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ORIGINAL ARTICLE

# Biallelic mutations in *DYNC2LI1* are a rare cause of Ellis-van Creveld syndrome

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Ellis-van Creveld syndrome (EvC) is a chondral and ectodermal dysplasia caused by biallelic mutations in the *EVC*, *EVC2* and *WDR35* genes. A proportion of cases with clinical diagnosis of EvC, however, do not carry mutations in these genes. To identify the genetic cause of EvC in a cohort of mutation-negative patients, exome sequencing was undertaken in a family with 3 affected members, and mutation scanning of a panel of clinically and functionally relevant genes was performed in 24 additional subjects with features fitting/overlapping EvC. Compound heterozygosity for the c.2T>C (p.Met1?) and c.662C>T (p.Thr221Ile) variants in *DYNC2LI1*, which encodes a component of the intraflagellar transport-related dynein-2 complex previously found mutated in other short-rib thoracic dysplasias, was identified in the 3 affected members of the first family. Targeted resequencing detected compound heterozygosity for the same missense variant and a truncating change (p.Val141*) in 2 siblings with EvC from a second family, while a newborn with a more severe phenotype carried 2 *DYNC2LI1* truncating variants. Our findings indicate that *DYNC2LI1* mutations are associated with a wider clinical spectrum than previously appreciated, including EvC, with the severity of the phenotype likely depending on the extent of defective DYNC2LI1 function.

**KEYWORDS**

*DYNC2LI1*, Ellis-van Creveld syndrome, genotype-phenotype correlations, Jeune syndrome, short-rib thoracic dysplasia

## 1 | INTRODUCTION

Ellis-van Creveld syndrome (EvC; MIM 225500) is a rare autosomal recessive chondral and ectodermal dysplasia with major features including short ribs, short limbs, congenital heart defects, postaxial polydactyly, and dysplastic teeth and nails.[1,2] Cognitive and motor development is normal. Heart defects occur in about 60% of affected subjects, and include abnormalities of atrial septation (common

atrium most commonly), atrioventricular canal defect, defects of the mitral and tricuspid valves, patent ductus arteriosus, ventricular septal defects, and hypoplastic left heart syndrome.[3,4]

EvC is caused by biallelic mutations in *EVC* (MIM 604831) or *EVC2* (MIM 607261).[5–7] These genes encode transmembrane proteins that localize to primary cilia and act downstream of the 7-transmembrane protein smoothened (SMO; MIM 601500) to promote hedgehog (Hh) signal transduction.[8,9] Consistently, accumulating evidence indicates that EvC is caused by impaired Hh signaling in cardiac, skeletal and orofacial tissues during embryonic development.[10]

While the clinical features of patients with EvC appear to be similar regardless of whether the disorder is caused by mutations affecting *EVC* or *EVC2*,[6,7] approximately 15% of individuals with clinical diagnosis of EvC do not carry disease-causing variants in the 2 genes, further supporting genetic heterogeneity.[11] Consistent with this observation, biallelic splicing variants in *WDR35* (MIM 613602), encoding retrograde intraflagellar transport protein 121, have recently been causally linked to a distinctive form of EvC.[12] WDR35 has been documented to be required for EVC/EVC2 recruitment into the ciliary compartment, and proper cell response to Hh signals, providing further evidence for defective Hh signaling as the pathogenetic mechanism underlying EvC.

Based on our previous finding that a significant proportion of patients with a clinical diagnosis of EvC do not have mutations in any of the identified disease genes,[11,12] we performed whole-exome sequencing (WES) in a family segregating EvC proved to be *EVC/EVC2/WDR35* mutation-negative, and analyzed a panel of candidate genes by targeted resequencing in 24 additional subjects also molecularly negative for known EvC genes, but diagnosed with EvC or having features overlapping with this condition, to investigate further the molecular genetics of this disorder. We report that a specific combination of variants in *DYNC2LI1*, a gene that had previously been shown mutated in other short-rib thoracic dysplasias (SRTDs), including Jeune syndrome, accounts for a small proportion of EvC cases. Our findings suggest that the severity of the phenotype associated with *DYNC2LI1* mutations depend on the extent of defective DYNC2LI1 function.

