Supplementary data – Materials and methods

In a subset of 50 individuals, molecular analysis included:

Variant analysis of ciliopathy patients.

Full ethics approval was obtained by the Northern and Yorkshire Research Ethics Committee (UK), the University of Michigan (US), Necker Hospital (France), and Duke University Medical Center (US), and informed written consent was obtained from patients and their relatives. DNA was extracted from whole blood or fetal tissue using standard techniques. The DNA of children with EVC was screened for variants using different custom Agilent SureSelect Target Enrichment libraries which captures 1/ 5.3 Mb covering 32,146 exons of 1,666 genes selected form cilia databases or 2/ 0,9 Mb Ciliopathy diagnostic panel covering 138 genes. Segregation analysis of rare candidate variants was performed by Sanger sequencing.

Customized Microarray

A customized 60K oligonucleotide microarray (Agilent Technologies Santa Clara, CA, USA) was used for this study according to manufacturer's recommendations. The microarray was spotted using 60,000 oligonucleotides corresponding to sequences across the whole genome (60,000 probes with a space of 60 kb between 2 consecutive probes). The *EVC* locus was enriched in oligonucleotides with an average distance between two probes of 2 kb in order to improve the detection of intragenic copy number variants. Genomic positions are relative to human genome Build NCBI38/hg38.

Targeted resequencing by NGS (capture by hybridization approach)

Genomic DNA was extracted from leucocytes. Illumina compatible barecoded genomic DNA libraries were constructed according to the manufacturer's sample preparation protocol (Ovation Ultralow V2, Nugen Technologies). Briefly, 400 ng to 3 µg of each patient's genomic DNA were mechanically fragmented to a median size of 200 bp using a Covaris. 100 ng of double strand fragmented DNA was end-repaired and adaptors containing barecodes were ligated to the repaired ends (one specific pair of barecode per patient). Ligated DNA fragments were PCR amplified to get precapture barecoded libraries that are pooled at equimolar concentrations. The capture process was performed using the SureSelect reagents (Agilent), 750 ng of this pool of precapture libraries and home-made biotinylated probes (similar method used by Benyelles M et coll., EMBO Mol Med. 2019; Venot Q et coll., Nature 2018 ; Rosain J et coll., J Clin Immunol. 2018 ; Vincent QB et coll., PLoS Negl Trop Dis. 2018 ; Grandin V et coll., Hum Mutat. 2017)

The biotinylated single strand DNA probes were designed and prepared to cover a 342,8 kb chromosomal region including the *EVC* and *EVC2* genes on chromosome 4 (chr4: 5,557,465-5,900,299, according to the GRCh38.p12 assembly of the human reference genome).

During the capture process, barecoded libraries molecules complementary to the biotinylated beads were retained by streptavidine coated magnetic beads on a magnet and PCR amplified to generate a final pool of postcapture libraries covering the targeted genomic regions. Pools of these final libraries were prepared and sequenced on an Illumina HiSeq2500 (Paired-End sequencing 130+130, production of ~20 millions of clusters per sample).

After demultiplexing, sequences were aligned to the reference human genome hg19 using the Burrows-Wheeler Aligner (Li and Durbin, 2010). The mean depth of coverage per sample was >=1000X to enable more accurate Copy Number Variant Analysis.

Downstream processing was carried out with the Genome Analysis Toolkit (GATK), SAMtools and Picard, following documented best practices (http://www.broadinstitute.org/gatk/guide/topic?name=best-practices). Variant calls were made with the GATK Unified Genotyper. The annotation process is based on the latest release of the Ensembl database. Variants were annotated and analyzed and prioritized using the Polyweb/PolyDiag software interface designed by the Bioinformatics platform of University Paris Descartes.

MLPA

The confirmation of deletions or duplications in the *EVC* and *EVC2* genes was performed by using the MRC-Holland's kit: SALSA MLPA Probemix P456 *EVC EVC2*.

To analyze MLPA data, we used Coffalyser, net software (freeware) developed by MRC-Holland.

