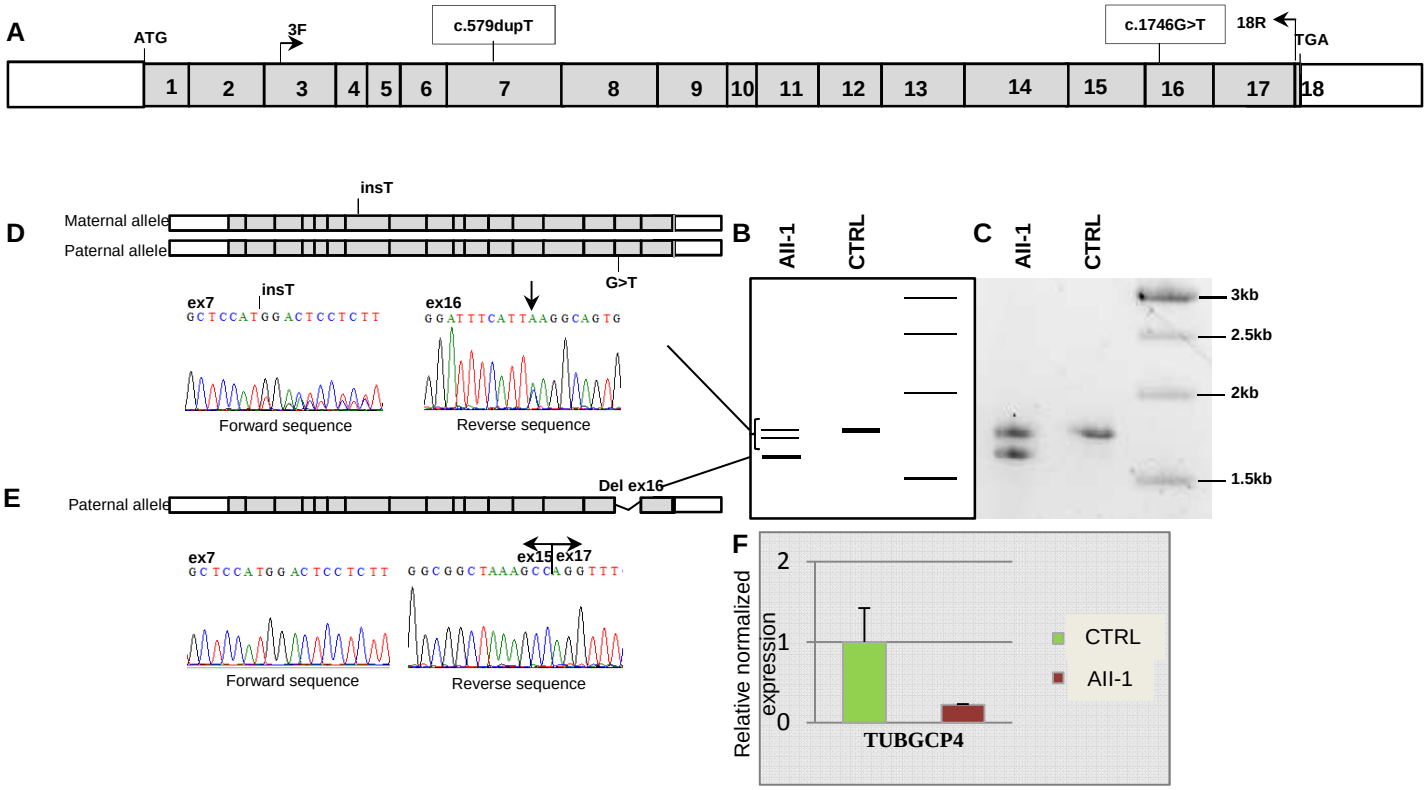
The American Journal of Human Genetics

Supplemental Data

Mutations in *TUBGCP4* Alter Microtubule Organization via the γ-Tubulin Ring Complex in Autosomal-Recessive Microcephaly with Chorioretinopathy

Sophie Scheidecker, Christelle Etard, Laurence Haren, Corinne Stoetzel, Sarah Hull, Gavin Arno, Vincent Plagnol, Séverine Drunat, Sandrine Passemard, Annick Toutain, Cathy Obringer, Mériam Koob, Véronique Geoffroy, Vincent Marion, Uwe Strähle, Pia Ostergaard, Alain Verloes, Andreas Merdes, Anthony T. Moore, and Hélène Dollfus

Figure S1: Synonymous mutation analysis showing residual expression of *TUBGCP4*.



cDNA was obtained from individual AII-1 skin fibroblasts and from a healthy control (CTRL).