## 2 | PATIENTS AND METHODS

### 2.1 | Patients

A cohort of 25 unrelated subjects with features fitting EvC or suggestive for this disease were included in the study. Clinical assessment was performed by experienced clinical geneticists, and all subjects had previously been tested negative for mutations in the *EVC*, *EVC2* and *WDR35* genes. In all subjects, chromosome and high-resolution CGH array analysis excluded the occurrence of clinically relevant CNVs. Clinical data and biological material collection, use and storage were attained after written informed consent was secured, in accordance with the ethical standards of the responsible institutional committees on human experimentation. Written informed consent was obtained from the parents for patients' photos publication.

### 2.2 | Whole-exome sequencing

WES was performed in a single affected individual, BL1304-12 (V-2, family 1), from a medium-sized family with 3 affected members having clinical diagnosis of EvC (Figure 1 and Table 1). DNA was obtained from leukocytes. Exome capture was carried out using Nextera rapid capture v1.2 (Illumina, San Diego, CA), and sequencing was performed on a NextSeq 500 platform (Illumina). WES data were processed and analyzed using an in-house implemented pipeline as previously described.[13–16] Briefly, high-quality variants were filtered against public databases (dbSNP147 and ExAC V.0.3) to retain novel and clinically associated variants, and annotated variants with unknown frequency or having MAF <0.1%, and occurring with a frequency <1% in an in-house database including frequency data from approximately 800 population-matched WES. SnpEff toolbox v.4.2 was used to predict the functional impact of variants, which were filtered to retain only those located in exons with any effect on the coding sequence, and splice site regions.[17] Functional annotation of variants was performed using SnpEff v.4.2 and dbNSFP V.2.9,[18] and their functional impact was analyzed by Combined Annotation Dependent Depletion (CADD) v.1.3,[19] and dbNSFP Support Vector Machine (SVM) v.2.9.[20] For sequencing statistics, see Table S1, Supporting Information. Validation of the *DYNC2LI1* variants (NM_001193464.1) and segregation analyses were performed by Sanger sequencing using ABI BigDye Terminator Sequencing chemistry (Applied Biosystems, Foster City, CA) and an ABI 3500 Genetic Analyzer (Applied Biosystems).

### 2.3 | Targeted resequencing analysis

A custom HaloPlexHS target panel (Agilent, Santa Clara, CA) was designed on RefSeq hg19 using the SureDesign tool (www.agilent.com/genomics/suredesign) to capture all coding exons and intronic flanking regions (±25 bp) of *DYNC2LI1* and other 67 genes of interest (size of target regions: 283.5 kbp; amplicons: 14 415; target coverage: 99.41%), including genes mutated in ciliopathies with skeletal involvement or primary ciliary dyskinesia, or coding proteins functionally related to EVC, EVC2 and DWR35 (Table S2). Sequence enrichment was performed using a HaloPlexHS target enrichment kit (Agilent), and sequencing was carried out on a MiSeq platform (Illumina) generating paired-end 150 bp reads. Variant calling and data analyses used the Sure Call software V.3.5.1.46 (Agilent). Validation of the *DYNC2LI1* variants and segregation analyses were performed by Sanger sequencing as indicated above.

### 2.4 | mRNA studies

Total RNA was extracted from leukocytes of both parents of case MGM14-1183 (family 3) using RNeasy Plus Mini kit (Qiagen, Hilden, Germany). RNA was retro-transcribed (Superscript IV, Invitrogen, Waltham, MA) using random hexamers, and the entire *DYNC2LI1* coding sequence and 3′ untranslated region (3′-UTR) were analyzed by Sanger sequencing.