Exome sequencing and data analysis

Exome capture was performed with the the Sure Select Human All Exon kit (Agilent Technologies). Agilent Sure Select Human All Exon (58 Mb, V6) libraries were prepared from 3 µg of genomic DNA sheared with an Ultrasonicator (Covaris) as recommended by the manufacturer. Barcoded exome libraries were pooled and sequenced with a HiSeq2500 system (Illumina), generating paired-end reads. After demultiplexing, sequences were mapped on the human genome reference (NCBI

build 37, hg19 version) with BWA. The mean depth of coverage obtained for the four proband's exome libraries was >150X with > =97% and >=94% of the targeted exonic bases covered at least by respectively 15 and 30 independent sequencing reads (>=97% at 15X >=96% at 30X). Variant calling was carried out with the Genome Analysis Toolkit (GATK), SAMtools, and Picard tools. Single-nucleotide variants were called with GATK Unified Genotyper, whereas indel calls were made with the GATK IndelGenotyper_v2. All variants with a read coverage %23 and a Phred-scaled quality %20 were filtered out. All the variants were annotated and filtered with PolyWeb, an in-house-developed annotation software.

Supplemental Table 1: Variants identified in our cohort in *EVC* (NM_153717.2), *EVC2* (NM_147127.4), *DYNC2LI1* (NM_001348913.1), *DYNC2H1* (NM_001080463.1), *PRKACB* (NM_002731.3) and *SMO* (NM_005631.4)

Families	Cs	No. Of affected cases	Array CGH 180K customized	Gene	SNVs						Status	CNVs	Inheritance
					Location	cDNA variant	Genomic coordinate (chr4)	Protein	Effect	ACMG classification			
1	unknown	1	No	EVC	Ex12	c.1715C>G	g.5785430C>G	p.Ser572*	NS	5 (PVS1, PM2, PM3, PP3, PP4)	ho		?
2	Yes	1	No	EVC	Ex9	c.1228G>T	g.5754692G>T	p.Glu410*	NS	5 (PVS1, PM2, PP4, BP4)	ho		paternal maternal
3	Yes	1	No	EVC	Ex9	c.1228G>T	g.5754692G>T	p.Glu410*	NS	5 (PVS1, PM2, PP4, BP4)	ho		paternal maternal
4	No	1 fetus	No	EVC	Ex7	c.873dupT	g.5747002dupT	p.Glu292*	NS	5 (PVS1, PM2, PM3, PP3, PP4, PP5)	he		?
					Ex15	c.2221G>T	g.5800436G>T	p.Glu741*	NS	5 (PVS1, PM2, PM3, PP3, PP4)	he		?
27	No	1 fetus	No	EVC	Ex10	c.1412_1413delGA	g.5755605delGA	p.Arg471Lysfs*5	FS	5 (PVS1, PM2, PP3, PP4)	he		paternal
					In6	c.801+30C>G	g.5743571C>G	?	Non coding variant	3 (PM2, PM3, PP4, BP4)	he		maternal
