Supplemental information

KIF7 (Costal2) mutations cause fetal Hydrolethalus and Acrocallosal syndromes

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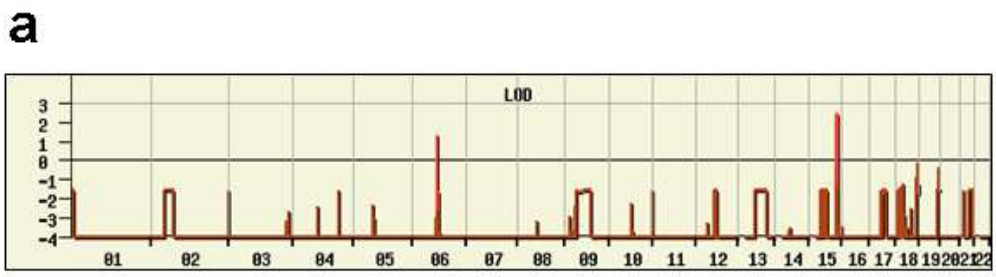
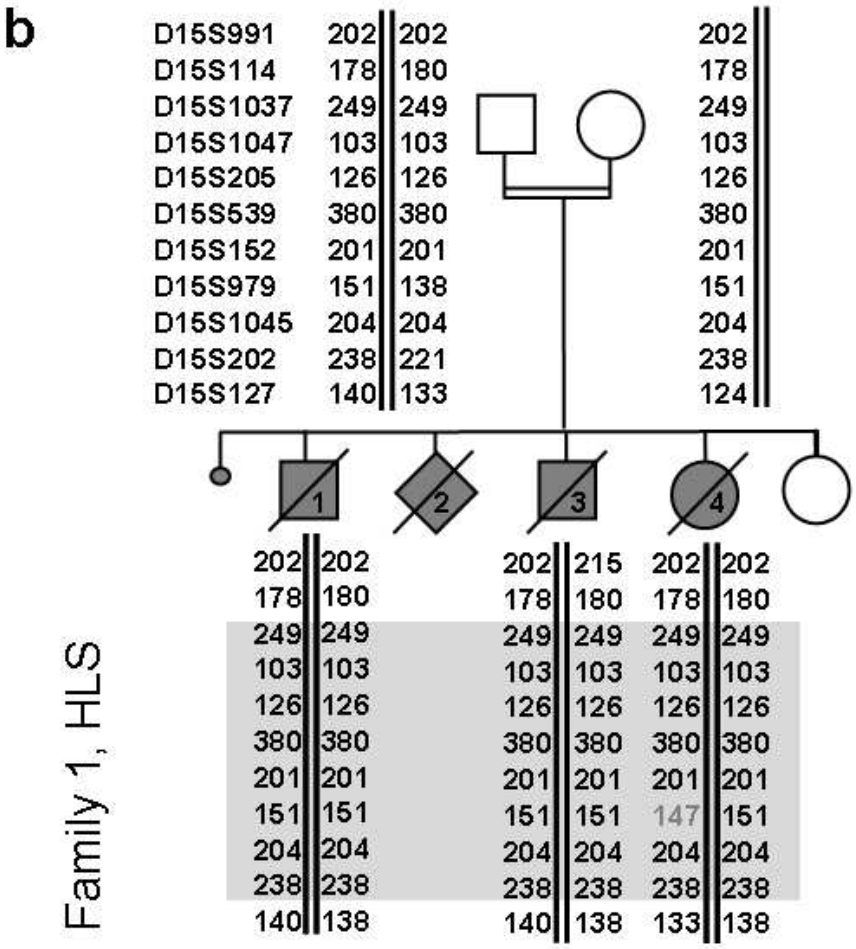
Supplementary figure 1: Results of linkage analysis in family 1

