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Supplemental Data

Mutations in *SEC24D*, Encoding a Component of the COPII Machinery, Cause a Syndromic Form of Osteogenesis Imperfecta

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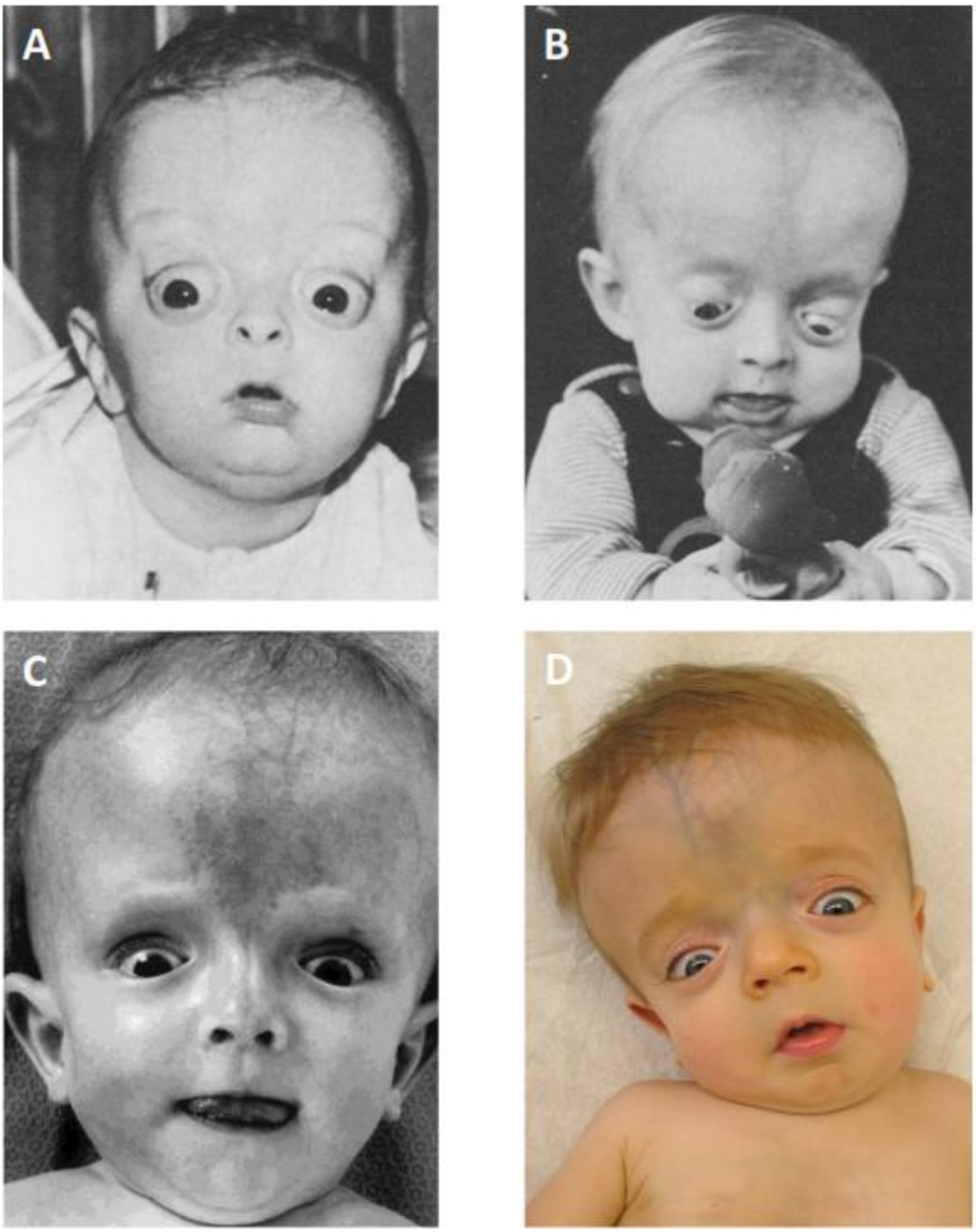
Figure S1. Comparison of the Facial Phenotype of Individuals with Cole-Carpenter Syndrome.



(A) Individual 1 of the original publication by Cole and Carpenter, 1986. (B) Individual 2 of the original publication by Cole and Carpenter.[1] (C) Individual published by Amor et al.[2] (D) Affected individual of family 1 at the age of 9 months.