28	No	1 fetus	No	EVC	Ex6	c.752dupA	g.5743488dupA	p.Lys252Glufs*4	FS	5 (PVS1, PM2, PP3, PP4, PP5)	ho		paternal maternal
29	Yes	1	No	EVC	In7	c.940-150T>G	g.5749725T>G	?	Non coding variant	3 (PM2, PP4, BP4)	ho		?
30	Yes	1 fetus	No	EVC	In13	c.1887-5_1904del	g.5798740delTC…TG (23bp)	?	Splice junction loss variant	5 (PVS1, PM2, PP3, PP4, PP5)	ho		paternal maternal
5	No	2 fetus	No	EVC2	Ex1	c.142_152del	g.5710089delTGGGGATCCCG	p.Arg48Glyfs*4	FS	5 (PVS1, PM2, PP4)	he		paternal
					In21	c.3659+2T>C	g.5566983A>G	?	Splicing variant	5 (PVS1, PM2, PM3, PP3, PP4, PP5)	he		maternal
6	Yes	1 fetus	No	EVC2	Ex10	c.1195C>T	g.5642516G>A	p.Arg399*	NS	5 (PVS1, PM2, PM3, PP3, PP4, PP5)	ho		paternal maternal
7	No	1 fetus	No	EVC2	Ex5	c.653delT	g.5690937delA	p.Val1218fs*12	FS	5 (PVS1, PM2, PP4)	he		?
					Ex6	c.788C>A	g.5687125G>T	p.Ala263Asp	MS	4 (PM2, PM3, PP3, PP4, BP1)	he		?
8	No	2 fetus	Yes	EVC2	Ex15	c.2656G>T	g.5620255C>A	p.Glu886*	NS	5 (PVS1, PM2, PM3, PP3, PP4)	he		paternal
					Ex1	c.142_151del	g.5710090delGGGGATCCCG	p.Asp49Trpfs*9	NS	5 (PVS1, PM2, PM3, PP4, PP5)	he		maternal
31	Yes	1 fetus	No	EVC2	Ex20	c.3533_3546del	g.5570182delCA…CT (14bp)	p.Glu1178Glyfs*82	FS	5 (PVS1, PM2, PM3, PP3, PP4)	ho		paternal maternal
32	Yes	1 fetus	No	EVC2	Ex10	c.1330C>T	g.5642381G>A	p.Gln444*	NS	5 (PVS1, PM2, PM3, PP3, PP4)	ho		?
33	Yes	1 fetus	No	EVC2	In3	c.451-2A>G	g.5693062T>C	?	Splicing variant	5 (PVS1, PM2, PM3, PP3, PP4, PP5)	ho		?
34	No	1	No	EVC2	Ex3	c.437_438dup	g.5696074dupTA	p.Thr147*	NS	5 (PVS1, PM2, PP3, PP4)	he		maternal
					Ex17	c.2885delG	g.5586522delC	p.Gly962Alafs*17	FS	5 (PVS1, PM2, PM3, PP4)	he		paternal
35	Yes	1 fetus	No	EVC2	Ex2	c.264C>A	g.5699339G>T	p.Cys88*	NS	5 (PVS1, PM2, PM3, PP3, PP5)	ho		paternal maternal
36	Yes	1 fetus	No	EVC2	Ex17	c.2995C>G	g.5586412G>C	p.Leu999Val	MS	3 (PM2, PP3, PP4, BP1)	ho		paternal maternal
37	No	1	No	EVC2	Ex11	c.1541_1542delTC	g.5633688delGA	p.Leu514Argfs*22	FS	5 (PVS1, PM2, PM3, PP3, PP4, PP5)	he		paternal
					Ex18	c.3194delA	g.5578045delT	p.Asp1065Valfs*14	FS	5 (PVS1, PM2, PM3, PP4)	he		maternal
38	No	1	No	EVC2	Ex14	c.2476C>T	g.5624289G>A	p.Arg826*	NS	5 (PVS1, PM2, PM3, PP3, PP4, PP5)	he		paternal
					Ex20	c.3405_3411del	g.5570317delGGCCCCG	p.Gly1136Argfs*6	FS	5 (PVS1, PM2, PM3, PP3, PP4, PP5)	he		maternal
39	Yes	1 fetus	No	EVC2	Ex2	c.264C>A	g.5699339G>T	p.Cys88*	NS	5 (PVS1, PM2, PM3, PP3, PP4, PP5)	ho		paternal maternal