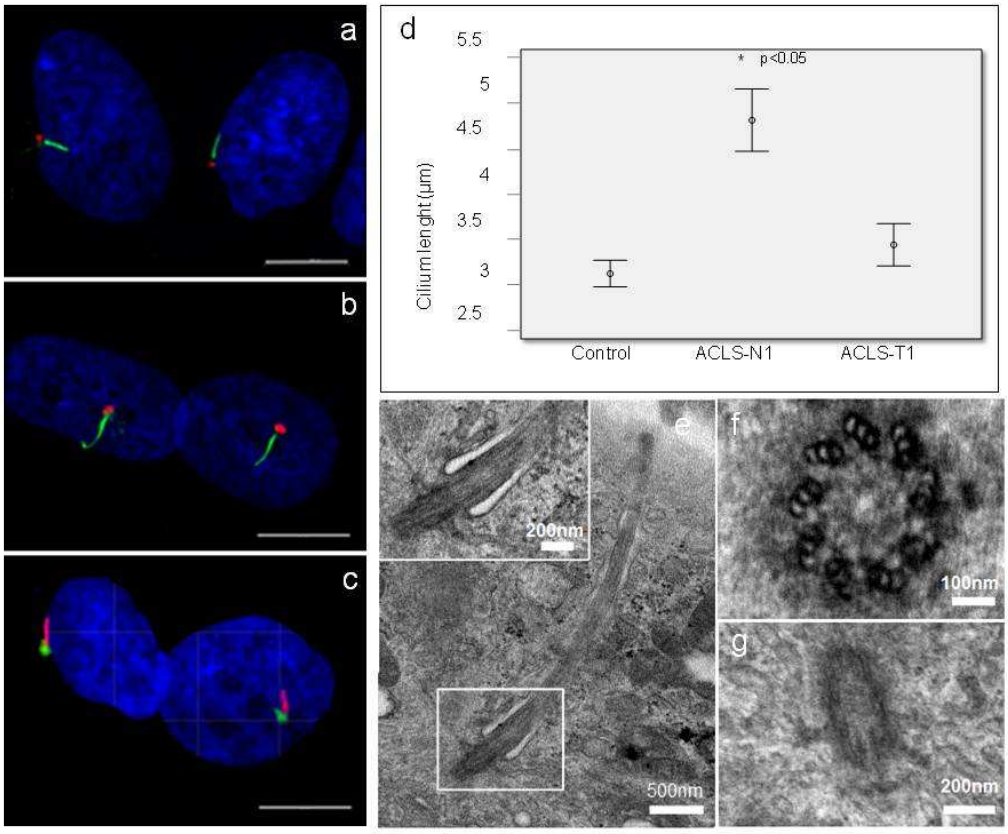
(**a**) Results of the multipoint linkage analysis with Affymetrix 250K SNP chips using MERLIN software assuming a fully penetrant recessive model with a disease allele of frequency 0.0001 and allowing for heterogeneity between families. The highest heterogeneity lodscore (Hlod 2,6) is found on chromosome 15. (**b**) Pedigree of family 1 and haplotype analysis confirming homozygosity on chromosome 15 between D15S114 and D15S127.





Supplementary figure 2: *KIF7* mutations result in impaired cilia length

(**a-c**) Confocal microscopy of two different *KIF7*-mutated fibroblast lines from affected individuals ACLS-T1 and ACLS-N1 marked by acetylated alpha tubulin (axoneme) and pericentrin (basal body). While cilia were apparently normal in human fibroblasts from control (**a**) and *KIF7* mutated patient ACLS-T1 (**c**), they seemed markedly longer in ACLS-N1 (**b**). Measurement of cilia length (**d**): Analysis of variance (Anova) showed a significant difference on cilium length between control and patient ACLS-N1 fibroblasts. Ultrastructural analysis of primary cilia from *KIF7* mutated ACLS-T1 shows apparently normal structural components: (**e**) Longitudinal section of a primary cilium growing into a ciliary pocketThe inset is a higher magnification of the primary cilium basal body. (**f**) Longitudinal section of a primary cilium thought the basal body showing the 9 microtubular triplets normally organized. (**g**) Longitudinal section through the transition zone of a primary cilium showing normal anchoring at the plasma membrane.



Supplementary Table 1 : Syndromes including cerebral anomalies and polydactyly.

OFD: orofaciodigital syndrome; HPE: holoprosencephaly; HPS: Holoprosencephaly-Polydactyly syndrome (pseudotrisomy 13); PHS: Pallister-Hall syndrome, DWM: Dandy-Walker Malformation, OE: occipital encephalocele; MTS: molar tooth sign; MR: mental retardation

