
Height SDS (cm/SDS)	99 cm (−0.7 SD)	136 cm (1.47 SD)	139.6 cm (0.15)	121 cm (1.1 SD)	167.5 cm (−1.26 SD)	165 cm (0.67 SD)	121.6 cm (−2.7 SD)	123.7 cm (−0.52 SD)	155.8 cm (0.89 SD)	
Weight SDS (kg/SD)	13 kg (−2 SD)	24.6 kg (−0.22)	25.8 kg (−1.53 SD)	24.6 kg (1.1 SD)	55.2 cm (−1.64 SD)	65 kg (1.09 SD)	22 kg (−2.6 SD)	22.4 kg (−0.93 SD)	57.8 kg (1.45 SD)	
BMI SDS	13.3 (−2.6 SD)	−1.41	−2.56	17 (0.9 SD)	19.7	0.99	14.9 (−1−1 SD)	−0.98	1.41	
Occipito-frontal circumference (cm/SDS)	45.6 (−3.2)	48.5 cm (−2.52 SD)	49.4 cm (−2.23 SD)	49 cm (−1.9 SD)	53.5 cm (−1.1 SD)	50 cm (−3.55 SD)	46 cm (−5.3 SD)	47.5 cm (−3.46 SD)	51.5 cm (−1.54 SD)	
Congenital heart disease	–	–	–	–	–	Mitral valve prolapse with insufficiency and patent foramen ovalis	–	–	Tetralogy of Fallot	5/19 (26.3%)
Neurodevelopmental issues										
Developmental delay (mild, moderate, severe)	Mild	Severe	Severe	Moderate	Mild	Moderate	Mild	Mild	Moderate	19/19 (100%)
Intellectual disability (mild, moderate, severe)	Mild	Moderate	Moderate	Moderate	Mild	Moderate	Mild	Moderate	Mild	19/19 (100%)
Speech delay (+/−)	+	+	+	+	+	+	+	+	+	19/19 (100%)
Motor delay (+/−)	+	+	+	+	+	+	+	+	+	19/19 (100%)

(Continues)

TABLE 2 (Continued)

	Case 11	Case 12	Case 13	Case 14	Case 15	Case 16	Case 17	Case 18	Case 19	Total and relative percentages
Behavioral issues										
Autistic features (+/−)	−	+	+	−	−	−	−	−	+	7/19 (36.8%)
ADHD (+/−)	−	−	−	−	−	+	−	+	−	7/19 (36.8%)
Aggression/self-mutilation (+/−)	+	+	−	−	−	+	−	+	−	7/19 (36.8%)
Sleep disturbances (+/−)	+	−	+	−	−	−	+	+	−	6/19 (31.6%)
Other	−	−	−	−	−	−	−	−	−	1/19, picacism (5.3%)
Movement disorders										
Truncal hypotonia (+/−)	+	−	−	−	−	−	+	−	−	4/19 (21%)
Lower limbs spasticity (+/−)	+	+	+	+	+	+	+	+	−	14/19 (73.7%)
Dystonia (+/−)	+	−	+	−	−	−	−	−	−	4/19 (21%)
Ataxic gait	+	+	−	−	+	+	−	−	−	8/19 (42%)
Other	−	−	Dysarthria	−	−	−	−	−	−	1/19 (5.3%)
Visual defects										
Strabismus (+/−)	+	+	+	+	+	+	−	+	−	14/19 (73.7%)
Nystagmus (+/−)	+	−	+	+	−	−	+	−	−	6/19 (31.6%)
Astigmatism (+/−)	−	−	+	+	−	+	+	+	+	13/19 (68.4%)
Myopia (+/−)	−	−	−	−	−	−	−	−	−	1/19 (5.3%)
Bilateral FEVR (+/−)	−	−	−	−	−	−	−	−	−	0/19
Hypermetropia (+/−)	+	−	+	+	+	+	−	+	−	10/19 (52.6%)
Cataract (+/−)	−	−	−	−	−	−	−	−	−	1/19 (5.3%)
Other	−	−	−	−	Oculomotor apraxia	−	−	−	−	1/19, oculomotor apraxia (5.3%)
Facial features (brief description)	Microcephaly, telecanthus, upslanting of palpebral fissure, long labial philtrum, prognathism	Microcephaly, almond-shaped eyes, narrow palpebral fissures, long and prominet nose, thin upper lip	Microcephaly, elongated face, arched eyebrows, almond shaped palpebral fissures, long and prominanet nose, flat philtrum, thin upper lip, slight retrognathia	Small OFC, elongated face, almond shaped palpebral fissures, thin upper lip, retrognathia, large auricles	Elongated face, almond shaped palpebral fissures, long and prominent nose, thin upper lip, prognathism, large auricles	Microcephaly, sparse eyebrows, elongated and narrow palpebral fissures, mild palpebral ptosis, prominent nose with high nasal bridge and broad nasal tip, prominent philtrum, thin upper lip, large ears	Microcephaly, broad and high forehead, elongated face, almond shaped palpebral fissures, protruding nose, smooth filter, thin upper lip	Microcephaly, broad and high forehead, elongated face, narrow palpebral fissures, esotropia, long and prominent nose, mooth filter, thin upper lip, arched and fleshy lower lip	Small OFC, horizontal eyebrows, hypotelorism, high nasal bridge, long and prominent nose, turned down nose tip, small mouth with thin upper lip, retrognathia	
Other (e.g.)										
IUGR	−	−	−	−	−	−	−	−	−	4/19 (21%)
Short stature	−	−	−	−	−	−	+	+	−	6/19 (31.6%)

TABLE 2 (Continued)