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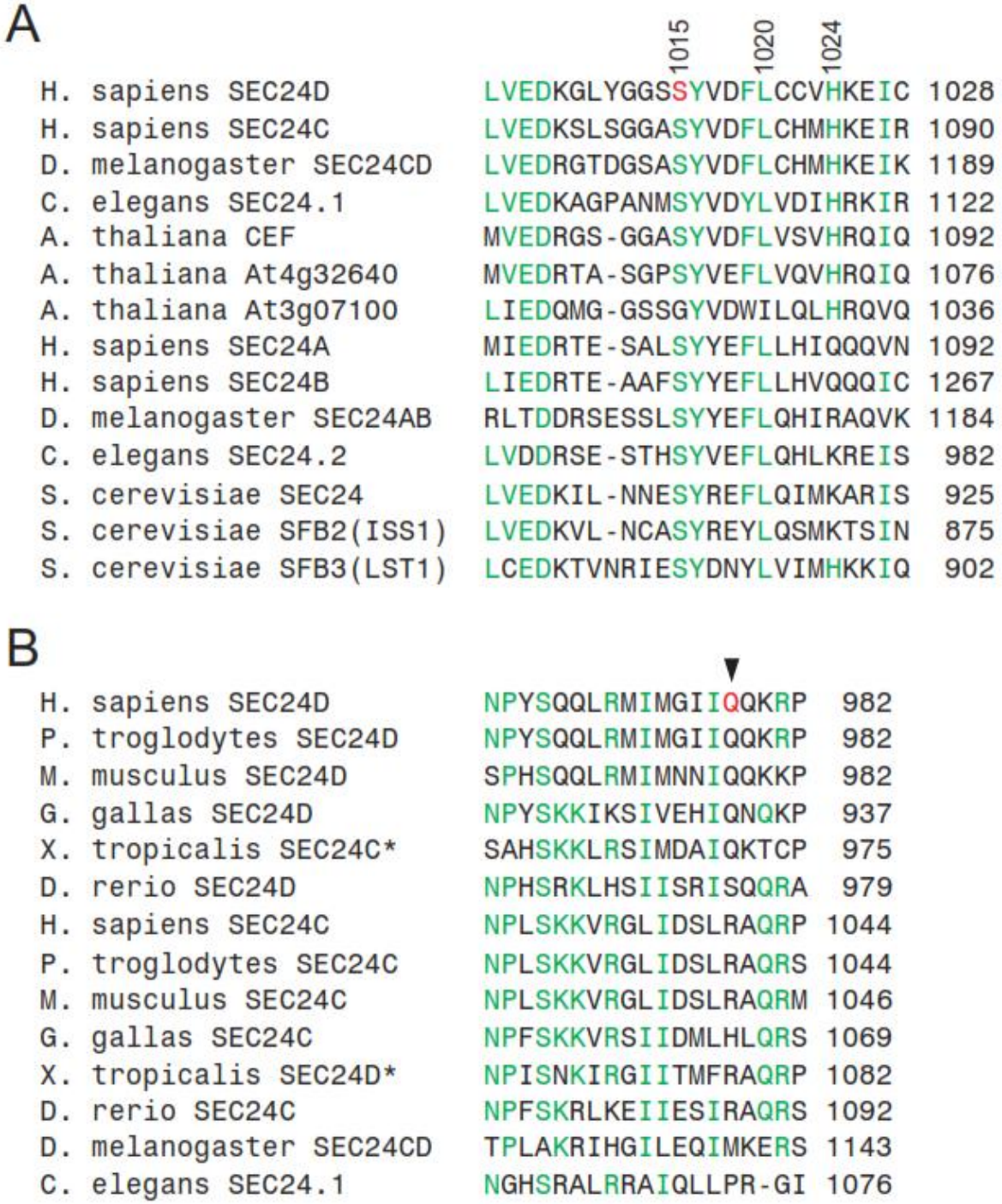