9	No	1 fetus	No	EVC2	In21	c.3659+2T>C	g.5566983A>G	?	Splicing variant	5 (PVS1, PM2, PP3, PP4, PP5)	he		maternal
											he	Deletion of exons 6 and 7 (MLPA)	paternal
10	Yes	1	Yes	EVC2							ho	arr[hg38] 4p16.2(5637778x2,5639292-5640893x1,5643966x2) (ex10)	paternal maternal
11	No	1 fetus	Yes	EVC	In17	c.2562-1G>C	g.5809927G>C	?	Splicing variant	5 (PVS1, PM2, PP3, PP4)	he		paternal
											he	arr[hg38] 4p16.2(5726379x2,5729225-5777675x1,5781917x2)	maternal
12	No	1	Yes	EVC	Ex7	c.873dupT	g.5747002dupT	p.Glu292*	NS	5 (PVS1, PM2, PP3, PP4, PP5)	he		paternal
											he	arr[hg38] 4p16.2(5726379x2,5729225-5777675x1,5781917x2)	maternal
13	No	1 fetus	Yes	EVC	Ex6	c.752dupA	g.5743488dupA	p.Lys252Glufs*4	FS	5 (PVS1, PM2, PP3, PP4, PP5)	he		paternal
											he	arr[hg38] 4p16.2(5726379x2,5729225-5777675x1,5781917x2)	maternal
14	No	1 fetus	Yes	EVC	In6	c.801+1G>A	g.5743542G>A	?	Splicing variant	5 (PVS1, PM2, PP3, PP4)	he		paternal
											he	arr[hg38] 4p16.2(5726379x2,5729225-5777675x1,5781917x2)	maternal
15	Yes	1 fetus	Yes	EVC	Ex10	c.1324C>T	g.5755520C>T	p.Gln442*	NS	5 (PVS1, PM2, PP3, PP4)	he		paternal
											he	arr[hg38] 4p16.2(5726379x2,5729225-5777675x1,5781917x2)	maternal
16	Yes	1	Yes	EVC							ho	arr[hg38] 4p16.2(5726379x2,5729225-5777675x1,5781917x2)	paternal maternal
40	Yes	1 fetus	No	EVC							ho	Deletion of exons 3 to 11 (MLPA)	paternal maternal
41	No	1	No	EVC							ho	Deletion of exons 3 to 11 (MLPA)	paternal maternal
42	No	1 fetus	No	EVC							ho	Deletion of exons 3 to 11 (MLPA)	paternal maternal
43	unkown	1 fetus	No	EVC							ho	Deletion of exons 3 to 11 (MLPA)	paternal maternal
17	Yes	1 fetus	Yes	STK32B/EVC2 and EVC							ho	arr[hg38] 4p16.2(5237432x2,5252144-5760305x1,5775056x2)	? maternal
18	No	1	Yes	EVC2/EVC							he	arr[hg38] 4p16.2(5623126x2,5624246-5731394x1,5734184x2)	maternal
				EVC								Duplication of exon 4, 5,6 and 7 (MLPA)	paternal
19	No	1 fetus	Yes	EVC2	In6	c.706+2T>C	g.5690882A>G	?	Splicing variant	5 (PVS1, PM2, PP3, PP4)	he		maternal
20	No	2 fetus	Yes	EVC2	Ex5	c.602C>A	g.5690988G>T	p.Ser201*	NS	5 (PVS1, PM2, PP3, PP4)	he		paternal
21	No	1 fetus	No	EVC2	Ex15	c.2635C>A	g.5620276G>T	p.Gln897Lys	MS	2 (PM2, BP1, BP4)	he		paternal
22	Yes	1 +2 fetus	No	DYNC2LI1	Ex6	c.513T>A (NM_001348913.1)		p.Asp171Glu	MS	3 (PM2, BP1, PP3)	ho		paternal maternal
23	No	1	Yes	DYNC2H1	In32	c.4968+5G>A	g.64535G>A	?	Non coding variant	3 (PM2, BP4)	he		paternal
											he	Deletion of exons 86 to 90	maternal
24	No	1	No	DYNC2H1	In32	c.4968+5G>A	g.64535G>A	?	Non coding variant	3 (PM2, PM3, BP4)	he		maternal
					Ex30	c.4567C>T	g.58673C>T	p.Arg1523*	NS	5 (PVS1, PM2, PP3)	he		paternal
44	No	1	No	DYNC2H1	In60	c.9440+4A>G	g.126920A>G	?	Non coding variant	3 (PM2, PM3, BP4)	he		paternal
					Ex37	c.10130delT	g.148921del	p.Leu3377Cysfs*35	FS	5 (PVS1, PM2, PP3, PP5)	he		maternal
25	No	1	No	PRKACB	Ex1	c.161C>T		p.Ser54Leu	MS	5 (PS1,PM1, PM2, PP2, PP3, PP5)	he		De novo mosaic
26	No	1	No	SMO	Ex10	c.1727G>A	g.27168G>A	p.Arg576Trp	MS	4 (PS2, PM2, PM5, PP2, PP3, PP5)	he		paternal
					Ex10	c.1726C>T	g.27167C>T	p.Arg576Gln	MS	4 (PS2, PM2, PM5, PP2, PP3, PP5)	he		maternal