	Case 11	Case 12	Case 13	Case 14	Case 15	Case 16	Case 17	Case 18	Case 19	Total and relative percentages
Scoliosis	–	–	+	+	–	+	–	–	–	*9/19 (47.4%)*
Brain MRI anomalies	+ (neurocranium-splanchnocranium disproportion; small arachnoid cyst in the right bulbocerebellar angle cistern)				–	+ (bilateral frontal cortical dysplasia)	+ (slight thinning of the isthmic portion of corpus callosum)	–	+ (calcification in the left caudate nucleus)	–
–	–	*6/19 (31.6%)*								
Truncal obesity	–	–	+	–	–	+	–	–	+	*6/19 (31.6%)*
Other somatic features	–	Long and tapering fingers	Inverted nipples, dorsal kyphosis, dorsal hypertrichosis, externally rotated lower limbs, clubfoot, hallux valgus	–	–	T5-L1 spinal foramen dilatation at spinal MRI; joint laxity, dorsal hypertrichosis, kyphoscoliosis, brachydactyly, persistent finger pads, bilateral valgus foot	–	Lacrimal ducts stenosis, long and tapering, 2nd toe of left foot under the 3rd toe	Fifth fingers clinodactyly, tapering fingers, sandal gap, 2nd-3rd toes syndactyly, fifth toes clinodactyly and flatfeet	

Note: The clinical features of the present cohort including the detected causative *CTNNB* variant, sex and auxological features, the eventual presence of CHD, neurodevelopmental issues, behavioral issues, movement disorders, visual defects and a brief phenotype description. This table modified from Ho et al.[49]

2.2 | Review of the literature

A literature review process aimed to explore previous reports dealing with *CTNNB1* associated CHDs up to April 2023 was performed. PubMed and EMBASE databases, Web of Science and Google Scholar Search engines were inspected for indexed published papers and queried through the keywords "*CTNNB1*" or "beta-catenin" or "β-catenin" and "heart" or "cardiac" or "congenital heart disease" or "congenital heart defect/defects" or "congenital heart disorder/disorders" using the Boolean operators "AND" and "OR." "*CTNNB1*" was also searched in conjunction to "echocardiography" or "echocardiogram" and to anatomical cardiac structures related terms such as "valve/valves" or "aortic" or "mitral" or "tricuspid" or "pulmonary" or "ventricular" or "atrial" or "septal," using the Boolean operators "AND" and "OR." Finally we searched for "*CTNNB1*" and "Review" and "congenital heart disease/diseases/disorder/disorders" or "neurodevelopmental disease/diseases/disorder/disorders" using the Boolean operator "OR" and "AND." Six papers reporting cases of *CTNNB1* pathogenic variations associated with CHDs cited in the text or in the "Supplemental Material" section[3] were displayed with a temporal coverage running from 2015, onwards.

2.3 | Genetics laboratory methods

Genomic DNA was extracted from circulating leukocytes according to the manufacturer's instructions. Molecular analyses were performed in the context of the clinical genetics diagnostic activity; case 5 was enrolled in an intramural research program dedicated to undiagnosed patients. Mutation scan was performed using either customized panels (SeqCap EZ Choice Enrichment Kit [Roche] or Twist Human Clinical Exome Panel [Twist Bioscience], or by exome sequencing; SureSelect AllExon V.7 [Agilent]), and analyzed on a NextSeq550 or NovaSeq6000 sequencing platform (Illumina, San Diego, CA). Reads were aligned to the reference genome UCSC GRCh37 using the DRAGEN Germline Pipeline and analyzed with Geneyx Analysis–Geneyx, variant prioritization software (gene panels) or using an in-house implemented pipeline previously described,[18] according to the GATK's Best Practices. All genetic variants detected in index cases were validated on re-extracted DNA and verified by bidirectional Sanger sequencing in available family members. Potential pathogenicity was predicted using SIFT, PolyPhen-2, PROVEAN, MutationAssessor, MutationTaster and CADD score (NCBI reference sequences: NG_032003.1; NG_013302.2; NP_037407.4) and following the American College of Medical Genetics and Genomics (ACMG) guidelines.[19] All variants behave as LoF and were predicted to affect proper transcript processing or result in a truncated protein. The detected variations are reported in Table 2. Nine novel previously unreported variants were detected.

3 | RESULTS

3.1 | *CTNNB1* patients with CHD: clinical description

3.1.1 | Case 5

He is an 8 year-old boy, single-born of healthy non-consanguineous parents. During pregnancy, echocardiographic diagnosis of isolated APV was reported. He was born at the 39th week by vaginal delivery. Birth weight was 3260 g (50th centile), length was 52 cm (50th centile), occipito-frontal circumference (OFC) was not available. Apgar was 7 and 7 (1′ and 5′, respectively). OFC at 7 weeks was 36.3 cm (below the 3rd centile). Echocardiography performed at birth showed pulmonary valve (PV) agenesis with IVS associated with large patent ductus arteriosus (PDA). The aortic valve had thickened leaflets, and a giant pulmonary trunk was observed (16 mm, *Z* score +4.1), compressing on the left pulmonary bronchus. At 14 days, he underwent the ductus arteriosus surgical closure and pulmonary trunk and left pulmonary artery diameters reduction. At age 2.9 years, during his follow-up, OFC was 47 cm (10th centile). A marked OFC reduction was reported since birth. Brain MRI showed a prevalence of the splanchnocranium over the neurocranium. Control echocardiography showed rudimentary PV resulting in regurgitation, no major stenosis (maximum gradient <20 mmHg). No significant further valve anomalies were observed. A severe dilation of the ascending aorta was identified (maximum diameter 19 mm, *Z* score +6.5). He managed to sit independently at 3 years, but he cannot stand without help nor walk alone. At 4 years, ophthalmic electrophysiology examination through flash visual evoked potentials and electroretinogram, and hearing test through brainstem auditory evoked potentials gave normal results. At present, he shows motor stereotypies. Language is evolving and he pronounces several words. Axial hypotonia with kyphotic attitude is evident. He shows head control and poor trunk control getting easily tired. Leiter 3 detected mild ID and mild developmental language disorder. Facial features included a long face with high forehead, horizontal eyebrows, hypotelorism, enophthalmos, narrow palpebral fissures, strabismus, eyelid ptosis, long nose, and retrognathia. He also showed 2nd–3rd toes brachydactyly, and limbs hypertonicity. Clinical exome sequencing revealed the de novo novel heterozygous pathogenic variant c.976_979delAATA (p.Asn326Ter) in the *CTNNB1*.