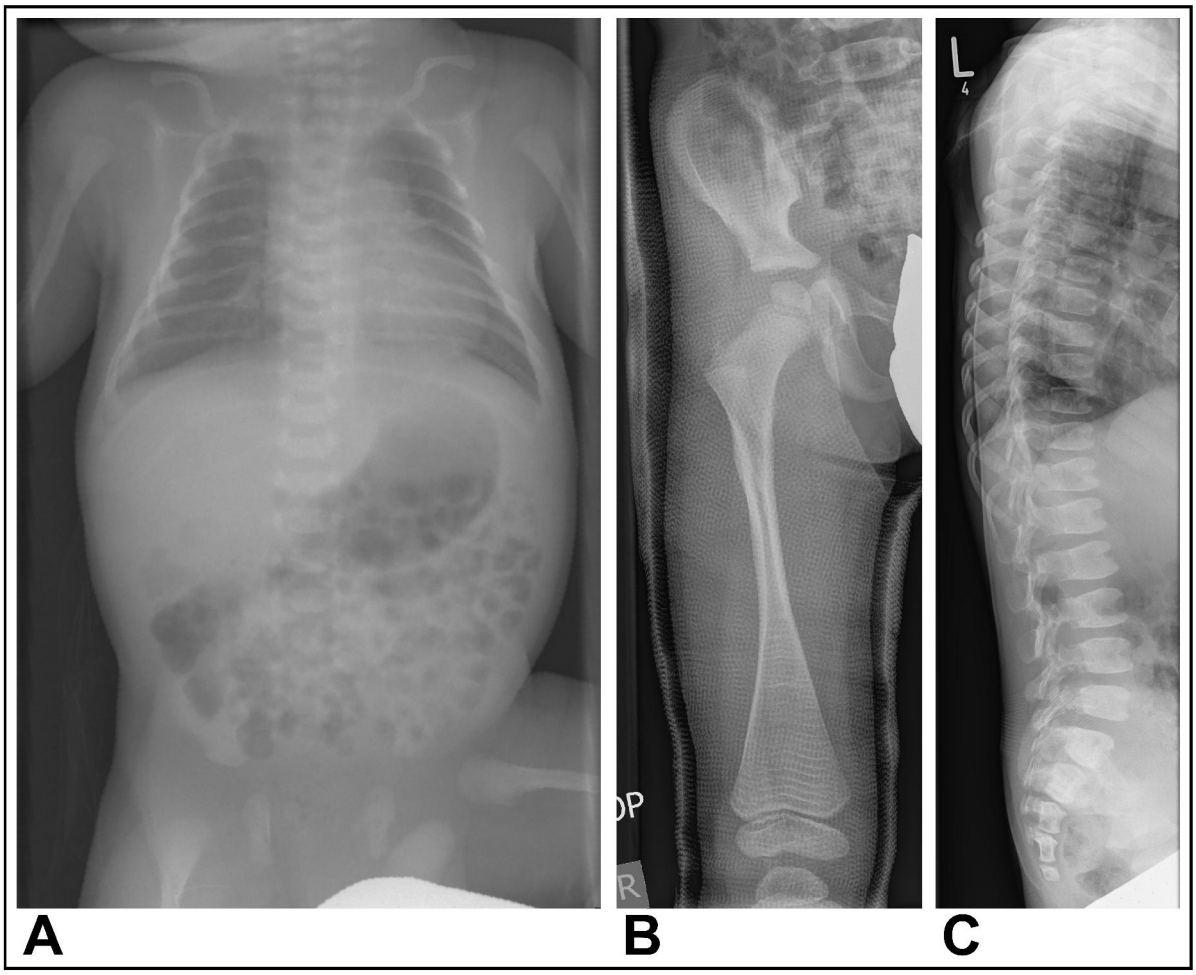
Figure S2. **SEC 24D Sequence Comparison.**



