Supplemental Data

Mutations in the Cilia Gene *ARL13B*

Lead to the Classical Form of Joubert Syndrome

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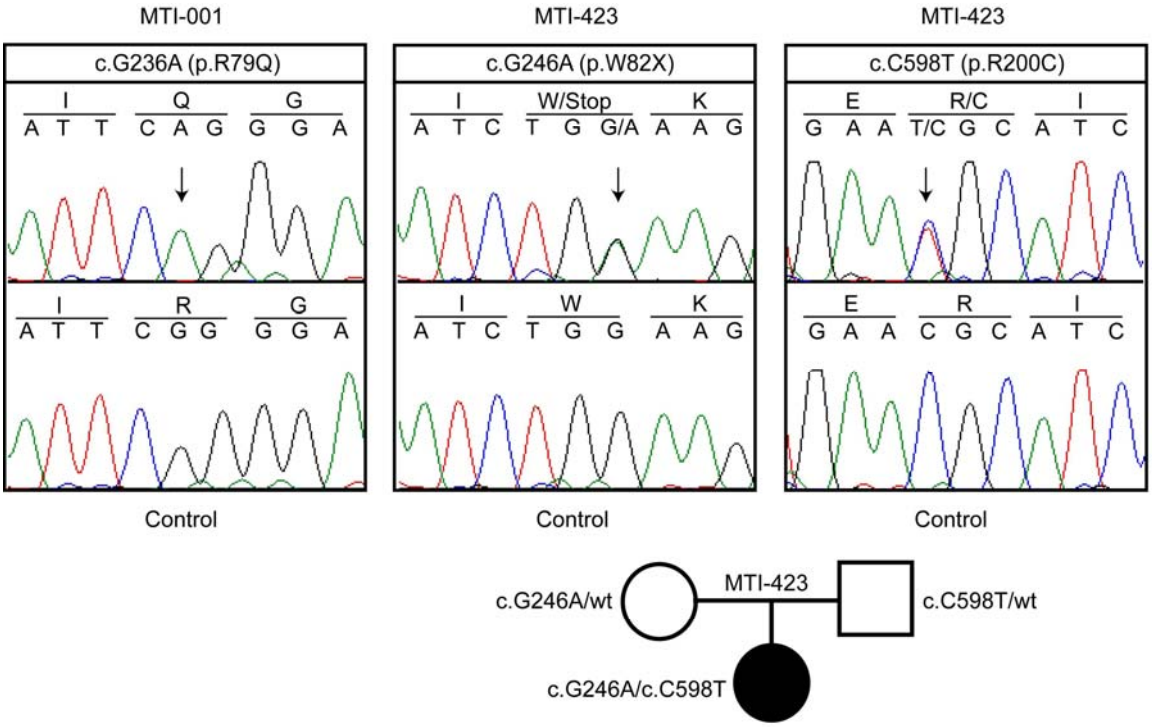

Figure S1. Sequence chromatograms two families with ARL13B mutations. Pedigree stucture for MTI-001 is provided in Fig. 1.
Left: Chromatogram for the homozygous c.G236A mutation in MTI-001, leading to an p.R79Q amino acid substitution. Middle: Chromatogram for the heterozygous c.G246A mutation, inherited from the mother, in MTI-423, leading to a premature stop codon (p.W82X).
Right: Chromatogram for the heterozygous c.C598T mutation, inherited from the father, in MTI-423, leading to an p.R200C amino acid substitution.
The affected girl has inherited both mutations. Control chromatograms at bottom.

Glutathione S-transferase

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFP
NLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVS
RIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALD
VVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGG
DHPPKSDLEVLFQGPLGSMFSLMASCCGWFKRWREPVRKVTLLMVGLDNAG
KTATAKGIQGEYPEDVAPTVGFSKINLRQGKFEVTIFDLGGGIRIRGIWKNYYA
ESYGVIFVVDSSDEERMEETKEAMSEMLRHPRISGKPILVLANKQDKEGALGE
ADVIECLSLEKLVNEHKCLCQIEPCSAISGYGKKIDKSIKKGLYWLLHVIARDFD
ALNERIQKETTEQRALEEQEKQERAERVRKLREERKQNEQEQAELDGTSGLA
ELDPEPTNPFQPIASVIIENEGKLEREKKNQKMEKDSDGCHLKHKMEHEQIET
QGQVNHNGQKNNEFGLVENYKEALTQQLKNEDETDRPSLESANGKKKTKKL
RMKRNHRVEPLNIDDCAPESPTPPPPPPPVGWGTPKVTRLPKLEPLGETHHN
DFYRKPLPPLAVPQRPNSDAHDVIS*

ARL13B

Figure S2. Sequence of the GST-ARL13B fusion, cloned into the pGEX-6P1 vector (see Methods section).
Blue shows GST sequence, black shows the polylinker sequence and red shows the ARL13B sequence. In green is the R79, which is mutated to Q in family MTI-001.