3.1.2 | Case 7

Case 7 is a 20 years-old girl born to healthy non-consanguineous parents. Her brother was reported to be healthy. She was born at 40 weeks and 5 days of gestation by vaginal delivery of an uneventful pregnancy. Not immediate crying was reported. Birth weight was 3100 g (50th centile), length 50 cm (50th centile), OFC 31 cm (below 3rd centile). Apgar was 6 and 7 (1′ and 5′, respectively). She presents dystonia, ataxia and cognitive disability. Psychomotor retardation was evident at 8 months when she cannot sit autonomously. First words were pronounced around the second year with improvements through rehabilitation. She walked at 30 months, mostly tip-toeing. An echocardiography performed when she was 11 years detected MVP with moderate mitral insufficiency. During years she was checked showing stable heart conditions. At 14 years hospitalization was requested at the Neurology Unit for refusal to walk due to worsening pain in the lower limbs for over 7 days. She already had similar episodes, after febrile episodes. At that time, she was mainly wheelchair-bound but she managed walking with bilateral support. Brain MRI resulted substantially normal. The symptomatology was considerably relieved with analgesic therapy. Cervical dystonia combined with mild bradykinesia, generalized rigidity and broad-based gait with pyramidal signs (ankle clonus and Babinski sign) were noted. IQ was evaluated with the Leiter International Performance Scale, 3rd Edition (Leiter-3) (IQ = 51). Episodes of agitation and aggression with poor tolerance to frustration were reported. Speech was dysarthric and simplified in content but communicative. Treatment with levodopa-carbidopa (up to 200 mg tid) and botulinum toxin A (BonT-A) injections, targeting cervical dystonia and lower limbs spasticity was beneficial. Valproic acid was effective for behavioral dysregulation. At the last evaluation, at 19 years and 8 months of age, she showed microcephaly (OFC 51.5 cm, <3rd centile), broad forehead, long face, turned down mouth corners, squared chin, retro-rotated *pinnae* with simple helix, mild nasal alar hypoplasia, arched palate, hypopigmented skin, scoliosis. Fingers brachydactyly and cutaneous syndactyly were noted. The movement disorder was stable under levodopa and regular BonT-A injections. Her mood was tendentially euphoric in the absence of a clear dysregulation, and valproic acid was discontinued at age 19. Clinical exome sequencing showed a de novo *CTNNB1* pathogenic variant (c.999delC, p.Tyr333Ter).[20]

3.1.3 | Case 9

Case 9 is a 15 year-old boy, single-born to healthy non-consanguineous parents. He was born at the 40th week by vaginal delivery of an uneventful pregnancy. Birth weight was 3480 g (50th centile), length 50 cm (50th centile), and OFC 36 cm (85th centile). Apgar was 8 and 10 (1′ and 5′, respectively). After perinatal asphyxia due to meconium ingestion, orotracheal intubation was required. He was transferred to the neonatal intensive care unit and ventilated for 2 weeks. At birth, cerebral and kidney ultrasounds were normal. Echocardiography revealed partial AVC with aortic coarctation and PDA, surgically corrected at 2 years of age. No significant cardiac sequelae were reported during his follow-up. He also presented bilateral cryptorchidism, surgically corrected at 6 years. He showed strabismus and astigmatism, corrected with lenses at 6 years and surgically at 9 years. A markedly reduced OFC was reported since birth. Brain MRI performed at 7 years showed microcephaly, in the absence of structural anatomical lesions. Clinical examination displayed severe psychomotor and language delay. He gradually developed lower limb hypertonia. Head was controlled at three and half months, uncertain control of the trunk was achieved at 12 months and from 18 months of age he

managed walking with unilateral support and tip-toeing. He shows signs of spastic paraparesis and dysarthria. At 11 years, Achilles tendon lengthening surgery was performed. He has moderate ID (IQ 49). Good adaptive skills are reported and no behavioral issues. At the last evaluation, he showed spasticity with cervical dystonia and mild bradykinesia. OFC at time of last evaluation at 15 years-old was 49 cm (−4.03 SD). He showed mild somatic features including elongated facies, large ears, narrow palpebral fissures, strabismus and astigmatism, elongated nose, open lips, spaced-out teeth, and retrognathia. Clinical exome sequencing identified a previously unreported de novo *CTNNB1* pathogenic variant (c.1081+1_1082-1)(2346+?)del, predicting an aberrant processing of the transcript (deletion of exons 8–15).

