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Supplemental Data

Mutations in *CKAP2L*, the Human Homolog of the Mouse Radmis Gene, Cause Filippi Syndrome

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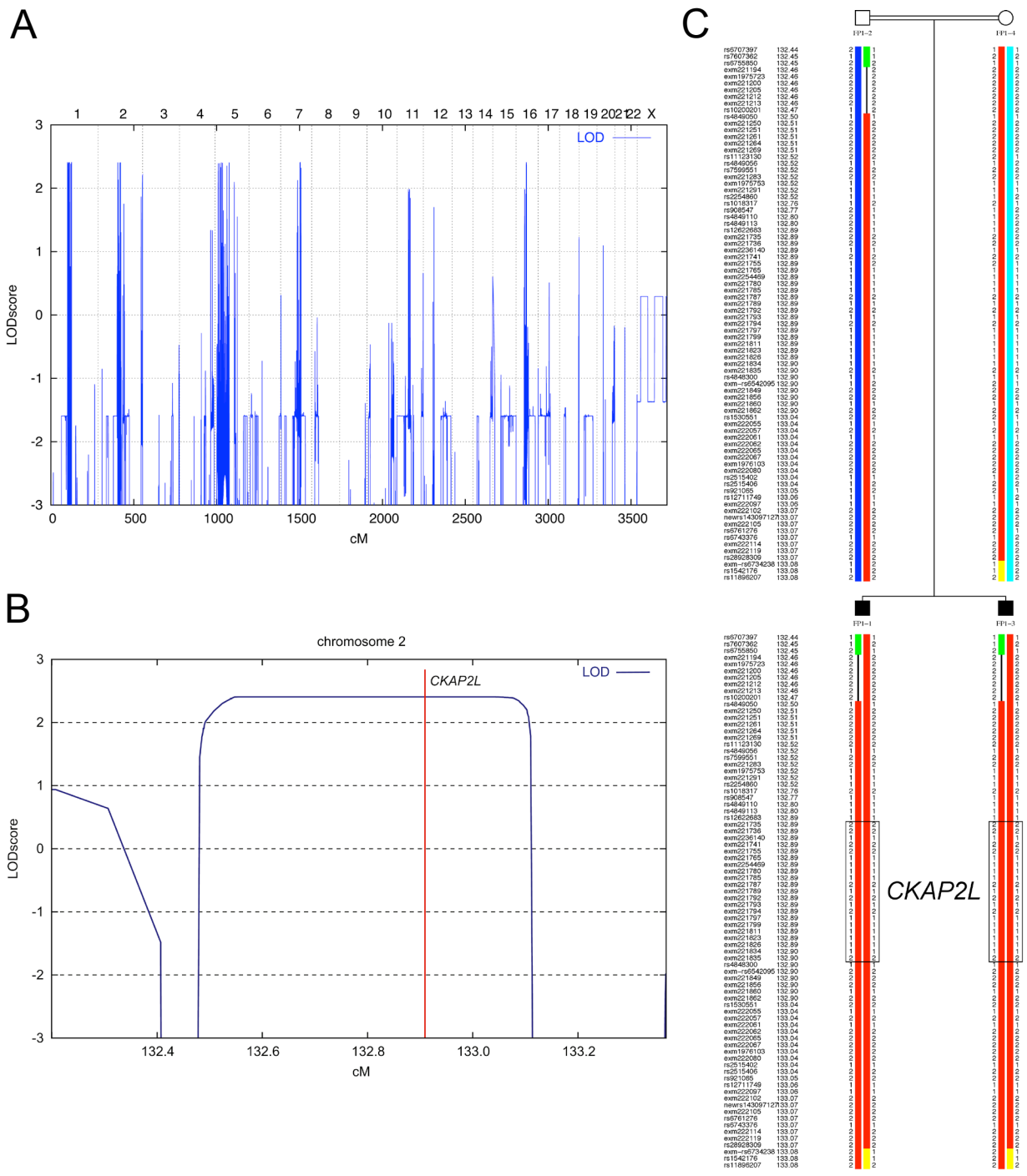
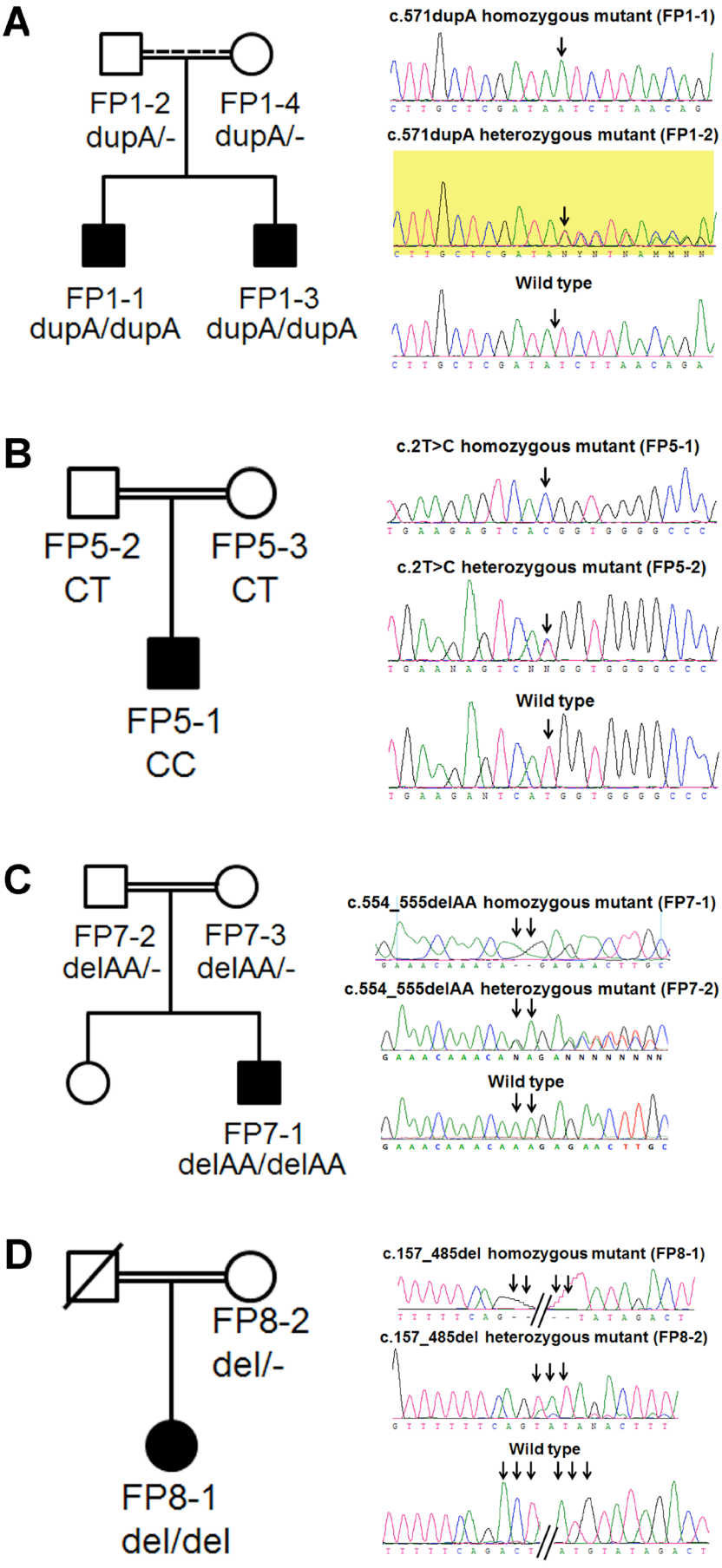