## 3 | RESULTS

### 3.1 | Molecular analyses

Data analysis of the WES performed in subject BL1304-12 allowed to identify 80 179 high-quality variants, of which 14 996 were

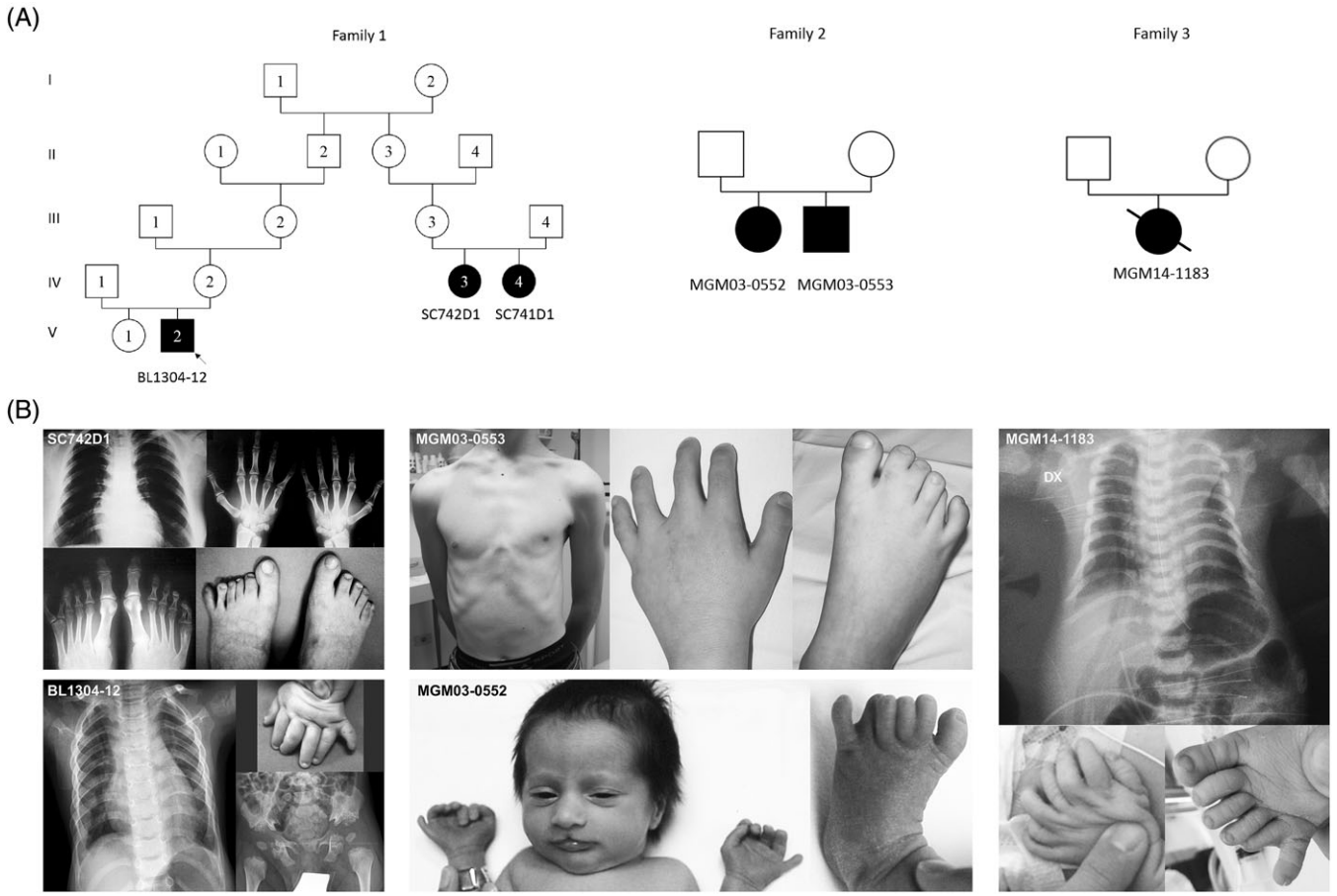


**FIGURE 1** Family trees and clinical phenotype of the subjects with biallelic *DYNC2LI1* mutations. A, Pedigrees of the 3 families included in the study. B, Features of SC742D1 (24 years, female) (left panels, above) and BL1304-12 (4 years, male) (left panels, below) (family 1), MGM03-0553 (13 years, male) (middle panels, above) and MGM03-0552 (20 days, female) (middle panels, below) (family 2), and MGM14-1183 (1 month, female) (right panels) (family 3) are shown. Note the long and narrow chest, postaxial polydactyly of hands (surgically corrected in SC742D1 and MGM03-0553) and feet, brachydactyly, dysplastic nails, and cleft lip

