

ARL13B and AR Joubert Syndrome

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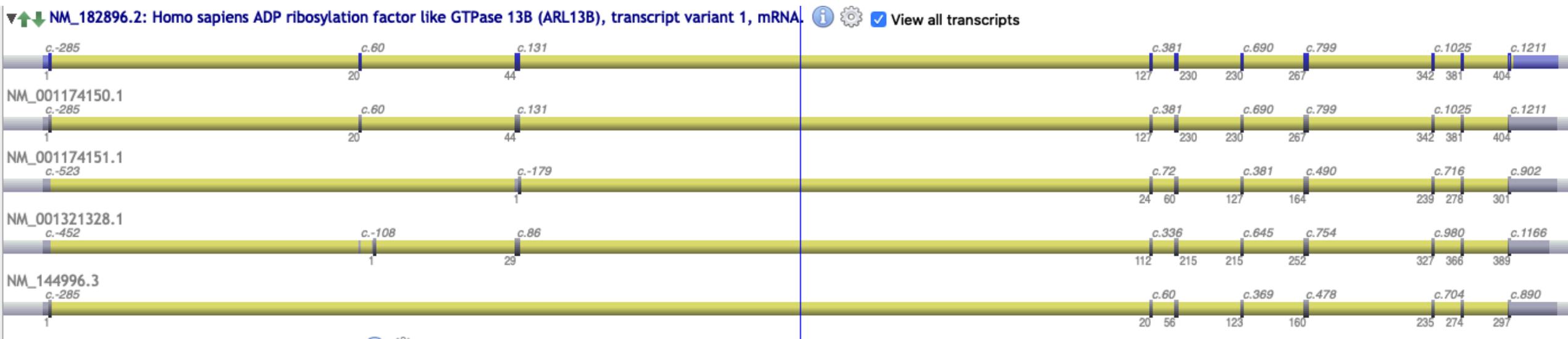
6.3.20

Syndromic Disorders GCEP

SOP version 7

ARL13B

- ADP-ribosylation factor like 13B
- Previous names: ARL2L1
- Member of the ARL family of small GTPases of the RAS super family. It is required for cilia formation.
- 5 curated RefSeq transcripts (longest is 11 exons/428 AA)



ARL13B

- Expression is ubiquitous in GTEx, but it was found to be expressed in all tissues affected by JS and localized to cilia in kidney, retina, and developing cerebellar granule neurons (Cantagrel 2008 PMID 18674751)
- Joubert syndrome can be caused by mutations in more than 30 genes. The proteins produced from these genes are known or suspected to play roles in primary cilia (GHR)

Joubert syndrome (AR)

- Congenital cerebellar ataxia
- Hypotonia
- Oculomotor apraxia
- “Molar tooth sign” (MTS)
Malformation of the midbrain-hindbrain junction