Figure S1. Genome-wide Linkage and Haplotype Analyses in an Italian Filippi Syndrome Family with Two Affected Children

(A) Genome-wide linkage analysis based on the genotypes of two siblings and their parents. Scores are plotted over genetic distance across the genome, where chromosomes are concatenated from p-ter to q-ter from left to right. For this pedigree, the highest possible LOD score of 2.4 was obtained for regions on chromosomes 1, 2, 5, 7 and 16.

(B) Detailed view of a linkage peak on chromosome 2. A maximum multipoint LOD score of 2.4 was reached over a region of about 0.63 cM corresponding to a chromosomal segment at cytoband 2q13 harboring *CKAP2L* (red line).

(C) Haplotypes of the linkage region including *CKAP2L*. The size of the homozygous region is 0.93Mb. The heterozygous markers flanking this interval are rs6755850 (112,145,144bp) at 132.45cM and exm-rs6734238 (113,083,453bp) at 133.08 cM. SNP markers of the *CKAP2L* region are boxed.


A
FP1-2
dupA/-
FP1-4
dupA/-
FP1-1
dupA/dupA
FP1-3
dupA/dupA
c.571dupA homozygous mutant (FP1-1)
c.571dupA heterozygous mutant (FP1-2)
Wild type
B
FP5-2
CT
FP5-3
CT
FP5-1
CC
c.2T>C homozygous mutant (FP5-1)
c.2T>C heterozygous mutant (FP5-2)
Wild type
C
FP7-2
delAA/-
FP7-3
delAA/-
FP7-1
delAA/delAA
c.554_555delAA homozygous mutant (FP7-1)
c.554_555delAA heterozygous mutant (FP7-2)
Wild type
D
FP8-2
del/-
FP8-1
del/del
c.157_485del homozygous mutant (FP8-1)
c.157_485del heterozygous mutant (FP8-2)
Wild type

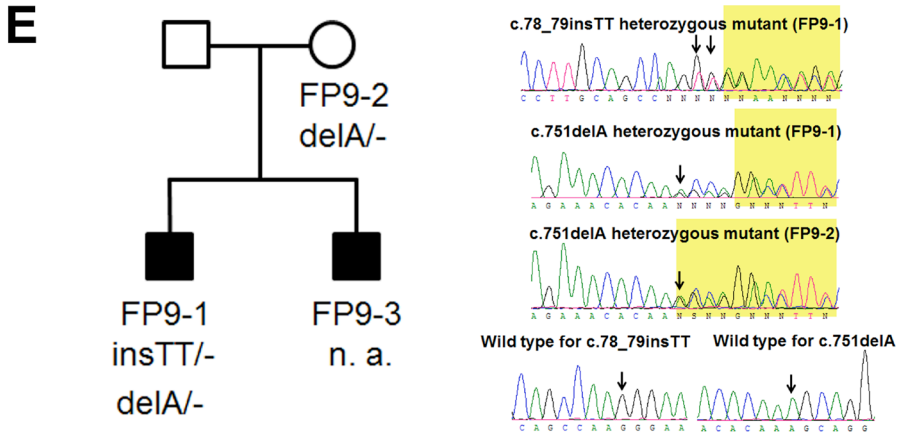


Figure S2. Segregation Analysis of *CKAP2L* Mutations in Five Filippi Syndrome Families

All available family members from index subjects in whom a mutation in *CKAP2L* had been identified were re-sequenced at the mutated site by Sanger sequencing. Pedigrees are shown along with the corresponding sequence chromatograms. Mutant sequences are juxtaposed to wild-type traces from a control individual.

(A) Italian family (FP1) with two affected brothers carrying the homozygous mutation c.571dupA. The parents are heterozygous. The broken line connecting the parents in addition to the solid one is to indicate consanguinity of unknown degree. For linkage analysis a second cousin marriage was assumed.

(B) Pakistani family (FP5) with a single affected male born to healthy consanguineous parents. The affected individual is homozygous for the mutation c.2T>C. The parents are heterozygous for this mutation.

(C) Turkish family (FP7) with an affected male born to consanguineous parents. He is the second child of the couple and is homozygous for the 2bp deletion c.554_555delAA. The parents are heterozygous for this mutation. DNA of the unaffected sister was not available for analysis.

(D) South Asian family (FP8) with a single affected female born to healthy consanguineous parents. The affected individual is homozygous for a 329bp deletion (c.157_485del). The mother is heterozygous. At the time of investigation, the father was deceased.

(E) British family (FP9) with two affected brothers one of them carrying two heterozygous mutations, c.78_79insTT and c.751delA. From the younger boy, no DNA sample was obtained. The mother was tested positive for the mutation c.751delA but negative for the mutation c.78_79insTT. A DNA sample from the father was not available. Compound heterozygosity of the affected children is the most likely explanation for the observed genotypes.