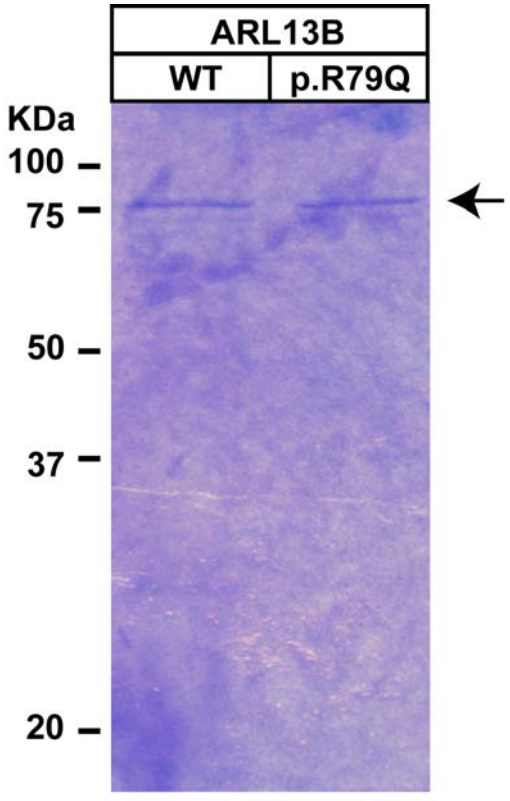

Figure S3. Coomassie-stained gel showing the purity of recombinant ARL13B wild-type and p.R79Q mutant protein (arrow).

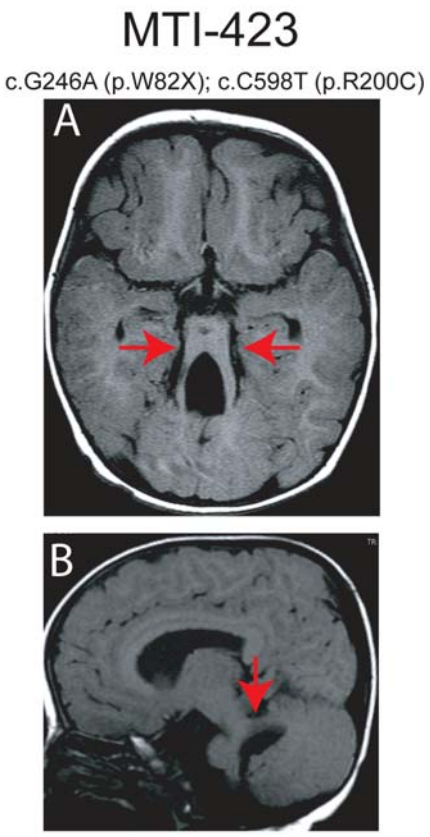

Figure S4. Molar tooth sign on brain MRI is evident in the affected girl from MTI-423 (red arrows in A).
Also evident is the horizontally-oriented superior cerebellar peduncle (red arrow in B), which is part of the JSRD diagnostic criteria.
Identified mutation is listed above the brain MRI.