JSRD (Joubert syndrome related disorders), can include

- Leber congenital amaurosis
- nephronophthisis
- encephalocele
- polydactyly

Molar tooth sign in Joubert syndrome

CLOSE X

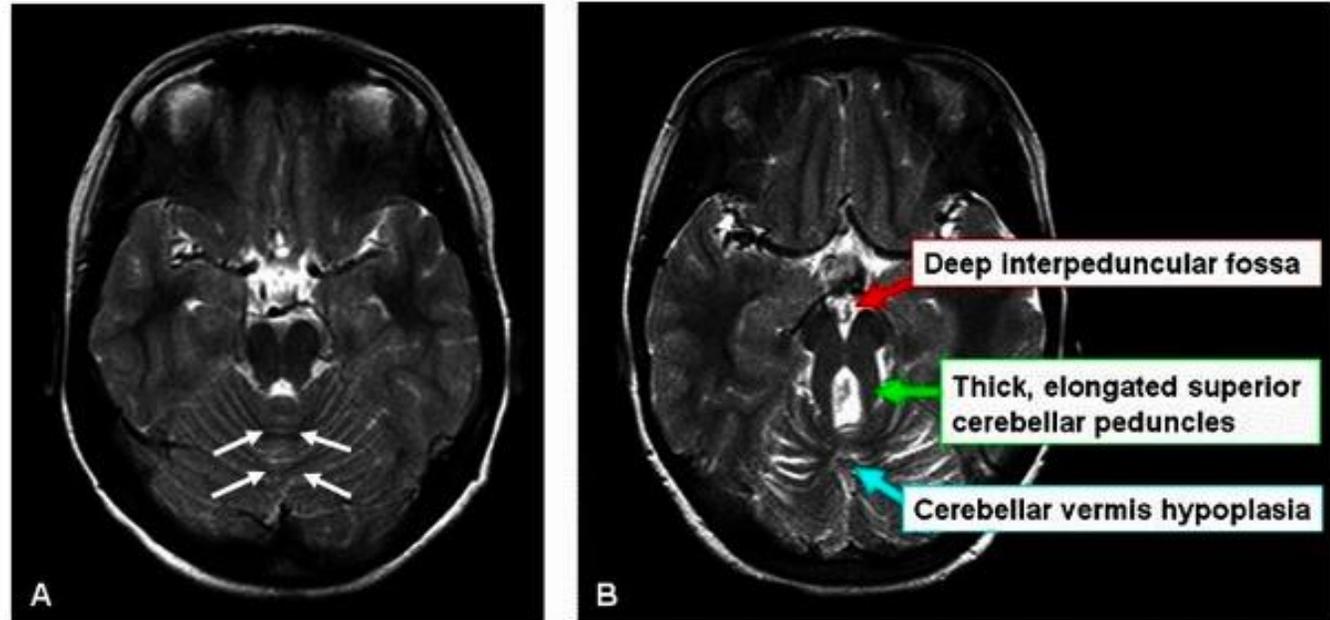
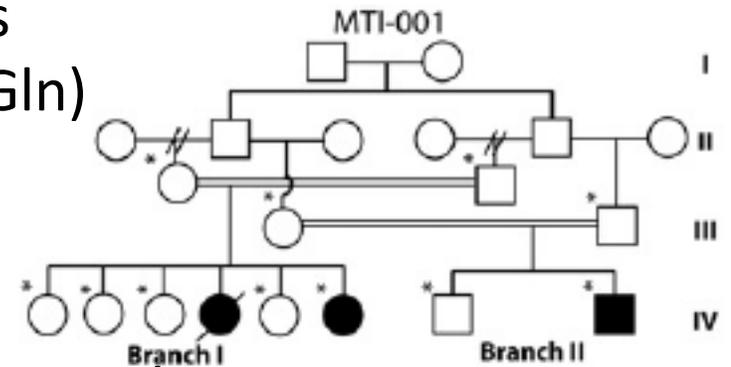


Image A shows an MRI image of a normal brain with an intact cerebellar vermis (indicated by white arrows). Image B shows an MRI image of the brain of a person with Joubert syndrome with arrows indicating the three components of the molar tooth sign.

First human paper: Cantagrel 2008 PMID 18674751

- Family MTI-001 (both affected had molar tooth sign on MRI, occipital encephalocele)
 - Pakistani consanguineous family, individuals were tested and negative for variants in known JS genes (NPHP1, AHI1, CEP290, MKS3, RPGRIP1L)
 - Linkage analysis was performed and candidate genes ARL6 AND ARL13B were sequenced (40 genes in the linkage interval), LOD 3.0
 - Both living affected individuals were homozygous for NM_001174150.2(ARL13B):c.236G>A (p.Arg79Gln) (present in only 1 Latino allele in gnomAD)



0.5 points (downgraded for consanguinity)