predicted to have functional impact (missense, nonsense and frameshift changes, small indels, and splice site changes). Among the latter, 526 private, rare and clinically associated variants were retained for further analysis (Table S1). Based on the occurrence of multiple affected individuals in the family, autosomal recessive transmission was considered as the most likely inheritance model. Filtering and prioritization of the 11 genes with biallelic variants pointed out to *DYNC2LI1* (dynein, cytoplasmic 2, and light intermediate chain 1; MIM 617083) as the most promising candidate gene for the disease. *DYNC2LI1* encodes a component of the dynein-2 microtubule motor protein complex with a role in the retrograde transport of cargo in primary cilia via the intraflagellar transport system. Compound heterozygosity for the c.662C>T (p.Thr221Ile) and c.2T>C (p.Met1?) *DYNC2LI1* variants was confirmed by Sanger sequencing, which also proved their co-segregation with the disorder in the family (Figure S1). The disruptive c.2T>C change had previously been reported (rs200859699; total MAF < 0.0001, ExAC), while the missense substitution, c.662C>T, had been described as disease-causing in a subject with a Jeune-like phenotype.[21] None of the 2 variants had previously been annotated in the available population-matched in-house database (approximately 800 exomes), and both variants were predicted to be damaging (Table 2).

To confirm the involvement of *DYNC2LI1* mutations in EvC and explore their prevalence in the disorder, 24 additional subjects with clinical features fully or partially overlapping EvC were screened for mutations in the entire coding sequence of *DYNC2LI1* by parallel sequencing. Besides *DYNC2LI1*, the panel included 67 genes that had been considered as disease gene candidates for EvC being either involved in primary ciliary dyskinesia or ciliopathies with skeletal involvement, and/or functionally related to the previously identified EvC genes. Biallelic variants in *DYNC2LI1* were identified in 1 family with 2 affected siblings (MGM03-0553 and MGM03-0552, family 2) with clinical diagnosis of EvC, and in a sporadic case (MGM14-1183, family 3) exhibiting a more severe phenotype (Figure 1 and Table 1). MGM03-0553 and MGM03-0552 were heterozygous for a maternally transmitted truncating variant (c.420delA, p.Val141*) and the previously identified missense change, c.662C>T (p.Thr221Ile), which was inherited from the father. MGM14-1183 was a compound heterozygote for a frameshift change (c.123_124insA, p.Gly42Argfs*12) and an intronic variant close to the exon 8 acceptor splice site (c.658-11delT). The variants, which were either rare or private, were validated by Sanger sequencing, and proved to co-segregate with disease (Table 2 and Figure S1). No gene with functionally relevant biallelic variants or de novo changes was identified in the other subjects.

**TABLE 1** Features in patients with biallelic *DYNC2LI1* mutations (present and previous studies) compared with those characterizing EvC and Jeune syndromes