3.1.4 | Case 16

Case 16 is a 13-year-old girl, born from healthy non-consanguineous parents. Her younger sister is healthy. She was born by vaginal delivery at the 36th week of an uneventful pregnancy. Birth weight was 2880 g (15th centile), length 48 cm (15th centile), and OFC 32.5 cm (below the 15th centile). Delayed motor milestones, delayed language, hypotonia, microcephaly and specific facial features were noted within the first months. The patient was evaluated at the age of 17 months. Weight was 12.100 kg (75th–90th centile), length 84 cm (90th–97th centile), OFC was 45 cm (below the 3rd centile). Microcephaly, strabismus, bilateral valgus foot, sparse eyebrows, elongated and narrow palpebral fissures, long and protruding nose, prominent philtrum, thin upper lip, large ears, joint laxity, brachydactyly, and persistent finger pads were evident. Cerebral ultrasound examination showed bilateral choroid plexus cyst. Neuropsychological evaluation at 6 years revealed mild ID, speech delay in both expression and comprehension, attention deficit disorder, and motor coordination difficulties. Broad-based gait was evidenced. Moderate ID worsened with an estimated IQ of 40 at 10 years of age. Behavioral problems with emotional dysfunction and aggressivity appeared. Aripiprazole was started at 12 years. Brain MRI with TC-scan only showed a punctiform calcification in the left caudate nucleus. Examination at 11 years showed long face, sparse eyebrows, up-slanted elongated and narrow palpebral fissures, mild palpebral ptosis, prominent nose with high nasal bridge and broad nasal tip, retrognathia, arched upper lip and everted lower lip, dental diastema, large ears, strabismus and hypermetropia, obesity, dorsal hypertrichosis, kyphoscoliosis, mild lower limbs asymmetry, valgus knee and feet, dry skin, keratosis pylaris of the trunk and upper limbs, melanocytic nevi. Weight was 60.500 kg (>97th centile), height 161 cm (97th centile), head circumference 49.5 cm (<3rd centile). Neurological examination showed broad-based gait and lower limbs hypertonia, in absence of overt spasticity. Abdominal ultrasound, ophthalmological and audiological examinations, and EEG were normal. Two-dimensional echocardiography revealed patent foramen ovalis (PFO) and MVP with moderate valve insufficiency. Clinical exome sequencing detected the novel de novo heterozygous *CTNNB1* pathogenic variant c.1852del (p.Leu618SerfsTer23).

3.1.5 | Case 19

He is a 12 year-old boy, second-born of healthy non-consanguineous parents. Prenatal diagnosis of ToF was performed by ultrasound examination at 32 weeks of gestational age, and was confirmed after birth. He was born at the 38th week by vaginal delivery. Birth weight was 2870 g (10th centile), length 45 cm (below 3rd centile), OFC 31.5 cm (below 3rd centile). Apgar was 8 and 9 (1′ and 5′, respectively). At 6 months of age, since pulmonary obstruction was demonstrated, he underwent surgical correction with ventricular septal defect closure plus trans-annular patch in order to release the outflow obstruction. The last echocardiography showed no residual shunts. Significant pulmonary insufficiency was evidenced with right ventricle dilation with preserved ejection fraction. A septal dyskinesia due to right volume overload was observed. No residual right outflow obstruction was shown. He walked alone at 24 months and babbling started around 18 months. He started speaking at 3 years with the first short sentences at four and a half years. Wechsler Intelligence Scale for Children-Fourth Edition was used to evaluate his neurodevelopment, resulting in a moderate ID (IQ = 46). At 12 years his OFC was 51.5 cm (−2 DS). Facial features examination included horizontal eyebrows, hypotelorism, high nasal bridge, long and prominent nose, turned down nose tip, small mouth with thin upper lip, and retrognathia. He had fifth fingers clinodactyly, tapering fingers, sandal gap, second-third toes syndactyly, fifth toes clinodactyly and flatfeet. Neurological examination was normal. Clinical exome sequencing detected a novel *CTNNB1* heterozygous truncating variant (c.2089G>T, p.Gly697Ter), not inherited from his mother. Paternal DNA was not available for testing.

3.2 | Molecular and cardiac results synopsis

The reported patients harbored heterozygous pathogenic/likely pathogenic (class 4/5 according to ACMG) *CTNNB1* variants. Nine novel previously unreported variations have been detected (47%).

In the studied cohort, CHD was diagnosed in 26% of patients (5/19) (three males and two females). Various types of cardiac anomalies were identified. In greater detail, case 5 presented APV with IVS and PDA; case 7 showed MVP with moderate mitral insufficiency; case 9 exhibited partial AVC with hypoplastic aortic arch; case 16 also had MVP with mitral insufficiency; case 19 presented ToF. In four individuals, the detected variations were previously unreported (case 5, case 9, case 16, and case 19).

The review of CHDs anatomic types diagnosed in patients with *CTNNB1*-NEDSDV from the literature in comparison with cases from the present series was performed (Table 1).

The molecular and full clinical characterization of the present cohort are summarized in Table 2.

4 | DISCUSSION

We present a series of 19 cases bearing *CTNNB1* pathogenic variants, documenting a relatively high prevalence of CHDs. All cases had LoF variants predicted to affect transcript processing or resulting in a truncated protein. Nine of these variants were not previously reported. Four novel variants were associated with CHDs.

Besides the higher prevalence of cardiac involvement in the present series (5/19 patients, 26%), a wide spectrum of CHDs were noted, including APV with IVS, ToF, AVC defect, and MVP with mitral insufficiency. When considering only the more severe cardiac malformations (APV with IVS, ToF, AVC), the prevalence was higher than 15% (3/19). Six articles reporting CHDs in *CTNNB1* patients were found in the literature,[3,13–17] four being included in the paper by Kayumi et al.[3,13–16] Two main clinical reviews about *CTNNB1*-syndrome were published to date and considered in the present report.[3,6] In the paper by Kayumi et al.,[3] 120/404 genetically diagnosed individuals having an adequate phenotype description were revised in detail. Accordingly, 10/120 (8.3%) cases had a CHD diagnosis (Table 1).[3,13–16] Overall, septal defects, PV anomaly, ToF, and anomalous pulmonary venous return have been reported.[3] Of note, a study by Morton et al.[17] evaluating the frequency of damaging variants in cancer risk genes among a large series of patients with CHDs, revealed three further *CTNNB1*-NEDSDV cases affected by heart defects. One case with atrial septal defect, one case with a dilated tricuspid valve and one case with ToF were reported.[17] In the present case-series, we describe the third case of ToF associated with a *CTNNB1* pathogenic variant (Case 19).