Family does not have enough affecteds to score segregation

Cantagrel 2008 PMID 18674751

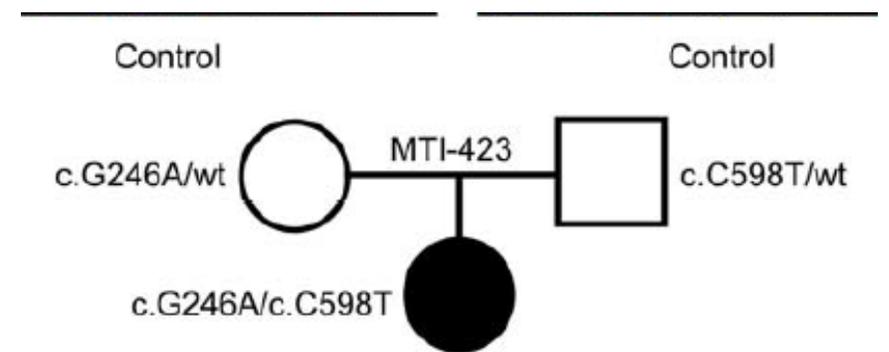
Family MTI-423 (from USA)

- Identified from a cohort of 182 individuals with JS and related disorders, sequenced for ARL13B only
- One affected female with molar tooth sign on MRI
- Compound het for NM_001174150.2(ARL13B):c.246G>A (p.Trp82Ter) and NM_001174150.2(ARL13B):c.598C>T (p.Arg200Cys)

(both variants absent from gnomAD)

- Confirmed in trans because parents tested

2 points, standard for LoF/missense in trans



Bachmann-Gagescu 2015 PMID 26092869

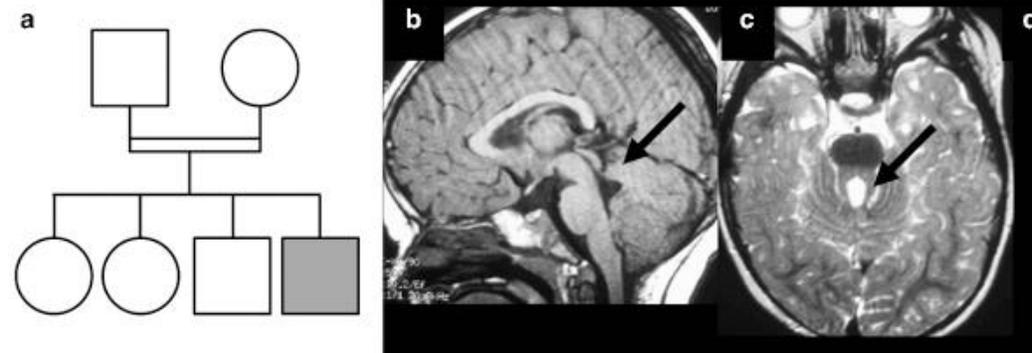
- 27 JS-associated genes were sequenced in 375 families
- 2 individuals were identified with variants in ARL13B
 - One had the same variants as the proband from MTI-423, so I did not score as likely that is the same person (both from USA, overlapping authors)
 - UW203-3 harbored two missense variants:
 - NM_182896.2:c.65T>G (p.Val22Gly) (absent from gnomAD)
 - NM_182896.2:c.461A>G (p.Asn154Ser) (only 2 alleles in gnomAD)
 - Present in a large supplementary table of variants, so no further phenotype information provided (family testing may have been done, but no information provided)
 - **1 point** (standard score, but we could downgrade because of lack of specific phenotype/or cis/trans data)

Thomas 2015 PMID 25138100

- Case report of a consanguineous family from Tunisia
- Linkage analysis was done and ARL13B only sequenced
- hypotonia, ataxia, breathing anomalies, oculomotor apraxia, abnormal eye movements, severe developmental delay and obesity, MTS
- Homozygous for (proband only tested for variant)