| Clinical features | SC742D1 (family 1) | SC741D1 (family 1) | BL1304-12 (family 1) | MGM03-0553 (family 2) | MGM03-0552 (family 2) | EvC (families 1 and 2) | MGM14-1183 (family 3) |
|---|---|---|---|---|---|---|---|
| Age | 42 y | 31 y | 4 y | 23 y | 16 y | | 1 mo |
| Sex | Female | Female | Male | Male | Female | | Female |
| Cleft lip | + (median) | + (median notch) | + (unilateral) | + | + | + (5/5) | – |
| Oral frenula | + | + | + | – | + | + (4/5) | – |
| Hypodontia | + | + | + | + | – | + (4/5) | NA |
| Oral hamartoma | – | – | + (SB) | – | – | + (1/5) | – |
| Narrow thorax | + | + | + | + | + | + (5/5) | + |
| Cardiac defect | – | AVCD, CA | – | PDA | AC | + (3/5) | HLHS |
| Postaxial polydactyly, hands | + | + | + | + | + | + (5/5) | + |
| Postaxial polydactyly, feet | + | + | – | + | + | + (4/5) | + |
| Brachydactyly, hands | + | + | + | – | – | + (3/5) | – |
| Dysplastic nails | + | + | + | – | – | + (3/5) | – |
| Stature/length | 156 cm, 25th-50th centile | 158 cm, 25th-50th centile | 98 cm, 25th-50th centile | 174 cm, 25th-50th centile | 158 cm, 50$^{th}$ centile | – (*n* = 5) | 47 cm, 25th-50th centile |
| Limb shortening | + | + | + | + | – | + (4/5) | + |
| Kidney anomalies | – | – | + (HK) | – | – | + (1/5) | – |
| Hydrometrocolpos | + | – | NA | – | NA | + (1/3) | NK |
| Agenesis/hypoplasia of epiglottis | – | – | – | – | – | – (*n* = 5) | – |
| Liver disease | – | – | – | – | – | – (*n* = 5) | – |
| Craniosynostosis | – | – | – | – | – | – (*n* = 5) | – |
| Retinal dystrophy | – | – | – | – | – | – (*n* = 5) | – |
| Alive | + | + | + | + | + | 5/5 | – |
| *DYNC2LI1* mutations/ disease gene(s) | p.Met1?/p.Thr221Ile | | | p.Val141*/p.Thr221Ile | | *DYNC2LI1* | p.Gly42Argfs*12/c.658-11delT |

| | Taylor et al[25] | | | Kessler et al[21] | | | EvC | SRTD1/Jeune |
|---|---|---|---|---|---|---|---|---|
| **Clinical features** | **R01-013A** | **R07-628A** | **R03-303A** | **P1** | **P2** | **P3** | | |
| Cleft lip | – | – | – | + | + | + | + | - |
| Oral frenula | NK | NK | NK | + | – | NK | + | - |
| Hypodontia | NA | NA | NA | NA | – | NA | + | - |
| Oral hamartoma | NK | NK | NK | – | – | NK | + | - |
| Narrow thorax | + | + | + | + | + | + | + | + |
| Cardiac defect | – | – | – | AVCD | – | NK | + | - |
| Postaxial polydactyly, hands | + | + | + | + | + | + | + | +/– |

(*Continues*)

**TABLE 1** (Continued)

| Clinical features | Taylor et al[25] | | | Kessler et al[21] | | | EvC | SRTD1/Jeune |
|---|---|---|---|---|---|---|---|---|
| | R01-013A | R07-628A | R03-303A | P1 | P2 | P3 | | |
| Postaxial polydactyly, feet | + | + | + | + | − | + | + | - |
| Brachydactyly hands | + | + | + | + | + | NK | + | - |
| Dysplastic nails | NK | NK | NK | + | | NK | + | - |
| Stature/length | NK | NK | NK | 50 cm[a] | 53 cm[b] | NK | short | short |
| Limb shortening | + | + | + | NK | − | NK | + | + |
| Kidney anomaly | − | − | − | NK | − | NK | + | + |
| Hydrometrocolpos | NK | NK | NK | NK | − | NK | + | - |
| Agenesis/hypoplasia of epiglottis | NK | NK | NK | + | − | NK | + | + |
| Liver disease | − | − | − | − | − | − | - | + |
| Craniosynostosis | − | − | − | − | − | − | - | - |
| Retinal dystrophy | NK | NK | NK | NK | − | NK | - | + |
| Alive | − (ETP) | − (ETP) | − (ETP) | − | + | − (ETP) | | |
| *DYNC2LI1* mutations/ disease gene(s) | *DYNC2LI1*, c.993+1G>A p.Leu117Val | *DYNC2LI1*, p.Leu117Val p.Trp124* | *DYNC2LI1*, c.996+3A>G p.Glu335* | *DYNC2LI1*, p.Arg208* p.Thr221Ile | *DYNC2LI1* (NA)[c] | *DYNC2LI1* (NA)[c] | *EVC*, *EVC2*, *WDR35* | *DYNC2H1*, *IFT80*, *TTC21B*, *WDR19* |

Abbreviations: AC, aortic coarctation; AVCD, atrioventricular canal defect; CA, common atrium; EvC, Ellis-van Creveld syndrome; ETP, elective termination of pregnancy; HK, horseshoe kidneys; HLHS, hypoplastic left heart syndrome; NA, not applicable; NK, not known; PDA, patent ductus arteriosus, SB, sublingual; SRTDs, short-rib thoracic dysplasias; +, present; −, absent. SD, standard deviation.