ToF, septal defects and PV anomalies are prevalent. PV involvement is reported as valve stenosis and as APV. PV stenosis is the prevalent CHD in Noonan syndrome (NS).[21] APV with IVS is a rare malformation characterized by dysplastic, hypoplastic, or absent PV leaflets. This cardiac defect is frequently associated with ToF, particularly in 22q11.2 deletion syndrome (DiGeorge/Velo-Cardio-Facial syndrome).[22] APV with IVS and PDA is rarer, with the quite specific association with the terminal deletion of the long arm of chromosome 18.[23] AVC defect is a genetically heterogeneous cardiac anomaly, prevalently associated with Down syndrome, 8p23 deletion syndrome including *GATA4*, ciliopathies and RASopathies.[24] Only the *CTNNB1*-NEDSDV patient we report in the present series associated with this type of cardiac defect has been diagnosed to date (Case 9). The defect is partial in our patient, associated with mild aortic arch hypoplasia. The combination of AVC defect and left-sided obstructions is rare in patients with Down syndrome, but described in patients with NS.[25]

The present results support the inclusion of *CTNNB1*-NEDSDV among genetic syndromes associated with ToF, AVC defect, PV stenosis, and absent PV with IVS.

Since mild cardiac anomalies, such as septal and valvular defects, are also reported in literature[3,13–17] and in two of the present cases (MVPs) (Case 7 and Case 16), we believe that all *CTNNB1*-NEDSDV patients should be evaluated for CHDs comprising an accurate and detailed echocardiogram at time of diagnosis. Cardiological evaluations should also be included in the follow-up management of affected patients. Indeed, it may be possible that previously diagnosed patients with *CTNNB1*-NEDSDV were prevalently identified through molecular screening of subjects referred for DD and not for CHDs. Therefore, the extensive use of exome and genome sequencing in a series of patients with syndromic CHD and neurodevelopmental disorders might lead to an increased diagnosis of *CTNNB1*-NEDSDV with cardiac malformations.

In the presently studied cohort, male *CTNNB1*-NEDSDV patients seem to be more frequently and more severely affected by CHDs compared to females (8 males out of 13 patients with information about sex provided). All the pathogenic variants identified in the present study were predicted to behave as LoF. No specific hot spots for CHD were detected, though the here reported patient with ToF (Case 19) and another previously described case with the same cardiac defect[17] harbor variants in exons 14 and 15, which are part of the β-catenin C-terminal domain. This region recruits both effectors and inhibitors of transcriptional regulation[26] and its direct role in β-catenin transcriptional activity has been demonstrated.[27,28]

B-catenin is recognized as a molecular multiplayer binding with various proteins and displaying different roles depending on its interface. It is mainly located at the membrane cytoplasmic-side where it binds to its interaction partners.[29] β-catenin role has mostly been studied in the context of the Wnt/β-catenin signaling.[29] Its role in establishing the anterior/posterior (A/P) body axis has been assessed in various systems. In mice, it was demonstrated that β-catenin participates in the control of the A/P axis identity and mesoderm formation, as embryos lacking β-catenin do not develop a proper A/P axis resulting in a defective gastrulation.[30] It is also known that β-catenin and the Wnt/β-catenin pathway play a biphasic function in heart development by functioning as a positive element at earlier stages in cardiomyogenesis and having an inhibitory influence at later stages.[32] LoF and GoF studies of the Wnt/β-catenin signaling in a spatiotemporally confined manner have provided evidence that it is essential for the expansion and development of precardiac and cardiac mesoderm in mouse.[31] Murine and human *CTNNB1* cardiac-specific deletion has shown to be deleterious for cells originating from the secondary heart fields (SHF), suggesting a spatial variation in the cardiac myocytogenesis controlled by this gene.[33] Indeed, β-catenin plays a central role for the Wnt pathway effectiveness and both Wnt and Wnts inhibitors are expressed in the developing heart cells suggesting a subtle control of the Wnt/β-catenin signaling in myocardium formation.[34] Islet1 (Isl1), a LIM-homeodomain transcription factor playing a crucial role in multiple organs during embryonic development including cardiovascular development, has been proposed as a marker of cardiac progenitor cells derived from SHF.[35] It was shown that Wnt/β-catenin directly regulates Isl1 expression in cardiovascular progenitors.[33] In mice, the outflow tract (OFT) myocytes mitosis rate was significantly reduced following β-catenin deletion in Isl1+ precursors. On the other hand, the β-catenin pathway constitutive activation within Isl1+ SHF cells progenitors leads to their massive accumulation followed by inhibition of myocytic differentiation, and supposedly to severe OFT defects.[33] Of note, in mice it was shown that mesenchymal *Ctnnb1* inactivation determines anomalies within the DiGeorge syndrome phenotype,

including great vessel abnormalities, hypoplastic pulmonary and aortic arch arteries and cardiac malformations such as ventricular and atrial septal defects.[36] These experiments testify the requirement of a proper β-catenin dosage for the correct OFT development.