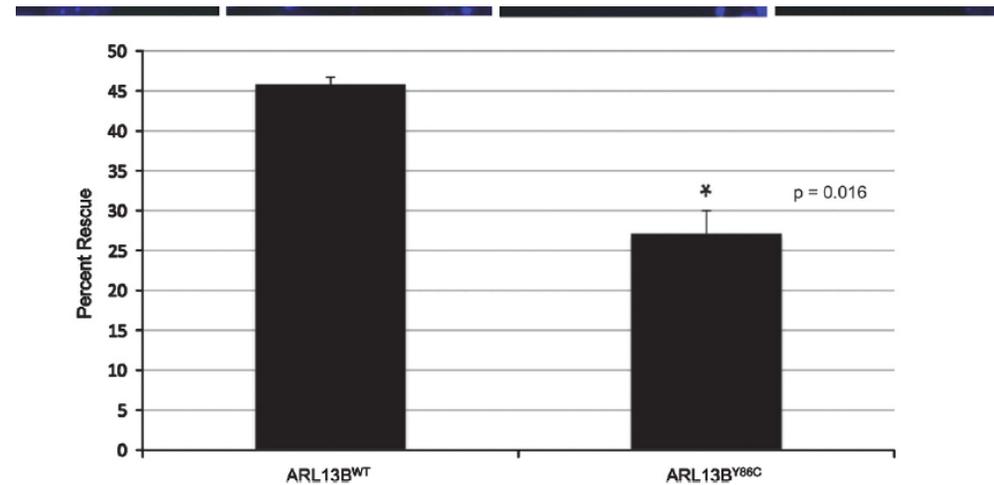
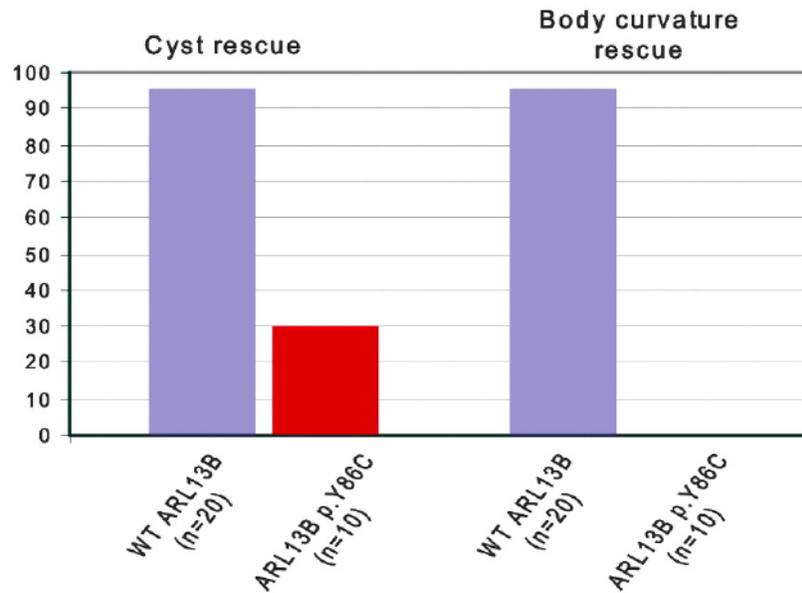
NM_182896.2:c.257A4G (p.Tyr86Cys)
(absent from gnomAD)

- 0.5pts downgraded for
Consanguinity



Thomas 2015 PMID 25138100

- The p.Tyr86Cys variant was injected into *scorpion* single cell embryos and did not significantly rescue the curled tail or kidney phenotype
- When transfected into MEFs from the *hennin* mouse, it did not fully rescue cilia formation either



Kang 2016 PMID 27491411

- 55 patients with Nephronophthisis-related ciliopathy (NPHP-RC) were exome sequenced and one variant was identified

K-10 (Korean):

- End stage renal disease at 3yrs of age, retinal dystrophy, hepatic fibrosis
- NM_001174151.1:c.259A>G (p.Ile87Val)
- Patient was present in a table and zygosity was not indicated. Phenotype also may not be appropriate to score for a JS curation?

- Did not score

Shaheen 2016 PMID 27894351

- 265 families with a range of ciliopathies were exome sequenced
F-170 (JS), Consanguineous
- Abnormal breathing, DD, dysmorphic features, polymicrogyria, hydrocephalus, MTS, hypotonia, oculomotor apraxia, scoliosis, renal failure
- Homozygous for NM_001174150.1:c.765T>A: p.(Asn255Lys)
(absent from gnomAD)

0.5pts downgraded for
Consanguinity

Shaheen 2016 PMID 27894351

F-188 (JS), Consanguineous

- Retinal dystrophy, DD, normal kidneys, bilateral hypermetropia, MTS-like
- Homozygous for NM_001174150.1:c.599G>A: p.(Arg200His)
(absent from gnomAD)