A) Schematic representation of the exon structure of human *TUBGCP4*, with indications of: positions of start codon (ATG), stop codon (TGA), mutations in exon 7 and exon 16 as well as the primers used for this assay forward primer (3F) and reverse primer (18R).

B) Schematic representation of the electrophoresis gel, the two bands identified on the drawing by the bracket cannot be distinguished on the gel (their lengths differ only by 1bp because of the insertion of one T).

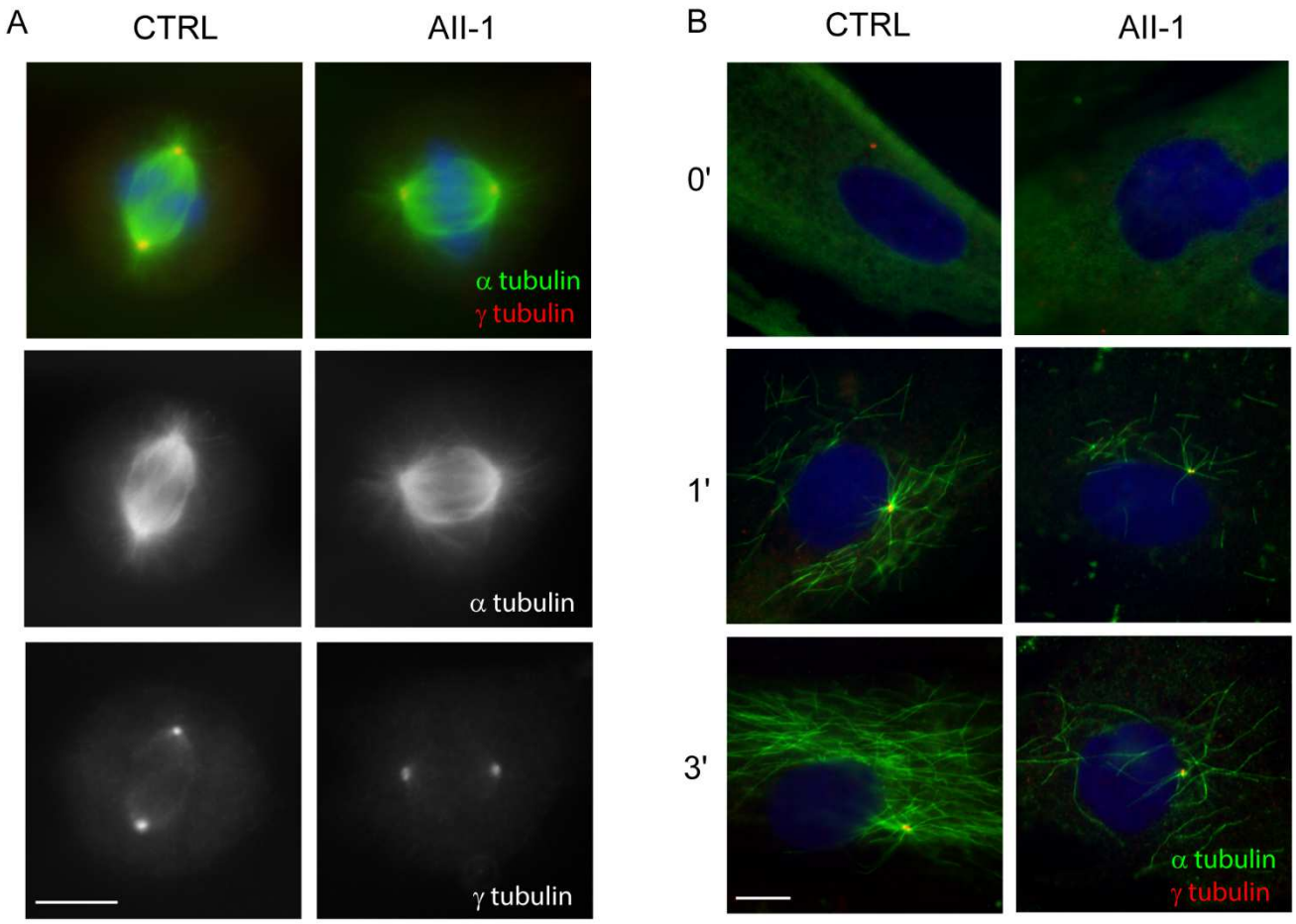
C) Electrophoresis gel showing the PCR amplification of 1.7kb cDNA using primers in exon 3 and exon 18.