Wnt/β-catenin cascade is also involved in the development of pathological and physiological heart remodeling, and maintain energetic metabolism in adult heart.[37] In particular, Wnt/β-catenin signaling may act as a metabolic regulator and sustain metabolic plasticity during adult heart homeostasis.[37] In human over 45-years old hearts, β-catenin expression is stronger than in younger individuals, indicating that the Wnt signaling is involved in the regulation of adult heart homeostasis in the process of age-related cardiac function.[38] In addition, β-catenin upregulated expression has been observed in patients with dilated cardiomyopathy[39] while mild cardiac hypertrophy was detected in β-catenin depleted adult hearts.[40]

Finally, the possible connections between β-catenin, GATA4 and NKX2-5 were considered. The NKX2-5 transcription factor is essential for heart development, and *NKX2-5* pathogenic variants were associated with a variety of CHDs.[41] In human fetal cardiac myocytes, an in vitro cell culture model of fetal cardiac myocytes in combination with antisense RNA or siRNA technology disclosed that *CTNNB1* and *GATA4* promoters harbor NKX2-5 binding sites.[42] It has been proposed that *NKX2-5* negatively regulate *CTNNB1* and positively regulate *GATA4* in cardiomyocytes suggesting the NKX2-5 modulation of both β-catenin and GATA4 transcriptional activities in developing human cardiac myocytes.[42] In murine embryonic stem cells, the cardiac differentiation has been shown to be exercised through the regulation of the Wnt/β-catenin pathway as well as the transcription factor *Gata4* through the action of another player, the transcriptional repressor RE-1-silencing transcription factor (REST).[43] *REST* is also involved in repressing the expression of fetal genes in the adult heart and its inhibition induces cardiac hypertrophy. It is ascertained that it regulates the expression of heart genes using multiple chromatin-modifying complexes.[43]

Since CHDs[44,45] and septal defect[46] have been associated with pathogenic variants in the NKX2-5 and GATA4 genes, their connection with *CTNNB1* could suggest a role of the latter in human CHDs, even if more evidences are needed.

Though further descriptions are required to accurately profile the nature and prevalence of CHDs in *CTNNB1*-NEDSDV, the present data strongly suggest including a complete cardiac evaluation and a detailed echocardiogram at the time of the diagnosis and at further follow-up into the multidisciplinary management of *CTNNB1*-NEDSDV.

5 | CONCLUSIONS

CHDs in patients with *CTNNB1*-NEDSDV are more common than previously expected. About a quarter of the cases in the present cohort showed CHDs with a spectrum ranging from severe (APV with IVS, AVCD, ToF) to milder (MVPs) cardiac anomalies. Affected patients reported to date show different cardiac anatomic defects, prevalently including ToF, PV anomalies, septal defects, AVC, abnormal pulmonary venous return, MPV. Based on the present findings, we suggest including an accurate and detailed cardiac assessment in the clinical management of *CTNNB1*-NEDSDV patients, both at diagnosis and during the follow-up management.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, writing: Lorenzo Sinibaldi, Maria Cristina Digilio, Giulio Calcagni. *Genetic and pediatric evaluation*: Lorenzo Sinibaldi, Maria Cristina Digilio, Maria Lisa Dentici, Marina Macchiaiolo, Roberta Onesimo, Rita Blandino, Valentina Trevisan, Mariella Iademarco, Giuseppe Zampino. *Neurological evaluation*: Giacomo Garone, Daria Diodato. *Neuropsychiatric evaluation*: Alessandra Mandarino. *Eye evaluation*: Giancarlo Iarossi. *Endocrine evaluation*: Laura Chioma. *Cardiac evaluation*: Angelica Bibiana Delogu, Gabriella De Rosa, Giulio Calcagni. *Formal analysis*: Giuseppe Merla, Emanuele Agolini, Alessia Micalizzi, Cecilia Mancini, Marcello Niceta, Antonio Novelli. *Supervision and revision*: Lorenzo Sinibaldi, Maria Cristina Digilio, Marco Tartaglia, Andrea Bartuli.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest is declared.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The present study was conducted according to the Declaration of Helsinki guidelines. It was reviewed and approved by the Institutional Review Board of Bambino Gesù Children's Hospital (RRC-2023-2 875 028). Written informed consent from the parents' patients for blood sampling for genetic testing was obtained as part of the diagnostic protocol. Written informed consent for scientific publication of the patients' data was also obtained.

ORCID

Lorenzo Sinibaldi https://orcid.org/0000-0002-1371-936X
Laura Chioma https://orcid.org/0000-0002-0388-4337
Maria Lisa Dentici https://orcid.org/0000-0002-9505-5906
Emanuele Agolini https://orcid.org/0000-0001-6543-6225
Marcello Niceta https://orcid.org/0000-0003-4766-7753
Marco Tartaglia https://orcid.org/0000-0001-7736-9672
Maria Cristina Digilio https://orcid.org/0000-0002-0205-9634