Syndromes	OMIM	Vermis	Corpus Callosum	Other brain anomalies	Upper limb Polydactyly	Lower limb Polydactyly	Cleft	Other	Molecular defect
Greig	175700	-	agenesis	-	Post-axial	Preaxial	-	Macrocephaly, Hypertelorism, Syndactyly	*GLI3*
PHS	146510	-	normal	hypothalamic hamartoblastoma	Post axial or central	Post axial or central	-	Facial dysmorphism, bifid uvula, syndactyly, cardiac malformation, renal anomalies, imperforate anus	*GLI3*
Acrocallosal	200990	Agenesis DWM	agenesis	Arachnoid cysts Anencephaly	Post-axial	Preaxial	-	Macrocephaly, Hypertelorism	*?*
HPS	264480	-	possible agenesis	HPE Hydrocephaly	Post-axial	Preaxial	+	Micrognathia, Genital anomalies in male	*?*
Hydrolethalus	236680	-	possible agenesis	Hydrocephaly Anencephaly	Post-axial	Preaxial	+	Cardiac anomalies Key hole foramen magnum Micrognathia, Polyhydramnios	*HYLS1*
Joubert	213300	MTS	normal	OE Polymicrogyria	Post-axial	Post-axial	-	MR, Cerebellar ataxia Abnormal eyes movements Retinal dystrophy, Renal anomalies	*JBS1-JBS10*
Meckel	249000	agenesis MTS	normal	OE Anencephaly	Post-axial	Post-axial	+/-	Cystic kidneys, Biliary dysgenesis Skeletal anomalies	*MKS1-MKS6*
OFD VI	277170	agenesis MTS	agenesis	Wide ventricles	Central	Preaxial	+	Lingual and dental anomalies Genital anomalies	*TMEM216/MKS2*

Supplementary Table 2: ***KIF7*** **Primers used for sequencing and RT-PCR**.

PCR primers were selected with primer3 program (http://frodo.wi.mit.edu/primer3/ input.htm) according to reference sequence NM_198525. PCR products were purified with the Exo-SAP cleanup kit (USB) and sequenced with BigDye chemistry and ABI 3100 (Applied Biosystems) automated sequencer. Sequences were analyzed with SeqScape® software.

Primers	Sequence	PCR size
KIF7-2F	AGGAGCTGCAGGATCTGG	591
KIF7-2R	CACAGATCCCTGTCTAGGAAATC	
KIF7-3F	CACTGCCTTCTCCATCCTAGAG	458
KIF7-3R	GCTAAAGCAAAACCTCCCAG	
KIF7-4F	GGTCAGGAAGGGCAGGTT	717
KIF7-4R	CCCAGCGAGTCTTTGAGGAT	
KIF7-5F	AGGTCCCCTGAGGTCTCC	826
KIF7-5R	AGCTGGAGACCTGAGACTCG	
KIF7-6F	CACCAGTGGGTCTGGAGTTC	376
KIF7-6R	GACTCTACACCCCTACCCCG	
KIF7-7F	TTTGACACTCCTCATTTTCAGC	494
KIF7-7R	ACAGCTGAAAGCAGCCTCTC	
KIF7-8F	CAGAGGGGTCATTTGAGCTG	386
KIF7-8R	CATCCTAGGACCCAGAGGC	
KIF7-9F	TCTGCCAGACTCACATCCTG	721
KIF7-10R	AGGCTGGGCTGAGTATCAAA	
KIF7-11F	CAAAGGCTTGGTGAAACCC	472
KIF7-11R	CTATACCAGCCTCACCCTGC	
KIF7-12F	CCGGCCTCTGTTATTATTATTGG	467
KIF7-12R	CAGGCTAAGTGACCTGCTTTC	
KIF7-13F	AGATTTGTGTGGGATGGGTC	742
KIF7-14R	TGGCAGGTTTATCACTTGGA	
KIF7-15F	GCTGAGCAGAACATTTGGAG	474
KIF7-15R	AGTGAAAACTTGGGTCTGCC	
KIF7-16F	GCTGACTTGGCCCTTGG	473
KIF7-16R	CTGCAGATGAGTTGGTCCTG	
KIF7-17F	GGGAAGACCCTGGTGATTTC	358
KIF7-17R	GCCGGGGTTGTGAGCCAT	
KIF7-18F	ACAGCCCCTGCACCTACA	398
KIF7-18R	AAGCAACCTGTGAATTGAGC	
KIF7-19F1	TGGGGAAAGCTTAGAGACCA	445
KIF7-19R1	ACAAAGGCCCAAAGTTCCAG	
KIF7-19F2	GACAGGCTCCTGGAAATGAA	473
KIF7-19R2	TTGATCCCAGTGAGGGTACAG	
RT-PCR		
KIF7-RT-14F	AGAAGACGGGGCTGGAGA	290
KIF7-RT-16R	GGCCTCATCCAACTGGAAC	
KIF7-RT-1F	CTGGGGATGGAAACCTGACT	614
KIF7-RT-4R	CTCCACGTCGACCTCCTTC	