(A) Sequence comparison near Ser1015 (red) was performed by ClustalW2. Highly conserved residues were colored green. Following sequences were used for comparison: *Homo sapiens* SEC24A (O95486), SEC24B (O95487), SEC24C (P53992) and SEC24D (O94855); *Drosophila melanogaster* SEC24AB (A1Z813) and SEC24CD (M9PC99); *Caenorhabditis elegans* SEC24.1 (Q19371) and SEC24.2 (Q23368); *Arabidopsis thaliana* CEF (Q9M291), At3g07100 (Q9SFU0), At4g32640 (Q9M081); *Saccharomyces cerevisiae* SEC24 (P40482),

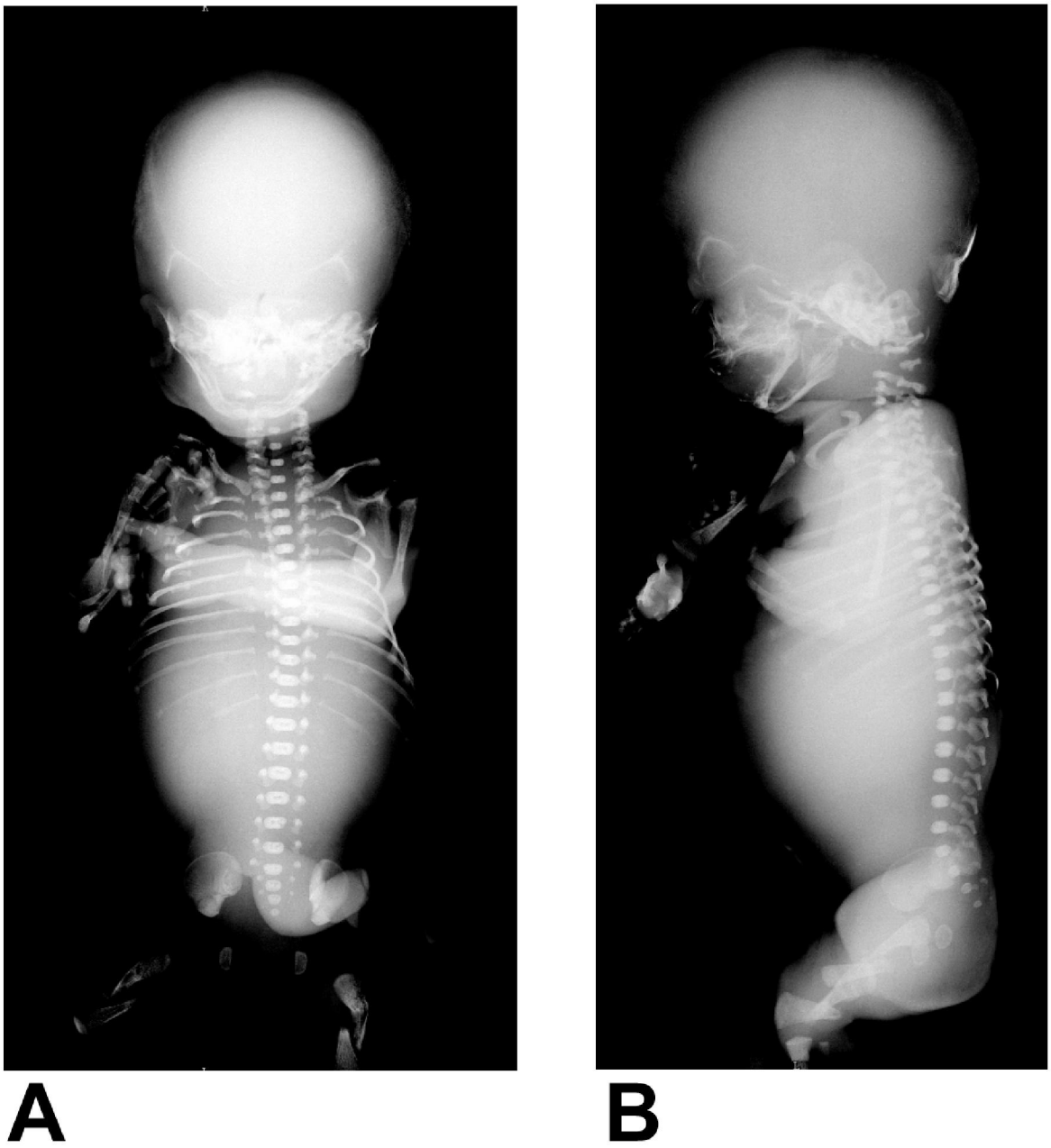
SFB3 (LST1, P38810) and SFB2 (ISS1, P53953). (B) SEC24C and SEC24D sequences were compared by ClustalW2. Highly conserved residues and Gln978 of SEC24D were colored green and red, respectively. The following sequences were used for comparison: *Homo sapiens* SEC24C (P53992) and SEC24D (O94855); *Pan troglodytes* SEC24C (H2Q241) and SEC24D (K7B5H4); *Mus musculus* SEC24C (G3X972) and SEC24D (Q6NXL1); *Gallus gallus* SEC24C (E1BUD8) and SEC24D (E1BSP8); *Xenopus tropicalis* SEC24C (F6ZIY6) and SEC24D (F6YFQ0); *Danio rerio* SEC24C (D5LHQ7) and SEC24D (F1R3A4); *Drosophila melanogaster* SEC24CD (M9PC99); *Caenorhabditis elegans* SEC24.1 (Q19371). Note that a phylogenic analysis of SEC24s indicates that SEC24C and SEC24D of *X. tropicalis* (asterisks) are actually close to SEC24D and SEC24C of other species, respectively (data not shown).