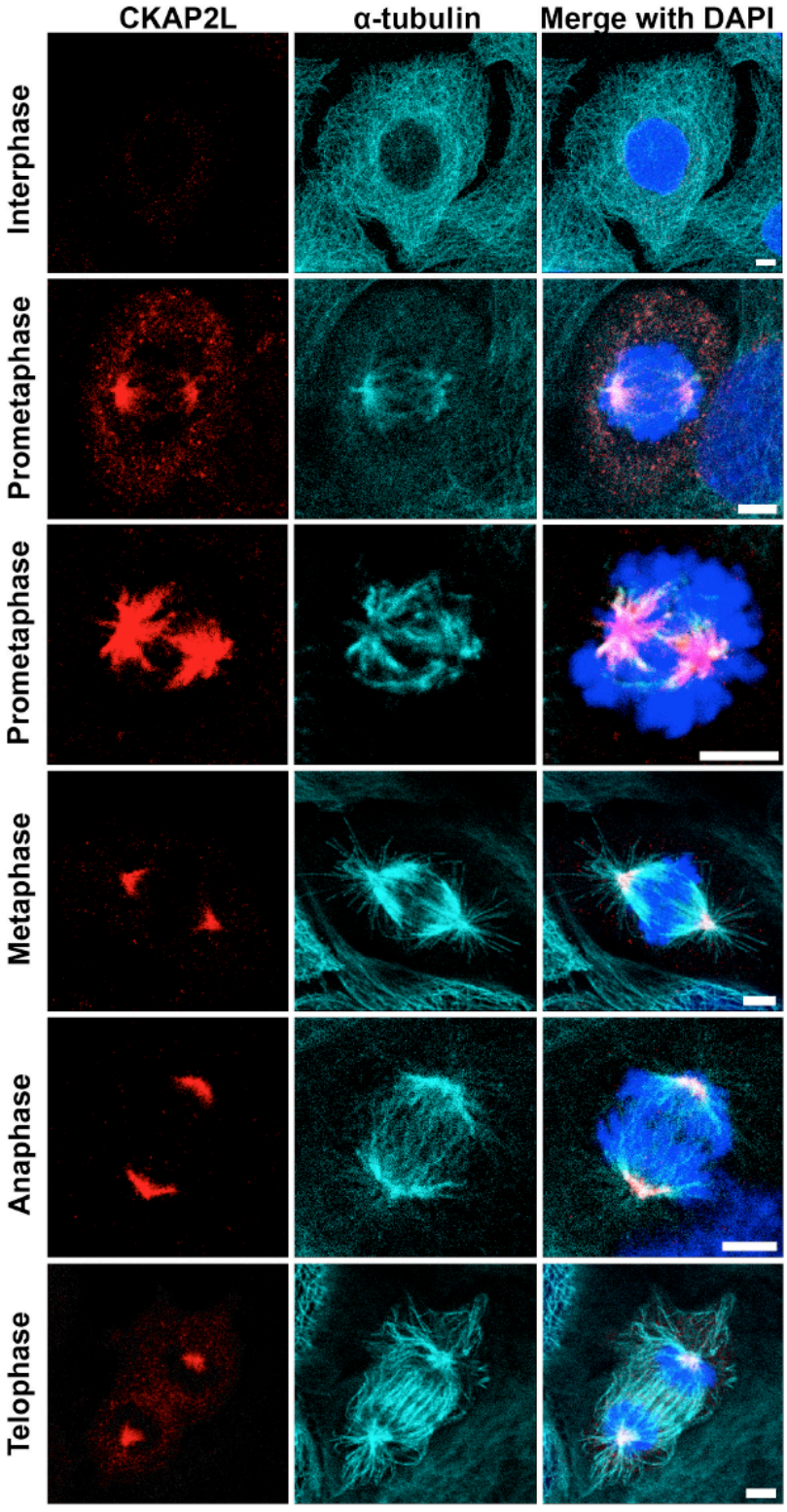


Figure S3. Subcellular Localization of CKAP2L in HaCaT Cells

Immunofluorescence analysis of HaCaT cells by using antibodies specific for CKAP2L (red) and α-tubulin (turquoise) revealed presence of CKAP2L at the spindle poles from prometaphase to telophase. Confocal microscopy also showed immunoreactivity of CKAP2L at spindle microtubules. Note, prometaphase cells show colocalization of CKAP2L with α-tubulin (spindle microtubules). Interphase cells did not show any reactivity for CKAP2L. Scale bar, 5 μm.