Abbreviations are as follows: Cs, consanguinity; ho, homozygote; he, heterozygote; ?, not defined; MS, missense; FS, frameshift; NS, nonsense

Supplemental Table 2: Clinical features of the 50 individuals with a clinical diagnosis of EVC syndrome

Features	**23**	**24**	**44**	**22 (F1)**	**22 (F2)**	**22 (F3)**	**26**	**25**	**45**
Ethnic origin	Morocco	France	European	Senegal	Senegal	Senegal	Togo/France	Morocco	European
Gender	M	F	M	F	F	F	M	M	F
Parental consanguinity	No	No	No	Yes	Yes	Yes	No	No	No
CHD	No	No	No	No	No	No	Yes Complete atrioventricular septal defect	Yes AVSD with single atrium and mitral anomaly	Yes Atrioventricular septal defect
Facial features									
Short / thin upper lip	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Yes
Short / multiple frenula	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Bifid tip of the tongue	No	No	No	Yes	No	No	No	No	No
Absence of upper mucobuccal fold	No	N/A	No	Yes	N/A	N/A	Yes	N/A	No
Serrated alveolar ridge	Yes	N/A	N/A	Yes	No	No	Yes	No	
Dental anomalies	Yes	one tooth agenesis	No	Yes	N/A	N/A	Yes Small, conical, abnormal enamel	Yes oligodontia	No
Short broad nose	No	No	Yes	Yes	N/A	N/A	Yes	Broad nose - not short	Yes
Long philtrum	No	No	Yes	Yes	Yes	Yes	No	No	Yes
Postaxial polydactyly	Yes And Syndactyly Hands and feet	Yes (right hexadactyly and left hand polydactyly with V-VI-VII syndactyly) hand	Yes Bilateral upper limbs	Yes	Yes	Yes	Yes Bilateral upper limbs	Yes Both upper and lower limbs	Yes Hands only
Skeletal features									
Brachydactyly	Yes	Yes	No	Yes	Yes	Yes	Yes Clinodactyly V-VI	Yes	Yes
Short limb	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Narrow chest	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes, moderate	Yes
Nail hypoplasia	Yes	Yes	Yes	Yes	Yes	Yes	Yes Small, brittle, friable, yellow-colored	No	No
Radiological features									
Short tubular bones	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Short ribs with narrow chest	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes
Small iliac bones with downward spike, trident aspect of the acetabular roof	No	No	Yes	Yes	Yes	Yes	Yes Y-shaped metaphysis	Small iliac bones No trident aspect	Yes
Other anomalies (sagital craniosynostosis, dolichocephany, liver, renal or retinal disease ……)	No Lumbar and sacral fusion	no renal, hepatic or ophthalmological involvement	Foot syndactyly	Occipital canal narrowness Normal hepatic, renal and retinal functions Obesity	None	None	Sparse hair Metacarpal fusion Normal hepatic, renal and retinal functions	Myopia Bilateral genu valga	None
Outcome (termination of pregnancy, age, death …)	Alive, Middle childhood (6–11y) Normal height Normal liver and kidney function/ morphology Normal Eye	Alive, Middle childhood (6–11y) Good health	Alive, Term neonatal (Birth-27d)	Alive, Middle childhood (6–11y) Stature – 1 SD	Preterm neonatal and foeto-foetal transfusion syndrome. Severe pulmonary insufficiency outcome. Died in hospital 1 month later	Preterm neonatal and foeto-foetal transfusion syndrome. Severe pulmonary insufficiency outcome. Died in hospital 2 months later	Alive, Middle childhood (6–11y) Normal cognitive development Stature +1SD	Alive, Early adolescence (12–18y) Normal height -1.8 SD (148.5 cm)	TOP
Mutated gene	*DYNC2H1*	*DYNC2H1*	*DYNC2H1*	*DYNC2LI1*	*DYNC2LI1*	*DYNC2LI1*	*SMO*	*PRAKCB*	none

CHD, congenital heart defect; TOP, Termination of Pregnancy; WG, Weeks of Gestation; AVSD, Atroventricular septal defect