Supplementary Table 3: Heterozygous missense variations found in the *KIF7* gene

KIF7 missense variations	Phenotype	Other mutated alleles	db SNP	1000 genomes	Controls	PolyPhen-2	SIFT	Human Splicing Finder
c.3345C>G, p.H1115Q	MKS HLS	-	-	-	0/384	Probably damaging PSIC: 0.995	Not tolerated	
c.2501A>G, p.Q834R	OFDVI BBS (x2)	*BBS10* : p.C91fsX95 heterozygous, *BBS7*: p.Q293P heterozygous	-	-	3/384	Possibly damaging PSCI: 0.550	Not tolerated	
c.1895C>T, p.P632L	PHS	-	-	-	0/384	Benign PSCI: 0.001	Not tolerated	
c.2105G>A, p.R702Q	ACLS	-	-	-	0/384	Probably damaging PSCI: 0.900	Tolerated	Possible splice modification
c.1921A>G, p.R641G	BBS	*BBS1*: p.M390R homozygous	-	-	0/384	Benign PSCI: 0.112	Not tolerated	Possible splice modification
c.2276T>C, p.L759P	BBS	-	-	-	0/384	Possibly damaging PSIC: 0.693	Not tolerated	
c.2981A>G, p.Q994R	BBS	*BBS1*: [p.M390R]+[p.R419X]	-	-	0/384	Possibly damaging PSCI: 0.813	Tolerated	
c.3202C>T, p.R1068W	BBS	*BBS9*: [p.K621fsX22]+[p.A665fsX13]	-	-	0/384	Probably damaging PSIC: 0.997	Not tolerated	

MKS: Meckel, HLS: Hydrolethalus, OFDVI: orofaciodigital type VI, BBS: Bardet-Biedl, PHS: Pallister-Hall, ACLS: acrocallosal syndrome

Supplementary Table 4: Zebrafish somite angle morphometrics for *kif7* mutant rescue assays

		Somite angle Measurements		p-values			
Injection	**n=**	**Mean (°)**	**S.E.M.**	**vs. WT RNA**	**vs. WT rescue**	**vs. MO**	**Pathogenicity**
Controls	59	84.1	0.5				
MO	58	99.1	1.0				
WT RNA	57	87.8	1.0				
MO + WT RNA	43	88.2	1.2				
P632L RNA	62	86.7	0.7	0.3758		<0.0001	Hypomorph
MO + P632L	63	94.1	0.8		0.0001	0.0001	
R641G RNA	63	85.9	0.7	0.1255		<0.0001	Hypomorph
MO + R641G	61	92.9	0.7		0.0005	<0.0001	
R702Q RNA	60	86.6	0.6	0.3045		<0.0001	Hypomorph
MO + R702Q RNA	65	93.8	0.8		0.0001	<0.0001	
L759P RNA	58	85.1	0.7	0.0273		<0.0001	Hypomorph
MO + L759P RNA	56	96.0	0.8		<0.0001	0.0174	
Q834R RNA	52	86.1	0.8	0.2027		<0.0001	Hypomorph
MO + Q834R RNA	59	94.3	1.1		0.0005	0.0015	
Q994R RNA	60	87.0	0.8	0.5301		<0.0001	Hypomorph
MO + Q994R	59	95.0	0.9		<0.0001	0.0029	
R1068W RNA	71	86.6	0.6	0.2870		<0.0001	Hypomorph
MO + R1068W RNA	60	94.8	1.4		0.0008	0.0115	
H1115Q RNA	58	88.0	0.8	0.8496		<0.0001	Hypomorph
MO + H1115Q RNA	55	95.0	1.1		0.0001	0.0056	
R1325Q* RNA	52	84.9	0.8	0.0279		<0.0001	Benign
MO + R1325Q* RNA	49	87.1	0.9		0.4167	<0.0001	

* rs73477443

Supplementary Table 5: List of genes deregulated in *KIF7* mutated fetuses

List of genes deregulated (fold change >1.2; p<0.05) in *KIF7* mutated fetuses as compared to age-matched controls. Work sheets 1 to 5 contain the lists of genes presented in the heat map. Work sheets 6 and 7 contain all the genes down or up regulated respectively in *KIF7* mutated fetuses as compared to age-matched controls. Work sheet 8 contains the qPCR results with theTaqMan Assays of the selected GLI targets used.

See Excel file online.