[a] −0.17 SD. The newborn died at the age of 3 days.

[b] 0.59 SD (at birth).

[c] DNA from P2 (sibling of P1) and P3 (fetus after terminated pregnancy from the same family) was not available for molecular analysis.

**TABLE 2** *DYNC2LI1* mutations associated with Ellis-van Creveld syndrome and clinically related phenotypes

| Subject(s) | Family/reference | Genomic location (hg19) | cDNA (NM_001193464.1) | Exon | Amino acid change (NP_001180393.1) | CADD score[a] | rs_ID/ ClinVar | Total MAF (ExAC) | Predicted disruptive effect |
|---|---|---|---|---|---|---|---|---|---|
| BL1304-12, SC741D1 and SC742D1 | 1 | Chr2:44001279 | c.2T>C | 1 | p.Met1? | 20.9 | rs200859699 | <0.0001[b] | Translation |
| | | Chr2:44027984 | c.662C>T | 9 | p.Thr221Ile | 23.0 | rs886037860 | NA | Protein function |
| MGM03-0553 and MGM03-0552 | 2 | Chr2:44021693 | c.420delA | 6 | p.Val141* | 34.0 | rs770155116 | 0.00008[c] | Truncating |
| | | Chr2:44027984 | c.662C>T | 9 | p.Thr221Ile | 23.0 | rs886037860 | NA | Protein function |
| MGM14-1183 | 3 | Chr2: 44004034 | c.123_124insA | 2 | p.Gly42Argfs*12 | 28.6 | — | — | Truncating |
| | | Chr2: 44027969 | c.658-11delT | Intron 8 | — | 9.0 | rs752971070 | 0.00001[d] | Transcript processing |
| Subjects 1 to 3 | Kessler et al[21] | Chr2:44023899 | c.622C>T | 9 | p.Arg208* | 45.0 | rs745930390 | 0.00004[e] | Truncating |
| | | Chr2:44027984 | c.662C>T | 9 | p.Thr221Ile | 23.0 | rs886037860 | NA | Protein function |
| R01-013A | | Chr2:44032386 | c.996+1G>A | Intron 12 | — | 25.0 | rs374356079 | 0.00002[f] | Transcript processing |
| | | Chr2:44021624 | c.349C>G | 6 | p.Leu117Val | 25.6 | rs201948500 | 0.00003[g] | Protein function |
| R07-628A | Taylor et al[25] | Chr2:44021647 | c.372G>A | 6 | p.Trp124* | 38.0 | rs769975073 | 0.00004[h] | Truncating |
| | | Chr2:44021624 | c.349C>G | 6 | p.Leu117Val | 25.6 | rs201948500 | 0.00003[i] | Protein function |
| R03-303A | | Chr2:44036850 | c.1003G>T | 13 | p.Glu335* | 50.0 | rs879255655 | NA | Truncating |
| | | Chr2:44032388 | c.996+3A>G | Intron 12 | — | 13.6 | rs879255656 | NA | Transcript processing |

[a] v.1.3

[b] MAF = 0.00014 for the European (non-Finnish) population (9/64 078 alleles, occurring as heterozygous changes in all cases). Total alleles: 11 6266.

[c] MAF = 0.000015 for the European (non-Finnish) population (1/66 732 alleles). Total alleles: 12 1372.

[d] MAF = 0.000015 for the European (non-Finnish) population (1/65 254 alleles). Total alleles: 118 080.