Features	**19**	**20**	**20**	**21**	**1**	**2**	**3**	**4**	**27**	**28**	**29**	**30**
Ethnic origin	European	European	European	European	Tchetchenia	Lebanese	Lebanese	Caucasian	China	Unknown	Morocco	Africa
Sex	F	F	M	M	F	F	M	M	M	F	F	M
Parental consanguinity	No	No	No	No	unknown	Yes	Yes	No	No	No	Yes	Yes
CHD	No	Yes Atrioventricular septal defect and single ventricle	Yes Atrioventricular septal defect Mitral atresia	Yes Complete mitral stenosis	No	No	No	Complex heart disease : right subclavian artery retro - esophageal with aortic hypoplasia and double superior vena cava	Yes Atrioventricular septal defect	Yes Single ventricle	Yes N/A	Yes Complete atrioventricular septal defect
Facial features												
Short / thin upper lip	No	No	No	No	Yes	Yes	No	Yes	N/A	N/A	N/A	N/A
Short / multiple frenula	N/A	No	No	No	No	Yes	Yes	No	N/A	N/A	N/A	N/A
Bifid tip of the tongue	No	No	No	No	No	Yes	Yes	No protruding tongue	N/A	N/A	N/A	N/A
Absence of upper mucobuccal fold	N/A	No	No	No	No	Yes	Yes	No	N/A	N/A	N/A	N/A
Serrated alveolar ridge	N/A	No	No	No	Yes	unknown	unknown	No	N/A	N/A	N/A	N/A
Dental anomalies	N/A	N/A	N/A	N/A	Yes	unknown	Yes	N/A	N/A	N/A	Yes	N/A
Short broad nose	Yes	No	No	No	No	Yes	Yes	Yes	N/A	N/A	N/A	N/A
Long philtrum	Yes	No	No	No	Yes	Yes	No	No	N/A	N/A	N/A	N/A
Postaxial polydactyly	Yes	Yes Hands only	Yes	Yes Upper and lower limbs	Yes	Yes	Yes	Yes	Yes Bilateral upper and lower limbs	Yes Bilateral upper limbs	Yes	Yes Bilateral upper limbs
Skeletal features												
Brachydactyly	N/A	Yes	No	N/A	Yes	No	Yes	Yes	No	Yes	Yes	Yes
Short limb	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Narrow chest	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	N/A	Yes
Nail hypoplasia	No	Yes	No	N/A	Yes	Yes	Yes	No	N/A	N/A	Yes	Yes
Radiological features												
Short tubular bones	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes with bowing humeri and femora	Yes	Yes	N/A	Yes
Short ribs with narrow chest	Yes	Yes	Yes	Yes	unknown	Yes	No	Yes	No	Yes	N/A	Yes
Small iliac bones with downward spike, trident aspect of the acetabular roof	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	N/A	Yes
Other anomalies (sagital craniosynostosis, dolichocephany, liver, renal or retinal disease ……)	None	None	None	Syndactyly Bilateral club feet Low set ears Absence of left fibulae Metaphyseal spikes Multicystic kidney Retrognathism Hypospadias	No	No	Genu valgum +++ Lumbar hyperlordosis	Cerebellum hypoplasia	Large proximal ulnary metaphyseal	IUGR	None	Left renal pyelic dilatation
Outcome (termination of pregnancy, age, death …)	TOP	TOP	TOP	TOP	Alive, Early adolescence (12–18y)	Alive	Alive	TOP	TOP	TOP	Alive Senior (60+ y)	TOP
Mutated gene	One event only in *EVC2*	One event only in *EVC2*	One event only in *EVC2*	One event only in *EVC2*	*EVC*	*EVC*	*EVC*	*EVC*	*EVC*	*EVC*	*EVC*	*EVC*

CHD, congenital heart defect; TOP, Termination of Pregnancy; WG, Weeks of Gestation; AVSD, Atroventricular septal defect