Figure S3. **Radiographic Findings of the Affected Individual from Family 1 at Different Ages.**



(A) Postnatal skeletal survey of thorax and abdomen demonstrating thin ribs and callus formation on the right thoracic side, normal clavicles and flattened vertebrae. (B) A.p. radiograph of the right femur and hip at the age of 5.7 years presenting zebralines after multiple cycles of intravenous bisphosphonate treatment, broadened distal epiphysis of the femur with an increased metaphyseal index (0.71) [3;4] as a sign of decreased remodelling after bisphosphonate treatment. The iliac wing is high and narrow. (C) Lateral radiograph of the spine showing flattened vertebrae with anterior notches and posterior wedging as well as mild kyphosis in the thoracic part.

Figure S4. Radiographic Findings of One Affected Fetus After Termination of Pregnancy in the 23rd Week of Gestation.



(A) Post mortem anteriorposterior skeletal survey of the 23rd week´s fetus demonstrating delayed skeletal development (reduced frontal calvarial ossification, no pubic bones are seen.[5] The fetus presents with thin ribs but without severe thoracic hypoplasia and callus formation, with short, fractured, and mildly bowed long bones, normal clavicles, and normal ossified vertebrae. Ossification of the calvarium is clearly reduced in contrast to the skull base. Remarkable is the discrepancy between skull ossification and ossification of the rest of the skeleton, a pattern which was also observed in the affected individual of family 1. (B) Post mortem lateral skeletal survey of the fetus confirming the discrepancy between skull base and calvarial ossification. The long bones of the upper extremity and vertebrae are well ossified and normal developed.

Table S1. Clinical Features of the Affected Individual from Family 1.

Clinical Findings	Affected Individual
Disease severity	moderate
Age at first presentation	7 years 4 months
Confirmed prenatal fractures	yes (both humeri and 2 rips)
Birth length / birth weight / head circumference at birth	47 cm /2800g/ 32cm all < - 2 SD
APGAR	7/7/9
Fractures before bisphosphonate treatment	Yes
Age at start of bisphosphonate treatment	2 months (i.v. pamidronate) (Glorieux et al. 1998)
Number of lower extremity fractures since birth	6
Number of upper extremity fractures since birth	3
Weight at visit kg/BMI (SD)	15.0/14.6
Length at visit cm/SD	101.5/- 4.5
Head circumference at visit (cm)	55.5
Sitting height at visit (cm)	78.5
Arm span at visit (cm)	99.0
Color of sclera	normal
Dentinogenesis imperfecta	no
Primary dentition	normal
Secondary dentition	mild misalignment of teeth
Hypermobility of joints	no
Spine	flat vertebrae (posterior wedging, anterior notches)
Pelvis	small hypoplastic acetabular roof; high and narrow iliac wings
Scoliosis	no
Chest deformities	pectus excavatum
Severe bowing of extremities	no
Hearing impairment	no
Cardiac ultrasound	lower normal
Intelligence	normal[a]
Mobility at visit (BAMF (Cintas et al. 2003))	9
Gross motor function measurement test (GMFM) (Russell et al. 1989)	96.6%
1 Minute walking test	120 m (without aids)
6 Minute walking test	520 m (without aids)
Deoxy-Pyridinolin-Diphosphat (marker for osteoclastic activity)	decreased
Bone density, DXA whole body without head (g/cm^2)/(z-score)	0.464 /- 2.0
Bone density, DXA ap spine (g/cm^2)/(z-score)	0.520 /- 2.0

[a] Tested with "Hannover-Wechsler Intelligence test for preschool kids" and " Kaufmann Assessment Battery for children"

Table S2. Genes Tested by Sanger Sequencing in Individuals with the Clinical Diagnosis of Cole-Carpenter Syndrome.