D) Sequence reads of the 2 indivisibles PCR products showing the presence of the mutated exon 7 maternal allele and the mutated exon 16 paternal allele ascertaining the residual presence of a functional exon 16 due to an alternative exon 16 skipping.

E) Sequence read of the exon 16 paternal allele with the total exon 16 skipping compared to the control.

F) Quantitative PCR (using exon 14 to exon 16 primers) showing the drastic reduction of the cDNA level for the affected individual compared to three controls.

Figure S2: Individual AII-1 fibroblasts have reduced staining of g-tubulin, bipolar spindles and show decreased microtubule nucleation

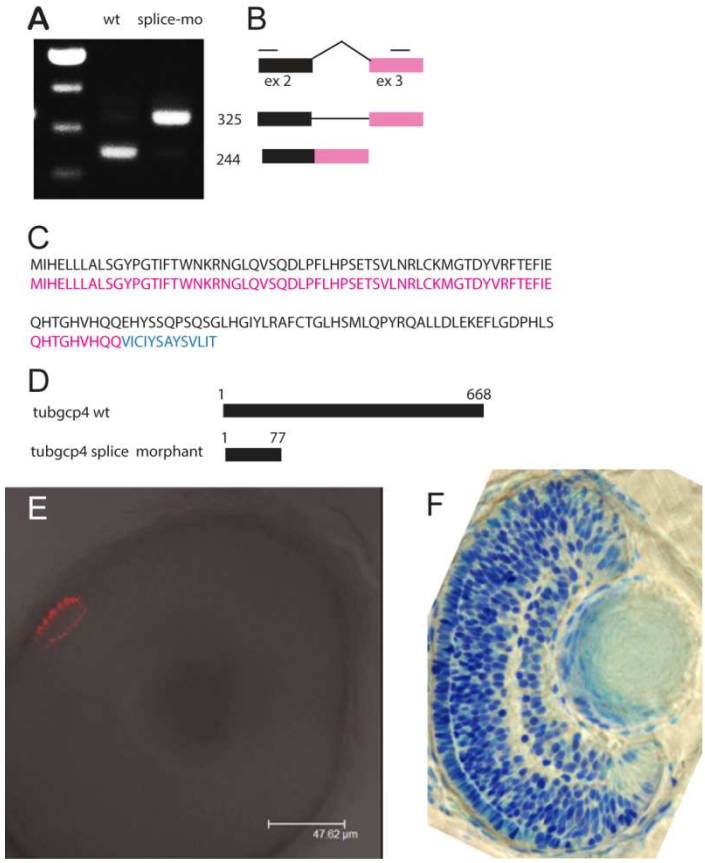


A) Immunofluorescence of microtubules (green) and γ tubulin (red) show bipolar spindles with reduced amounts of g-tubulin in AII-1 mitotic fibroblasts compared to control.

B) Microtubule regrowth assay show decreased microtubule nucleation in AII-1 fibroblasts compared to control. Cells were exposed to cold and reheated for 0, 1 or 3 minutes at 25°C before permeabilisation, fixation and immunofluorescence of microtubules (green) and γ tubulin (red). Microtubules nucleated from the centrosome and from the Golgi apparatus are visible.

DNA is blue in merged images, scale bars: 10 µm.

Figure S3: splice morpholinos fail to generate a continuous photoreceptors layer



A-D) tubgcp4 splice morpolino generate a truncated protein. A : PCR made with cDNA of controls embryos (wt) or tubgcp4 splice morpholinos injected embryos (splice-mo) amplify a bigger band in the morphant (325 bp) compared to the control (244 bp). B : 244 corresponds to correct splicing of the intron 2-3, whereas in morphant, the intron is kept. thin black bars indicate the place of primers. C : the failed splicing generates a premature stop codon. Blue indicates the amino acid that diverge from the control sequence. D : a 77 amino acids protein is generated in morphant whereas the full length sequence should contain 668 amino acids.

E-F) Zpr1 staining showing only single patch of photoreceptor in splice morphant at 72 hpf (E). Scale bar : 47mm. F : 5 mm plastic section of E showing presence of photoreceptors layers.