Features	**5 (F1)**	**5 (F2)**	**6**	**7**	**8 (F1)**	**8 (F2)**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**9**	**10**
Ethnic origin	Caucasian	Caucasian	Tunisian	Mother Mali Father Madagascar	French	French	Turkish	Unknown	Guiana	France	Mali	Algeria	France	European	N/A	France/ Portugal/ Belgium	Mali
Sex	F	F	F	M	M	M	M	M	F	M	F	F	F	M	F	M	F
Parental consanguinity	No	No	Yes first cousin	No	No	No	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	No	Yes
CHD	No	No	Yes Atrial septal defect	Yes Partial Atrioventricular canal defect	Yes Single auricle, Atrioventricular ring	Yes Atrioventricular defect	Yes Atrioventricular septal defect and single atrium	Yes Complete atrioventricular septal defect and single atrium	Yes Atrial septal septal defect and single atrium	Yes Tetralogy of Fallot	Yes Atrioventricular septal defect and atrial septal defect	Yes Atrioventricular septal defect	Yes Complete atrioventricular septal defect and left-sided superior vena cava	Yes Atrioventricular septal defect	N/A	Yes Atrial septal defect	No
Facial features																	
Short / thin upper lip	No	No	N/A	N/A	Yes	Yes	Yes	N/A	Yes	Yes	N/A	Yes	No	Yes	Yes	No	Yes
Short / multiple frenula	No	No	Yes	Yes	Yes	Yes	N/A	N/A	No	Yes	Yes	N/A	Yes	Yes	No	No	Yes
Bifid tip of the tongue	No	No	N/A	No	No	No	No	N/A	No	No	N/A	N/A	No	No	No	No	Yes
Absence of upper mucobuccal fold	No	No	N/A	Yes	No	No	N/A	N/A	N/A	No	N/A	N/A	Yes	Yes	Yes	No	Yes
Serrated alveolar ridge	No	No	N/A	Yes	N/A	N/A	N/A	N/A	No	No	N/A	N/A	Yes	Yes	No	No	Yes
Dental anomalies	N/A	N/A	N/A	N/A	N/A	N/A	Yes	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Yes	N/A	Yes
Short broad nose	No	No	N/A	Yes	Yes	Yes	No	N/A	Yes	Yes	N/A	N/A	No	Yes	Yes	No	Yes
Long philtrum	No	No	N/A	N/A	Yes	Yes	No	N/A	No	Yes	N/A	Yes	No	Yes	Yes	No	Yes
Postaxial polydactyly	Yes Hands only	Yes Upper and lower limbs	Yes	Yes Hands only	Yes	Yes	Yes Bilateral upper limbs	Yes Bilateral upper limbs	Yes Bilateral upper and lower limbs	Yes Bilateral upper limbs	Yes Bilateral upper limbs Left lower limb	Yes Bilateral upper limbs	Yes Bilateral upper limbs	Yes Bilateral upper limbs	Yes Bilateral upper and lower limbs	Yes upper limbs	Yes
Skeletal features																	
Brachydactyly	N/A	N/A	N/A	Yes	Yes	Yes	Yes	N/A	N/A	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Short limb	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Narrow chest	Yes	No	Yes	Yes	Yes	N/A	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Nail hypoplasia	N/A	N/A	N/A	Yes	N/A	N/A	Yes	N/A	No	Yes	Yes	No	Yes	Yes	Yes	N/A	Yes
Radiological features																	
Short tubular bones	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Short ribs with narrow chest	Yes	Yes	Yes	Yes	Yes	N/A	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Small iliac bones with downward spike, trident aspect of the acetabular roof	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Other anomalies (sagital craniosynostosis, dolichocephany, liver, renal or retinal disease ……)	No	Horseshoe kidney	No	Dolichocephaly	No	Yes Abnormal lung segmentation	Nephrocalcinosis, stature -4 SD, microcephaly (head circumference -3.5 SD), pectus excavatum	Cerebellar vermis hypoplasia, necrotizing enterocolitis, disseminated intravascular coagulation,	Right suprathalamic arachnoid cyst	Microspherophakia	IUGR	Dandy Walker malformation	IUGR, cystic renal tubular dilatation	Pulmonary hypoplasia	N/A	Hypospadias	Sparse hair
Outcome (termination of pregnancy, age, death …)	TOP	TOP	TOP	TOP	TOP	TOP	Alive, Toddler (13mo–2y)	Preterm neonatal, death at 2 days of life	Alive, Early childhood (2-5y)	TOP	Alive, Early childhood (2-5y)	Death at 2 hours of life	TOP	TOP	Alive	TOP	Alive, Toddler (13mo–2y) Normal cognitive development Stature - 4SD
Mutated gene	*EVC2*	*EVC2*	*EVC2*	*EVC2*	*EVC2*	*EVC2*	*EVC2*	*EVC2*	*EVC2*	*EVC2*	*EVC2*	*EVC2*	*EVC2*	*EVC2*	*EVC2*	*EVC2*	*EVC2*

CHD, congenital heart defect; TOP, Termination of Pregnancy; WG, Weeks of Gestation; AVSD, Atroventricular septal defect