REFERENCES

1. Kuechler A, Willemsen MH, Albrecht B, et al. De novo mutations in beta-catenin (CTNNB1) appear to be a frequent cause of intellectual disability: expanding the mutational and clinical spectrum. *Hum Genet*. 2015;134(1):97-109. doi:10.1007/s00439-014-1498-1
2. de Ligt J, Willemsen MH, van Bon BW, et al. Diagnostic exome sequencing in persons with severe intellectual disability. *N Engl J Med*. 2012;367(20):1921-1929. doi:10.1056/NEJMoa1206524
3. Kayumi S, Pérez-Jurado LA, Palomares M, et al. Genomic and phenotypic characterization of 404 individuals with neurodevelopmental disorders caused by CTNNB1 variants. *Genet Med*. 2022;24(11):2351-2366. doi:10.1016/j.gim.2022.08.006
4. Jin SC, Lewis SA, Bakhtiari S, et al. Mutations disrupting neuritogenesis genes confer risk for cerebral palsy. *Nat Genet*. 2020;52(10):1046-1056. doi:10.1038/s41588-020-0695-1
5. Moreno-De-Luca A, Millan F, Pesacreta DR, et al. Molecular diagnostic yield of exome sequencing in patients with cerebral palsy. *Jama*. 2021;325(5):467-475. doi:10.1001/jama.2020.26148
6. Miroševič Š, Khandelwal S, Sušjan P, et al. Correlation between phenotype and genotype in CTNNB1 syndrome: a systematic review of the literature. *Int J Mol Sci*. 2022;23(20):12564. doi:10.3390/ijms232012564
7. Clevers H. Wnt/beta-catenin signaling in development and disease. *Cell*. 2006;127(3):469-480. doi:10.1016/j.cell.2006.10.018
8. Sjöstedt E, Zhong W, Fagerberg L, et al. An atlas of the protein-coding genes in the human, pig, and mouse brain. *Science*. 2020;367(6482):eaay5947. doi:10.1126/science.aay5947
9. Chenn A. Wnt/beta-catenin signaling in cerebral cortical development. *Organogenesis*. 2008;4(2):76-80. doi:10.4161/org.4.2.5852
10. Wisniewska MB. Physiological role of β-catenin/TCF signaling in neurons of the adult brain. *Neurochem Res*. 2013;38(6):1144-1155. doi:10.1007/s11064-013-0980-9
11. Hirabayashi Y, Itoh Y, Tabata H, et al. The Wnt/beta-catenin pathway directs neuronal differentiation of cortical neural precursor cells. *Development*. 2004;131(12):2791-2801. doi:10.1242/dev.01165
12. *Human Protein Atlas version 22*. https://www.proteinatlas.org/ENSG00000168036-CTNNB1/tissue/heart+muscle
13. Homsy J, Zaidi S, Shen Y, et al. De novo mutations in congenital heart disease with neurodevelopmental and other congenital anomalies. *Science*. 2015;350(6265):1262-1266. doi:10.1126/science.aac9396
14. Ke Z, Chen Y. Case report: a de novo CTNNB1 nonsense mutation associated with neurodevelopmental disorder, retinal detachment, polydactyly. *Front Pediatr*. 2020;8:575673. doi:10.3389/fped.2020.575673
15. Monies D, Abouelhoda M, AlSayed M, et al. The landscape of genetic diseases in Saudi Arabia based on the first 1000 diagnostic panels and exomes. *Hum Genet*. 2017;136(8):921-939. doi:10.1007/s00439-017-1821-8
16. Rossetti LZ, Bekheirnia MR, Lewis AM, et al. Missense variants in *CTNNB1* can be associated with vitreoretinopathy-seven new cases of CTNNB1-associated neurodevelopmental disorder including a previously unreported retinal phenotype. *Mol Genet Genomic Med*. 2021;9(1):e1542. doi:10.1002/mgg3.1542
17. Morton SU, Shimamura A, Newburger PE, et al. Association of damaging variants in genes with increased cancer risk among patients with congenital heart disease. *JAMA Cardiol*. 2021;6(4):457-462. doi:10.1001/jamacardio.2020.4947
18. Lin YC, Niceta M, Muto V, et al. SCUBE3 loss-of-function causes a recognizable recessive developmental disorder due to defective bone morphogenetic protein signaling. *Am J Hum Genet*. 2021;108(1):115-133. doi:10.1016/j.ajhg.2020.11.015
19. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. doi:10.1038/gim.2015.30
20. Sun W, Xiao X, Li S, Jia X, Wang P, Zhang Q. Germline mutations in CTNNB1 associated with syndromic FEVR or Norrie disease. *Invest Ophthalmol Vis Sci*. 2019;60(1):93-97. doi:10.1167/iovs.18-25142
21. Pierpont ME, Digilio MC. Cardiovascular disease in Noonan syndrome. *Curr Opin Pediatr*. 2018;30:601-608. doi:10.1097/MOP.0000000000000669
22. Unolt M, Versacci P, Anaclerio S, et al. Congenital heart diseases and cardiovascular abnormalities in 22q11.2 deletion syndrome: from well-established knowledge to new frontiers. *Am J Med Genet A*. 2018;176:2087-2098. doi:10.1002/ajmg.a.38662
23. Versacci P, Digilio MC, Sauer U, Dallapiccola B, Marino B. Absent pulmonary valve with intact ventricular septum and patent ductus arteriosus: a specific cardiac phenotype associated with deletion 18q syndrome. *Am J Med Genet A*. 2005;138A(2):185-186. doi:10.1002/ajmg.a.30916
24. Pugnaloni F, Digilio MC, Putotto C, De Luca E, Marino B, Versacci P. Genetics of atrioventricular canal defects. *Ital J Pediatr*. 2020;46(1):61. doi:10.1186/s13052-020-00825-4
25. Digilio MC, Marino B, Picchio F, et al. Noonan syndrome and aortic coarctation. *Am J Med Genet*. 1998;80(2):160-162.
26. Städeli R, Hoffmans R, Basler K. Transcription under the control of nuclear arm/beta-catenin. *Curr Biol*. 2006;16(10):R378-R385. doi:10.1016/j.cub.2006.04.019
27. Dar MS, Singh P, Singh G, et al. Terminal regions of β-catenin are critical for regulating its adhesion and transcription functions. *Biochim Biophys Acta*. 2016;1863(9):2345-2357. doi:10.1016/j.bbamcr.2016.06.010
28. Vleminckx K, Kemler R, Hecht A. The C-terminal transactivation domain of beta-catenin is necessary and sufficient for signaling by the LEF-1/beta-catenin complex in Xenopus laevis. *Mech Dev*. 1999;81(1–2):65-74. doi:10.1016/s0925-4773(98)00225-1
29. Valenta T, Hausmann G, Basler K. The many faces and functions of β-catenin. *EMBO J*. 2012;31(12):2714-2736. doi:10.1038/emboj.2012.150
30. Huelsken J, Vogel R, Brinkmann V, Erdmann B, Birchmeier C, Birchmeier W. Requirement for beta-catenin in anterior-posterior axis formation in mice. *J Cell Biol*. 2000;148(3):567-578. doi:10.1083/jcb.148.3.567
31. Kwon C, Arnold J, Hsiao EC, Taketo MM, Conklin BR, Srivastava D. Canonical Wnt signaling is a positive regulator of mammalian cardiac progenitors. *Proc Natl Acad Sci U S A*. 2007;104(26):10894-10899. doi:10.1073/pnas.0704044104
32. Kwon C, Cordes KR, Srivastava D. Wnt/beta-catenin signalling acts at multiple developmental stages to promote mammalian cardiogenesis. *Cell Cycle*. 2008;7(24):3815-3818. doi:10.4161/cc.7.24.7189
33. Qyang Y, Martin-Puig S, Chiravuri M, et al. The renewal and differentiation of Isl1+ cardiovascular progenitors are controlled by a Wnt/-beta-catenin pathway. *Cell Stem Cell*. 2007;1(2):165-179. doi:10.1016/j.stem.2007.05.018
34. Eisenberg LM, Eisenberg CA. Wnt signal transduction and the formation of the myocardium. *Dev Biol*. 2006;293(2):305-315. doi:10.1016/j.ydbio.2006.02.014