Family 1 *(Index patient tested)*	*MIM*	*Family 2* *(one of two fetuses tested)*	*MIM*
COL1A1	*120150*	*COL1A1*	*120150*
COL1A2	*120160*	*COL1A2*	*120160*
ALPL	*171760*	*ALPL*	*171760*
CRTAP	*605497*	*BMP1*	*112264*
FKBP10	*607063*	*CRTAP*	*605497*
LEPRE1	*610339*	*FKBP10*	*607063*
PPIB	*123841*	*LEPRE1*	*610339*
SERPINF1	*172860*	*PPIB*	*123841*
SERPINH1	*600943*	*SERPINF1*	*172860*
SP7	*606633*	*SERPINH1*	*600943*
		SP7	*606633*

Table S3. Candidate Genes and Variants Identified by Whole-Exome Sequencing in Family 1.

Position	*Ref*	*Alt*	*Gene*	*OMIM*	*Transcript*	*cDNA*	*protein*
chr4:119644725	***G***	***A***	***SEC24D***	***607186***	***NM_014822***	***c.C3044T***	***p.S1015F***
chr4:119736666	***G***	***A***	***SEC24D***	***607186***	***NM_014822***	***c.C613T***	***p.Q205X***
chr6:137325901	*TAA*	*TA*	*IL20RA*	*605620*	*NM_014432*	*IVS*	*altered splicing*
chr7:139611038	*G*	*A*	*TBXAS1*	*274180*	*NM_030984*	*c.G254A*	*p.R85H*
chr7:139715531	*G*	*A*	*TBXAS1*	*274180*	*NM_030984*	*c.G1238A*	*p.R413Q*
chr11:1275486	*G*	*A*	*MUC5B*	*600770*	*NM_002458*	*c.G15382A*	*p.A5128T*
chr11:1270475	*C*	*T*	*MUC5B*	*600770*	*NM_002458*	*c.C12365T*	*p.T4122M*
chr13:76427413	***C***	***A***	***LMO7***	***604362***	***NM_015842***	***c.C3851A***	***p.P1284H***
chr13:76429387	***T***	***C***	***LMO7***	***604362***	***NM_005358***	***c.3816-9T>C***	***altered splicing***

Filtering for deleterious variants was performed under an autosomal recessive disease model as described in the main text. The variant in *IL20RA* was homozygous. Candidate genes which were also identified in family 2 are marked in bold.

Table S4. Candidate Genes and Variants Identified by Whole-Exome Sequencing in Family 2.