Table S1: Summary of the exome sequencing results from Family A

Individuals	**AII-1**		**AII-3**		**AII-2**		**AI-2**	
Type of sequence variant	**SNV**	**Indel**	**SNV**	**Indel**	**SNV**	**Indel**	**SNV**	**Indel**
Total number of variants	51723	8318	52151	8279	53804	8393	51998	8207
After exclusion of non-pathogenic variants (as determined from the ClinicalSignificance field in dbSNP) validated by at least 2 methods in dbSNP (as determined from the “Validation Status” field)	7103	5991	7082	5983	9498	6083	7060	5909
After exclusion of variants with an allele frequency > 1% (extracted from the dbSNP database and the Exome Variant Server)	6731	5257	6696	5289	9133	5371	6687	5237
After exclusion of variants found in the homozygous state + exclusion of variants found more than once in the heterozygous state in 48 control exomes	1418	1084	1354	1107	4175	1250	1299	1083
After exclusion of 5'UTR, 3'UTR, downstream, upstream and intron locations without local splice effect prediction (from the “localSpliceEffect” field of Alamut-Batch)	614	215	537	200	2112	315	529	193
After exclusion of synonymous variants without local splice effect prediction (from the “localSpliceEffect” field of Alamut-Batch)	487	215	430	200	1655	315	423	193
After selection of variants consistent with recessive transmission (compound heterozygous, homozygous variants).	**3 compound heterozygous (in the *TUBGCP4*, *TRPM2*, and *ERICH6B* genes)**							

Table S2: Primers table

RefSeq Gene	Gene	Forward (5’-3’)	Reverse (5’-3’)	size
NM_014444	TUBGCP4-ex1	GTTGAGCTGCCGAACTTCC	GCCTCTCTAGGTGTCGCATC	472bp
	TUBGCP4-ex2-3	GGATAGGGAACCCCTTTGAA	ACTGTACCTTCCAGCCATGC	831bp
	TUBGCP4-ex4	ATAAGAGCCCTGGCTTGGTT	GGCTCTGAATCCAGAAGAAAAA	346bp
	TUBGCP4-ex5	CTGGCCAAAATGGTGAAAC	AAAAACTACTAAAAACTAGGGTCTTCG	580bp
	TUBGCP4-ex6	CTGCTGATGGGAGAGAGGTC	TCCTCTGCTCATGGAGCTTT	396bp
	TUBGCP4-ex7	GGCTGCAAATATGGAAATTCA	TGATGGAAGGTGCTCAGCTA	489bp
	TUBGCP4-ex8	TGTGCAACACCAGATTTGAAG	TTATCGACCACTCGCTCTGA	396bp
	TUBGCP4-ex9	TCAGAGCGAGTGGTCGATAA	GCCTCCCAAAGTGTTGAGAC	453bp
	TUBGCP4-ex10-11	TTTTTCTATGTGGGGCAAGG	CAAGAGCCTTCCATCTCTGG	850bp
	TUBGCP4-ex12	ATGTCCACTGTGTCCTGCTG	AGCCTCTCTGGACTTCTGGA	400bp
	TUBGCP4-ex13	TTTTAGTAGAGATGGGGTTTCACTG	GCTCTGGACTAGCTGGGATT	400bp
	TUBGCP4-ex14	GGGTGGCACAAAGTCCTAGA	CCCAAACCCCACCATTTTAT	380bp
	TUBGCP4-ex15	AGGGAGGGGGTTCAGAATTA	GAGACGGGTTTTCACCATGT	589bp
	TUBGCP4-ex16	TCAGATTTGGGAGGTCAGCTA	TGCTTATTTGATGGGCTGAA	481bp
	TUBGCP4-ex17	GAAGGCCAGGTGGTTCTGTA	TTGGACTAAAGTCTTCTTCCAGTTC	360bp
	TUBGCP4-ex18	CAGAAGAGTCAAAAGAAACTCTTCAGT	TCCTGGATGTTCAGTAACTGCT	300bp
	TUBGCP4-RT (ex14->16)	ATCACATGGCATTTTTGGTG	ATCCAGTGGGCCTAGGTTCT	269bp
	TUBGCP4-RT-EX3F-18R	TGCACAGGGCTGGATTCTGT	GAAACTGCCCAGAGTTCCAC	1720bp

Table S3: Morpholinos sequences

tubgcp4-mo start	AAGCCAACAGCAACTCGTGAATCAT
tubgcp4-mo splice	GCAGAGTAAATGCAGATTACCTGTT
cont1	GATAGATTCACTTCACCTGCCGCAT
cont 2	CACGCACAGTTTACTGATCATTTGT
cont3	ATCAAAACGATATTTCGGTATGACT