35. Ren J, Miao D, Li Y, Gao R. Spotlight on Isl1: a key player in cardiovascular development and diseases. *Front Cell Dev Biol*. 2021;9:793605. doi:10.3389/fcell.2021.793605
36. Huh SH, Ornitz DM. Beta-catenin deficiency causes DiGeorge syndrome-like phenotypes through regulation of Tbx1. *Development*. 2010;137(7):1137-1147. doi:10.1242/dev.045534
37. Balatskyi VV, Sowka A, Dobrzyn P, Piven OO. WNT/β-catenin pathway is a key regulator of cardiac function and energetic metabolism. *Acta Physiol*. 2023;237(3):e13912. doi:10.1111/apha.13912
38. Kasacka I, Piotrowska Z, Niezgoda M, Lewandowska A, Lebkowski W. Ageing-related changes in the levels of beta-catenin, CacyBP/SIP, galectin-3 and immunoproteasome subunit LMP7 in the heart of men. *PloS One*. 2020;15(3):e0229462. doi:10.1371/journal.pone.0229462
39. Perriard JC, Hirschy A, Ehler E. Dilated cardiomyopathy: a disease of the intercalated disc? *Trends Cardiovasc Med*. 2003;13(1):30-38. doi:10.1016/s1050-1738(02)00209-8
40. Baurand A, Zelarayan L, Betney R, et al. Beta-catenin downregulation is required for adaptive cardiac remodeling. *Circ Res*. 2007;100(9):1353-1362. doi:10.1161/01.RES.0000266605.63681.5a
41. Schott JJ, Benson DW, Basson CT, et al. Congenital heart disease caused by mutations in the transcription factor NKX2-5. *Science*. 1998;281(5373):108-111. doi:10.1126/science.281.5373.108
42. Riazi AM, Takeuchi JK, Hornberger LK, et al. NKX2-5 regulates the expression of beta-catenin and GATA4 in ventricular myocytes. *PloS One*. 2009;4(5):e5698. doi:10.1371/journal.pone.0005698
43. Aksoy I, Marcy G, Chen J, et al. A role for RE-1-silencing transcription factor in embryonic stem cells cardiac lineage specification. *Stem Cells*. 2016;34(4):860-872. doi:10.1002/stem.2304
44. Dixit R, Narasimhan C, Balekundri VI, Agrawal D, Kumar A, Mohapatra B. Functionally significant, novel GATA4 variants are frequently associated with tetralogy of Fallot. *Hum Mutat*. 2018;39(12):1957-1972. doi:10.1002/humu.23620
45. Sheng W, Qian Y, Wang H, et al. DNA methylation status of NKX2-5, GATA4 and HAND1 in patients with tetralogy of fallot. *BMC Med Genomics*. 2013;6:46. doi:10.1186/1755-8794-6-46
46. Sarkozy A, Conti E, Neri C, et al. Spectrum of atrial septal defects associated with mutations of NKX2.5 and GATA4 transcription factors. *J Med Genet*. 2005;42(2):e16. doi:10.1136/jmg.2004.026740
47. Kharbanda M, Pilz DT, Tomkins S, et al. Clinical features associated with *CTNNB1* de novo loss of function mutations in ten individuals. *Eur J Med Genet*. 2017;60(2):130-135. doi:10.1016/j.ejmg.2016.11.008
48. Yan D, Sun Y, Xu N, Yu Y, Zhan Y, Mainland Chinese League of NEDSDV Rare Disease. Genetic and clinical characteristics of 24 mainland Chinese patients with *CTNNB1* loss-of-function variants. *Mol Genet Genomic Med*. 2022;10(11):e2067. doi:10.1002/mgg3.2067
49. Ho S, Tsang MH, Fung JL, et al. CTNNB1-related neurodevelopmental disorder in a Chinese population: a case series. *Am J Med Genet A*. 2022;188(1):130-137. doi:10.1002/ajmg.a.62504

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