Position	*Ref*	*Alt*	*Gene*	*OMIM*	*Transcript*	*cDNA*	*protein*
chr1:144873964	*G*	*A*	*PDE4DIP*	*608117*	*NM_001198832*	*c.C4861T*	*p.Q1621X*
chr1:144857691	*C*	*T*	*PDE4DIP*	*608117*	*NM_001198832*	*c.G6045A*	*p.M2015I*
chr1:156268808	*A*	*G*	*VHLL*	*---*	*NM_001004319*	*c.T173C*	*p.I58T*
chr1:156268845	*G*	*A*	*VHLL*	*---*	*NM_001004319*	*c.C136T*	*p.R46C*
chr2:61719303	*C*	*A*	*XPO1*	*602559*	*NM_003400*	*c.G1754T*	*p.C585F*
chr2:61719303	*C*	*A*	*XPO1*	*602559*	*NM_003400*	*c.G1754T*	*p.C585F*
chr2:196741296	*A*	*T*	*DNAH7*	*610061*	*NM_018897*	*c.T6089A*	*p.V2030D*
chr2:196756535	*GAA*	*GA*	*DNAH7*	*610061*			*---*
chr3:9786696	*A*	*C*	*BRPF1*	*602410*	*NM_004634*	*c.A2907C*	*p.L969F*
chr3:9785283	*T*	*G*	*BRPF1*	*602410*	*NM_004634*	*c.T2315G*	*p.V772G*
chr4:119649741	***T***	***G***	***SEC24D***	***607186***	***NM_014822***	***c.A2933C***	***p.Q978P***
chr4:119644725	***G***	***A***	***SEC24D***	***607186***	***NM_014822***	***c.C3044T***	***p.S1015F***
chr5:90101200	*G*	*A*	*GPR98*	*602851*	*NM_032119*	*c.G14761A*	*p.A4921T*
chr5:89933700	*C*	*G*	*GPR98*	*602851*	*NM_032119*	*c.C2175G*	*p.I725M*
chr6:90494849	*C*	*T*	*MDN1*	*---*	*NM_014611*	*c.1335-4G>A*	*splicing*
chr6:90405631	*C*	*T*	*MDN1*	*---*	*NM_014611*	*c.G9464A*	*p.R3155Q*
chr11:65402768	*T*	*G*	*PCNXL3*	*---*	*NM_032223*	*c.T5033G*	*p.V1678G*
chr11:65403969	*C*	*T*	*PCNXL3*	*---*	*NM_032223*	*c.C5701T*	*p.R1901W*
chr12:644443	*A*	*C*	*B4GALNT3*	*612220*	*NM_173593*	*c.273+8A>C*	
chr12:644374	*G*	*A*	*B4GALNT3*	*612220*	*NM_173593*	*c.G212A*	*p.R71K*
chr13:76430676	***C***	***T***	***LMO7***	***604362***	***NM_015842***	***c.C3997T***	***p.P1333S***
chr13:76397701	***A***	***G***	***LMO7***	***604362***	***NM_015842***	***c.A1942G***	***p.M648V***
chr14:75134206	*G*	*C*	*KIAA0317*	*615380*	*NM_001039479*	*c.C2006G*	*p.A669G*
chr14:75134206	*G*	*C*	*KIAA0317*	*615380*	*NM_001039479*	*c.C2006G*	*p.A669G*
chr17:21318731	*C*	*T*	*KCNJ12, KCNJ18*	*602323*	*NM_021012*	*c.C77T*	*p.S26L*
chr17:21318896	*G*	*A*	*KCNJ12, KCNJ18*	*602323*	*NM_021012*	*c.G242A*	*p.R81Q*
chr17:39635147	*C*	*T*	*KRT35*	*602764*	*NM_002280*	*c.G812A*	*p.C271Y*
chr17:39636977	*G*	*C*	*KRT35*	*602764*	*NM_002280*	*c.C373G*	*p.L125V*
chr17:73569700	*C*	*G*	*LLGL2*	*---*	*NM_004524*	*c.C2864G*	*p.P955R*
chr17:73569700	*C*	*G*	*LLGL2*	*---*	*NM_004524*	*c.C2864G*	*p.P955R*
chr17:26694784	*C*	*A*	*VTN*	*193190*	*NM_000638*	*c.G1276T*	*p.V426L*
chr17:26694784	*C*	*A*	*VTN*	*193190*	*NM_000638*	*c.G1276T*	*p.V426L*
chr19:9066140	*T*	*G*	*MUC16*	*606154*	*NM_024690*	*c.A21306C*	*p.L7102F*
chr19:9064309	*AGGGG*	*A*	*MUC16*	*606154*	*NM_024690*	*c.23133_23137T*	*---*

Filtering for deleterious variants was performed as described in the main text. We assumed autosomal recessive inheritance of the phenotype. Note that the non-affected parents and not the affected fetuses were analyzed, which increased the number of candidates. We searched for genes harboring potential mutations in a heterozygous state in both the mother´s and the father´s dataset. Candidate genes which were also identified in family 1 are marked in bold. The genotype of the affected fetuses was only determined for the variants in *SEC24D* and *LMO7*.

Table S5. Detailed Comparison of Clinical and Radiological Findings in the Affected Individual from Family 1 and in the Six Published Individuals with Cole-Carpenter Syndrome.