[e] MAF = 0.00006 for the European (non-Finnish) population (4/66 670 alleles, occurring as heterozygous changes in all cases); MAF = 0.0001 for the African population (1/10 384 allele). Total alleles: 121 274.

[f] MAF = 0.00003 for the European (non-Finnish) population (2/66 486 alleles, occurring as heterozygous changes in both cases). Total alleles: 120 802.

[g] MAF = 0.000045 for the European (non-Finnish) population (3/66 642 alleles, occurring as heterozygous changes in all cases); MAF = 0.0001 for the African population (1/10 106 alleles). Total alleles: 120 948.

[h] MAF = 0.000075 for the European (non-Finnish) population (5/66 704 alleles, occurring as heterozygous changes in all cases). Total alleles: 120 164.

[i] MAF = 0.000045 for the European (non-Finnish) population (3/66 642 alleles, occurring as heterozygous changes in all cases); MAF = 0.0001 for the African population (1/10 106 alleles). Total alleles: 120 948.

To confirm pathogenicity of c.658-11delT, the functional impact of the variant on *DYNC2LI1* transcript processing was assessed. As biological material of subject MGM14-1183 was not available for the study, total RNA extracted from leukocytes of her parents was retro-transcribed and used to scan the entire *DYNC2LI1* coding sequence and 3′-UTR, demonstrating allele-specific transcript decay associated with the c.658-11delT variant. Sequencing of the *DYNC2LI1* cDNA in both parents, identified 2 informative SNPs that served to document loss of the maternal *DYNC2LI1* mRNA transcribed from the allele containing the splice site change, thus providing direct evidence for the disruptive impact of the variant on transcript processing (Table S3).

### 3.2 | Clinical features of *DYNC2LI1* mutation-positive subjects

Clinical data of the 6 affected subjects are summarized in Table 1, and are compared with the features characterizing EvC and Jeune syndrome.

As anticipated, affected members in families 1 and 2 had features within the clinical spectrum of EvC. In family 1, the phenotype of the 3 affected subjects was homogeneous. The 2 siblings, SC741D1 and SC742D1, had previously been clinically described.[22] The older sister, SC742D1, was 42-year-old and exhibited postaxial polydactyly of hands and left foot, brachydactyly, nail dysplasia, narrow thorax, median cleft of upper lip, oral frenula, and absence of 2 lower molars. Her 31-year-old sister, SC741D1, had signs of skeletal dysplasia (elongated and narrow thorax, short ribs, highly positioned short clavicles, congenital stenosis of the spinal canal with short pedicles, wedging of the vertebral bodies at the thoracolumbar junction, bilateral postaxial polydactyly of hands and feet), median notch of the upper lip, oral frenula, and absence of a molar. In addition, she had a partial atrioventricular canal defect with a common atrium. After surgical correction of polydactyly and cardiac defect, she appeared in good health with the exception of spinal disorders (disc herniation operated at 24 years, and multiple intraspongious herniations) and mild scoliosis; recently, she gave birth to a healthy child. The 4-year-old relative, BL1304-12, showed macrocephaly, central cleft lip, gingival frenula, hypodontia, narrow elongated thorax, mesomelic shortening of limbs, postaxial polydactyly of both hands, complete cutaneous syndactyly between the second and third toes, nail dysplasia, and horse-shoe kidneys. Of note, height was within the normal range in all affected members of the family.

Similarly, MGM03-0553 (family 2), a 23-year-old male subject, displayed short ribs and narrow thorax, short limbs, bilateral hand and feet polydactyly, aortic coarctation, supernumerary teeth, oral frenula, and stature within the normal range. His 16-year-old sister, MGM03-0552, had comparable features, including mild narrow thorax, short limbs, bilateral postaxial polydactyly of hands and feet, patent ductus arteriosus, hypodontia, central cleft lip, and normal stature.

Subject MGM14-1183 (family 3), a female child of healthy parents, showed a more severe condition, which was characterized by prenatal and postnatal evidence of short limbs, bilateral postaxial polydactyly of hands and feet, severely narrowed thorax, and congenital heart defect (hypoplastic left ventricle). The baby died in the first month of life due to cardiorespiratory complications.