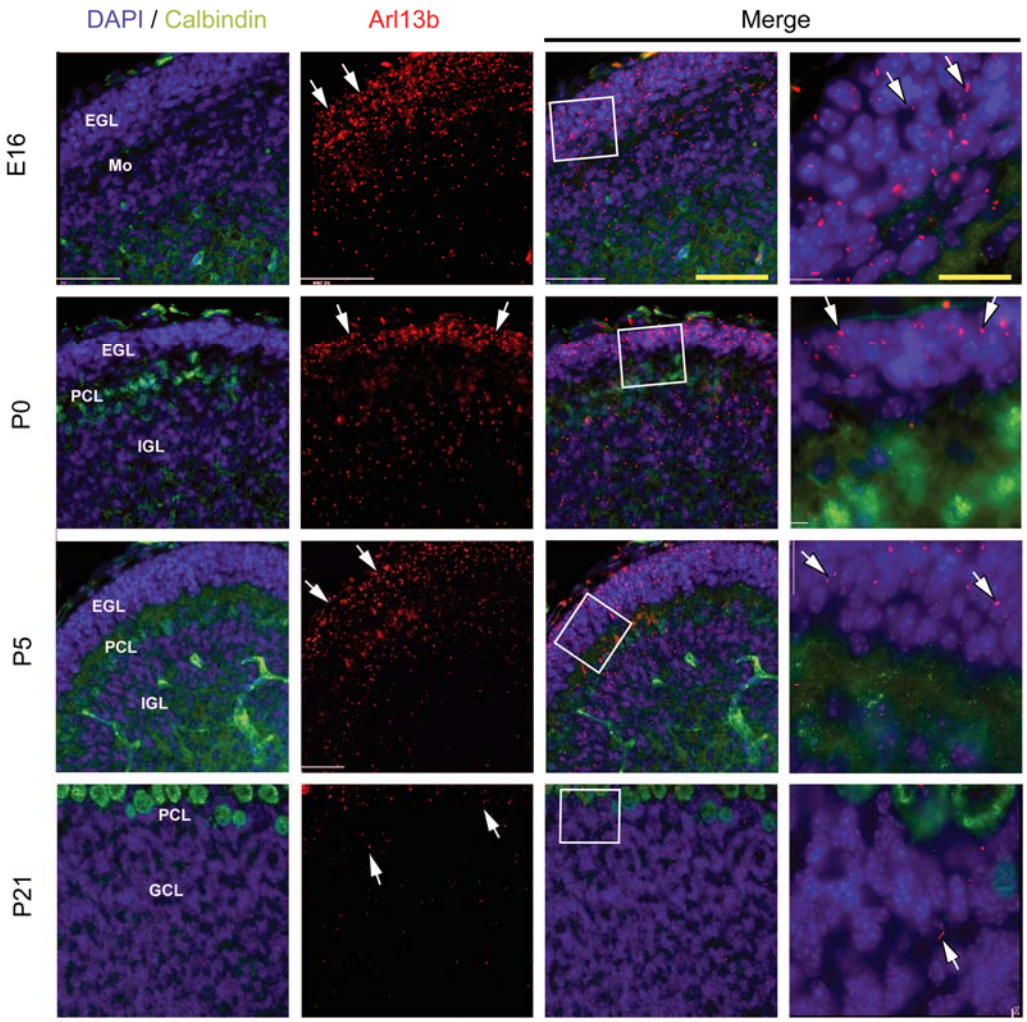

Figure S5. Arl13b is localized to cilia during the developmental spectrum of murine cerebellar development.
Wildtype cerebellar parasagittal sections at ages shown on left, stained with Dapi (nuclei, blue), Calbindin (Purkinje cells, green), and Arl13b (red). (Note that some blood vessels have stained positive for calbindin in the IGL at P5). Boxed region indicates area of higher power magnification at right. At E16, Arl13b-positive cilia-like structures are noted for about 40% of granule cells in the EGL (arrows). The percentage of cells with Arl13b-positive cilia-like structures drops appreciably during postnatal ages, and by P21, fewer than 1% of granule cells in the IGL show a notable cilia. GCL - granule cell layer, EGL - external granule layer, IGL - internal granule cell layer, Mo - Molecular layer, PCL - Purkinje cell layer, Scale bar (yellow) = 100 um (low power), 40 um (high power).

Supplementary Table 1. Clinical findings of patients with *ARL13B* mutations

Demographic information		
Family ID	MTI-001	MTI-423
Country of origin	Pakistan	USA
Patient (sex)	Three affecteds (IV-4, -6 and -8, see Fig. 1a)	One affected (female)
Death	IV-4 child died from complications of disease	N
Documented consanguinity	Y	N
Genetic results		
Nucleotide change (exon) amino acid change Allele 1	c.G236A (exon 3) p.R79Q	c.G246A (exon3) p.W82X
Nucleotide change (exon) amino acid change Allele 2	c.G236A (exon 3) p.R79Q	c.C598T (exon 5) p.R200C
Mutation type	Homozygous	Compound heterozygous
Neurological signs		
Hypotonia/ataxia	Y	Y
Psychomotor delay	Y	Y
Mental retardation	Y	Y
OMA	Y	Y
Breathing abnormalities	N	Y
Ocular signs		
Retinopathy	IV-4 (deceased) had retinopathy. Both IV-6 and IV-8 have normal retinas.	Normal exam
Other abnormalities	N	Ptosis
Coloboma	N	N
Renal signs		
NPH/UCD	N	N
Kidney ultrasound	Normal at around 5 years of age in all.	Normal at 13 months of age.
Other organs		
Liver abnormalities	N	N
Polydactyly	N	N
MRI reading		
MTS	Y	Y
Other abnormalities	IV-4 and IV-6 with small occipital encephalocele, not seen in IV-8	Mildly enlarged ventricles
Previous negative genetic screening		
Gene tested	*NPHP1 -, AHI1 -, CEP290 -, RPGRIP1L -, TMEM67 -*	*NPHP1 -, AHI1 -, CEP290 -, RPGRIP1L -, TMEM67 -*

Legend: ERG - electroretinogram, MTS - Molar tooth sign, NPHP - nephronophthisis, OMA Oculomotor apraxia, UCD - urinary concentrating defect, *AHI1 - ableson helper integration site 1, CEP290 - centrosomal protein 290, NPHP1 - nephrocystin 1, RPGRIP1L - RPGRIP1-like, TMEM67 (i.e. MKS3) - Meckel syndrome 3* .