Features	**11**	**12**	**13**	**14**	**15**	**16**	**40**	**41**	**42**	**43**	**17**	**18**
Ethnic origin	European	European	European	French	European	Algeria	N/A	Tunisian	European	N/A	Morocco	Lebanese
Sex	M	M	F	M	M	F	F	M	M	M	M	M
Parental consanguinity	No	No	No	No	No	Yes	Yes	No	No	No	First cousins	No
CHD	No	Yes Atrial ventricular septal canal defect	Yes	Yes Left heart hypoplasia	Yes	Yes Atrioventricular septal defect	Yes Atrioventricular septal defect	Yes Atrioventricular septal defect	Yes Atrioventricular septal defect	Yes Ventricular septal defect, hypoplastic left heart, and interrupted aortic arch	Yes Atrial defect and atrioventricular canal	Yes
Facial features												
Short / thin upper lip	Yes	Yes	Yes	No	No	Yes	N/A	Yes	Yes	N/A	Yes	No
Short / multiple frenula	Yes	No	N/A	No	N/A	Yes	N/A	N/A	Yes	N/A	N/A	Yes
Bifid tip of the tongue	No	No	N/A	No	No	No	N/A	No	No	N/A	No	Yes
Absence of upper mucobuccal fold	No	No	N/A	No	No	Yes	N/A	Yes	Yes	N/A	N/A	No
Serrated alveolar ridge	Yes	Yes	N/A	No	N/A	N/A	N/A	N/A	Yes	N/A	N/A	N/A
Dental anomalies	N/A	Yes	N/A	N/A	N/A	N/A	N/A	N/A	Yes	N/A	N/A	No
Short broad nose	Yes	Yes	Yes	No	Yes	Yes	N/A	Yes	Yes	N/A	Yes	No
Long philtrum	Yes	Yes	N/A	Yes	No	Yes	N/A	Yes	Yes	N/A	N/A	Yes
Postaxial polydactyly	Yes Upper and lower limbs	Yes	Yes	Yes	Yes	Yes Bilateral	Yes Bilateral upper limbs	Yes Bilateral upper limbs	Yes Bilateral upper limbs	Yes Right hand	Yes Bilateral upper limbs	Yes
Skeletal features												
Brachydactyly	Yes	Yes	N/A	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes P3 hypoplasia	Yes
Short limb	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Narrow chest	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Nail hypoplasia	Yes	Yes	N/A	No	Yes	Yes	N/A	Yes	Yes	Yes	Yes	Yes
Radiological features												
Short tubular bones	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Short ribs with narrow chest	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Small iliac bones with downward spike, trident aspect of the acetabular roof	Yes	N/A	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Other anomalies (sagital craniosynostosis, dolichocephany, liver, renal or retinal disease ……)	Kidney cysts	None Hip dislocation	Club feet left	Micropenis Bifidity of the supernumerary 6th digit	None	None	None	IUGR, pulmonary hypoplasia	None	None	Ureteral dilataion Corpus callosum hypoplasia	Club feet
Outcome (termination of pregnancy, age, death …)	TOP	Alive, Early childhood (2–5y)	TOP	TOP	TOP	Death at 4 months	TOP	Death at 2 days of life	TOP	Death at 2 days of life	TOP	Alive
Mutated gene	*EVC*	*EVC*	*EVC*	*EVC*	*EVC*	*EVC*	*EVC*	*EVC*	*EVC*	*EVC*	*STK32B/EVC2 and EVC*	*EVC2/EVC*

CHD, congenital heart defect; TOP, Termination of Pregnancy; WG, Weeks of Gestation; AVSD, Atroventricular septal defect

Supplemental Table 3: Analysis done for each family

Families	Ciliome	Cildiag	Array CGH 180K customized	MLPA EVC/EVC2	Targeted resequencing EVC/EVC2 by NGS	Whole Exome sequencing
1	Yes	No	No	No	No	No
2	Yes	No	No	No	No	No
3	Yes	No	No	No	No	No
4	Yes	No	No	No	No	No
27	No	Yes	No	No	No	No
28	No	Yes	No	No	No	No
29	No	Yes	No	No	No	No
30	No	Yes	No	No	No	No
5	Yes	No	No	No	No	No
6	Yes	No	No	No	No	No
7	Yes	No	No	No	No	No
8	Yes	No	No	No	No	No
31	No	Yes	No	No	No	No
32	No	Yes	No	No	No	No
33	No	Yes	No	No	No	No
34	No	Yes	No	No	No	No
35	No	Yes	No	No	No	No
36	No	Yes	No	No	No	No
37	No	Yes	No	No	No	No
38	No	Yes	No	No	No	No
39	Yes	No	No	No	No	No
9	Yes	No	No	No	No	No
10	Yes	No	Yes	Yes	No	No
11	Yes	No	Yes	Yes	No	No
12	Yes	No	Yes	Yes	No	No
13	Yes	No	Yes	Yes	No	No
14	Yes	No	Yes	Yes	No	No
15	Yes	No	Yes	Yes	No	No
16	Yes	No	Yes	Yes	No	No
40	No	Yes	No	Yes	No	No

41	No	Yes	No	Yes	No	No
42	No	Yes	No	Yes	No	No
43	No	Yes	No	Yes	No	No
17	Yes	No	Yes	Yes	No	No
18	Yes	No	Yes	Yes	Yes	No
19	Yes	No	Yes	Yes	Yes	No
20	Yes	No	Yes	Yes	Yes	No
21	Yes	No	No	Yes	No	No
22	Yes	No	No	No	No	Yes
23	Yes	No	No	Yes	No	Yes
24	Yes	No	No	No	No	No
44	No	No	No	No	No	Yes
25	Yes	No	No	Yes	No	Yes
26	Yes	No	No	Yes	No	Yes
45	Yes	No	No	Yes	No	No