## 4 | DISCUSSION

Here, we report on the identification of biallelic *DYNC2LI1* mutations as the molecular event underlying EvC in 5 affected subjects from 2 unrelated families, and confirmed the severity of the clinical phenotype resulting from loss of DYNC2LI1 function in a sixth individual.

*DYNC2LI1* is a widely expressed gene coding a component of the intraflagellar transport-related dynein-2 complex, a machinery mediating retrograde traffic along the cilium, and whose function is required for cilium assembly and function, including signal transduction.[23] This complex is composed of at least 5 components, including DYNC2H1 (MIM 603297), WDR34 (MIM 515633) and WDR60 (MIM 615462), whose coding genes are mutated in SRTDs with or without polydactyly.[24] Among these disorders, features differentiating the individual nosologic entities are the variable presence of craniofacial, ocular, renal, hepatic and cardiac malformations. Of note, the severity of the disease varies significantly among conditions, with a number of disorders, including SRTD15 (MIM 617088) and Jeune syndrome (MIM 208500), being usually lethal due to the profound respiratory insufficiency occurring in the neonatal age. By contrast, this complication generally does not occur in patients with EvC, who instead are distinctively characterized by extra-skeletal features, including cardiac, genitourinary and ectodermal anomalies most commonly.

Biallelic inactivating mutations in *DYNC2LI1* had previously been identified as the cause of SRTD15 with 3 reported fetuses from 3 unrelated families identified by prenatal ultrasound showing shortened long bones, postaxial polydactyly of the upper and lower extremities, a long narrow thorax with very short horizontal ribs, irregular metaphyseal borders with lateral spikes, without any significant involvement of other tissues and organs (Table 1).[25] Clinical features of these cases were considered to be similar to those occurring in SRTD3 (MIM 613091) and SRTD11 (MIM 615633), which are respectively caused by mutations in the *DYNC2H1* and *WDR34* genes.[26,27] Independently, compound heterozygous *DYNC2LI1* mutations have been reported in a single family with 3 affected siblings sharing features suggestive of Jeune syndrome and clinically overlapping EvC (Table 1).[21] In the present series, almost 5 of 6 affected individuals showed an apparent homogeneous, milder phenotype fitting EvC. Shared/recurrent features in these patients included congenital heart defect (particularly atrioventricular canal defect), median cleft lip with multiple oral frenula, and ectodermal defects, all of which are typical signs for EvC. In these subjects, chest narrowing was less evident compared with that occurring in patients with Jeune syndrome. Consistently, 4 subjects reached adulthood and none of them suffered respiratory complications during infancy. In addition, polydactyly and oral features, including oral fenula, cleft lip, hypodontia and abnormal teeth, and ectodermal defects, such as dysplastic nails, do not usually occur in Jeune syndrome.[24] Overall, the clinical phenotype of affected subjects from families 1 and 2 of this report strongly resembled EvC, and comparison between these subjects and *EVC/EVC2* mutation-positive EvC cases did not reveal any significant difference in clinical features except for the stature that was observed within the normal range in the 5 subjects with biallelic *DYNC2LI1* mutations. Remarkably, besides the occurrence of a truncating

mutation, the c.662C>T missense change was a common finding of patients sharing the EvC phenotype, including the cases previously reported by Kessler et al.[21] The nucleotide substitution affects a highly conserved threonine among vertebrate orthologues, and was suggested to have a disruptive impact on nucleoside triphosphate hydrolase function.[21] Overall, this finding suggests that compound heterozygosity for this specific missense change, which probably acts as a hypomorphic mutation, can result in a variable clinical spectrum having Jeune syndrome and EvC as recognized nosologic entities.

In conclusion, the present findings demonstrate that biallelic *DYNC2LI1* mutations account for a small proportion of EvC. Together with previously reported observations, these data also document that *DYNC2LI1* mutations are associated with a wide phenotypic spectrum, and suggest the presence of genotype-phenotype correlations, with the c.662C>T being specifically associated with a distinctive form of EvC.

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## Conflict of interest

The authors declare that they have no competing financial interests.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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