	This report, index patient from family 1	***Cole and Carpenter [1] pat. 1***	***Cole and Carpenter [1] pat.2***	***Marwaha et al. [8]***	***Stopfer et al. [9]***	***McDermot et al. [7]***	***Amor et al. [2]***
Birth parameters	Decreased	Normal	Normal	Decreased	N. d.	Decreased	Decreased
Long bone deformities	Yes	Yes	Yes	Yes	yes	N. d.	Yes
Fractures first two years	Yes	Yes	Yes	N. d.	yes	Yes	Yes
Metaphyseal abnormalities	Yes	Yes	Yes	N. d.	yes	Normal	Yes
Spondylodysplastic changes	Yes	Yes	Yes	Yes	N. d.	normal	Yes
Disturbed ossification of the skull[a]	Yes	Yes	Yes	Yes	Yes	N. d.	Yes
Increased head circumference	Yes	Yes	N. d.	Yes	N. d.	No	Yes
Skull erosions	Yes	Yes	N. d.	N. d.	N. d.	N. d.	No
Craniosynostosis	Yes	Yes	Yes	Yes	Yes	No	Yes
Hydrocephalus	Yes	Yes	Yes	Yes	N. d.	Yes	No
VP-Shunt	No	Yes	No	Yes	N. d.	No	No
Increased fontanella	Yes	N. d.	Yes	Yes	N. d.	N. d.	No
Wide sutures	Yes	N. d.	Yes, at age 2 months	Yes	N. d.	N. d.	Yes
Wormian bones	Yes	Yes	No	no	N. d.	-	Yes
Frontal bossing	Yes	Yes	Yes	N. d.	Yes	Yes	Yes
Occular proptosis	Yes	Yes	Yes	Yes	Yes	No	Yes
Short stature	Yes	Yes	Yes	Yes	N. d.	Yes	Yes
Mid-facial hypoplasia	Yes	Yes	Yes	N. d.	N. d.	N. d.	N. d.
Micrognathia	Yes	Yes	Yes	Yes	N. d.	N. d.	Yes
Fine motor skills	Normal	Normal	Normal	N.d.	N. d.	Delayed	Delayed
Normal speech	Yes	Yes	Yes	Yes	N. d.	Delayed	Yes
High voice	Yes	Yes	Yes	Yes	N. d.	N. d.	Yes
Blue sclera	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Walking impaired	Yes	Yes	N. d.	N. d.	N. d.	Yes	Yes
Muscle hypotonia	No	N. d.	Yes	Yes		Yes	Yes
Teeth abnormalities (Colour / eruption)	No, only misalignment	Yes	N. d.	N. d.	N. d.	Yes	Yes
Reason for referral	Fractures	Fractures	Hypotonia, failure to thrive	Increased head circum-ference at age 2 weeks	N. d.	Fractures	Growth failure
Ear abnormalities	Yes	N. d.	N. d.	N. d.	Yes	N. d.	N. d.
Osteopenia	Yes	Yes	Yes	Yes	Yes	Yes	Yes

N. d. = not described

[a]This term is used as a general term for craniosynostosis and / or skull ossification defects.

Supplemental Note: Clinical Report of the Affected Individual from Family 1 with Suspected Diagnosis of Cole-Carpenter Syndrome.

The boy was the second child of unrelated and healthy parents. His sister was also healthy. During pregnancy, an oligohydramnion was diagnosed. The individual was born after 41 weeks of gestation by vaginal delivery. The birth weight was 2800 g (< 3rd centile), length was 47 cm (< 3rd centile) and head circumference 32,5 cm (< 3rd centile). The following dysmorphic features were noted postnatally: turricephalus, exophthalmus, down-slanting palpebral fissures, an angular root of the nose, retrognathia, and gaping fontanelles. Ophthalmological analysis was normal. Sonographical and X-ray analysis confirmed new as well as older fractures of the right clavicula, multiple ribs, and both radii, and documented extensive ossification defects of the cranium and craniosynostosis. The presence of multiple fractures lead to Osteogenesis imperfecta as a putative diagnosis, and a treatment with cyclical application of pamidronate, a biphosphonate, was initiated. The affected individual´s weight with 8 month was 5.2 kg (< 3rd centile), and his head circumference was 43.5 cm (> 3rd centile). At the age of 9 months, his height was 62.2 cm (0.5 cm < 3rd centile). He tried to stand up, and there were no signs of neurodevelopmental delay. The boy´s facial features at this age were described as follows: He had a wide fontanelle, a triangle shape of the face with a receding forehead, hypertelorism, proptosis of the eyes, “sunset” eyes, mid-face hypoplasia, a high palatine, microretrognathia and a slight dysplasia of the right concha. At the age of 15 months, the boy was seen again with a weight of 6140 g (< 3rd centile) and a length of 67 cm (< 3rd centile). The last visit prior to the recruitment for the genetic study was at the age of 4 4/12 years, when the affected individual presented with a weight of 11300 g (< 3rd centile), a length of 87 cm (< 3rd centile) and a head circumference of 54 cm (>97 centile). His motor development was delayed, possibly due to recurrent fractures of the extremities, but he was now able to walk, to run, and to climb stairs. He presented with a good general condition and normal mental development. A cranial CT scan documented dilated ventricles and subarachnoid spaces, in addition to the skull ossification defects. Brain parenchyma was normal. Two month later, a nasolacrimal duct stenosis was operated.

Supplemental References

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