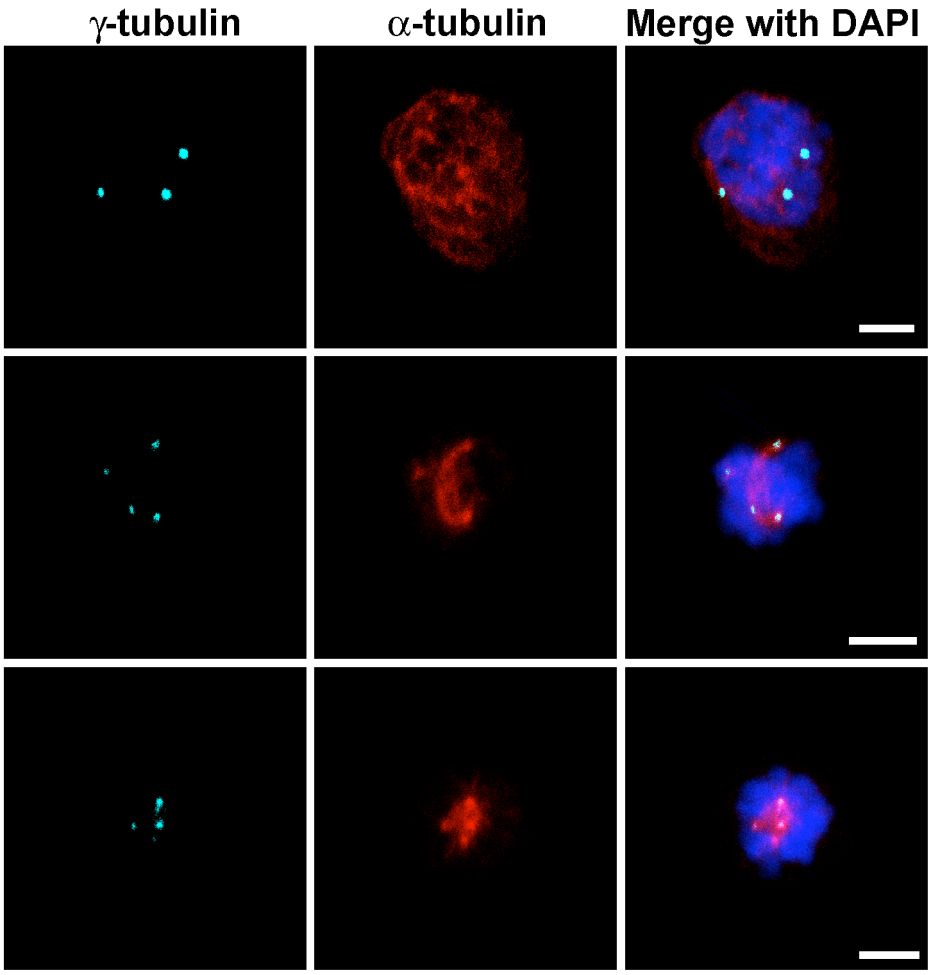


Figure S4. Supernumerary Centrosomes Identified in LCLs Carrying the c.571dupA Mutation
Confocal microscopy of mitotic LCLs of Filippi syndrome family 1 stained with antibodies against γ-tubulin (turquoise) and α-tubulin (red) revealed supernumerary centrosomes leading to multipolar spindle configuration. These cells also revealed the disorganization of the spindle microtubules. Scale bar, 5 μm.

Table S1. Parameter Settings for Variant Filtering with VARBANK

Filter Parameter	Values
Frequency range of reads showing the variation in contrast to wild type reads	75-100%
Maximal number seen in Epilepsy InhouseDB (n=511)	10
Maximal population variation frequency (taken from 1000genomes build 20110521 and EVS build ESP6500)	1%
Minimal read coverage	6
Minimal variation quality	10
Maximal target distance	100
Transcript biotypes	Protein coding transcripts
Variations overlap runs of homozygosity (ROH)	YES
Consequence types	Protein structure affected; Strong 5' or 3' splice site effects
Variation seen in both affected siblings	YES
Chromosomes	Autosomes