0.5pts downgraded for
Consanguinity

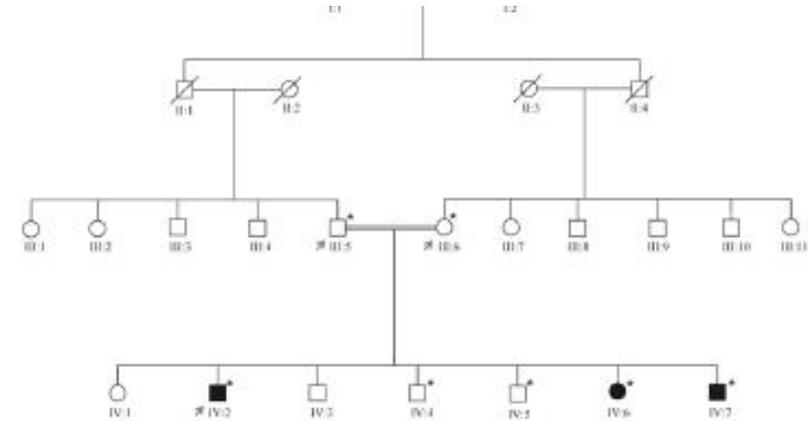
Rafiullah 2017 PMID 29255182

Family A

- Case report of two consanguineous families from Pakistan. Patients from both families have ID and ocular abnormalities. MTS in Fam A
- (both homozygous for the NM_001174150.1 :c.223G>A (p.(Gly75Arg) variant), WES performed (absent from gnomAD)

Family A: 3 affecteds, 2 unaffecteds

0.5 points for variant in consanguineous family



IV:2

IV:2

IV:6

IV:7

Rafiullah 2017 PMID 29255182

- Family B
- NM_001174150.1
:c.223G>A (p.(Gly75Arg))

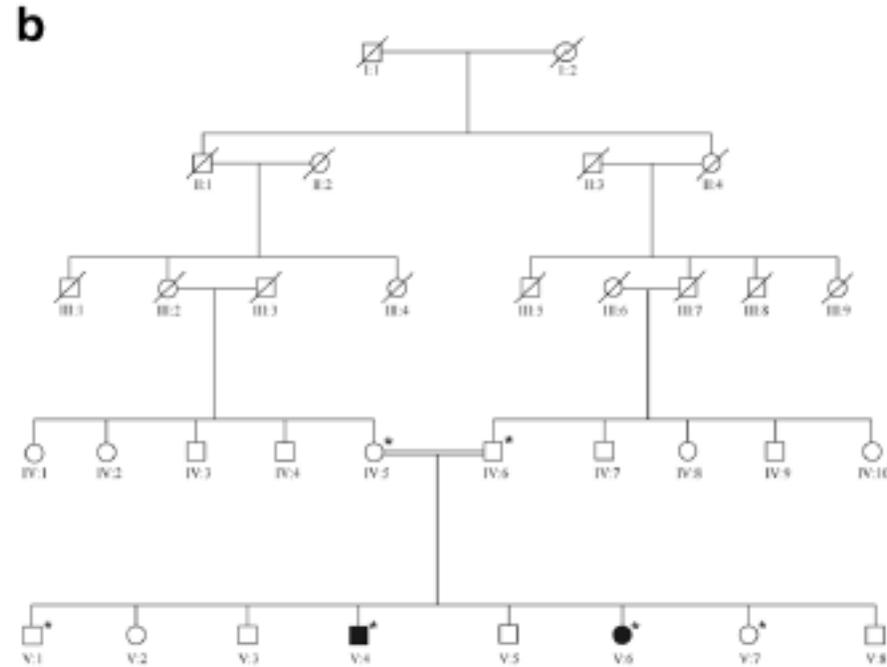
- 2 affecteds
- 2 unaffecteds tested

Scored variant only once

- Combined Fam A and B for Segregation

LOD 2.61 (calculated)

- 1 point



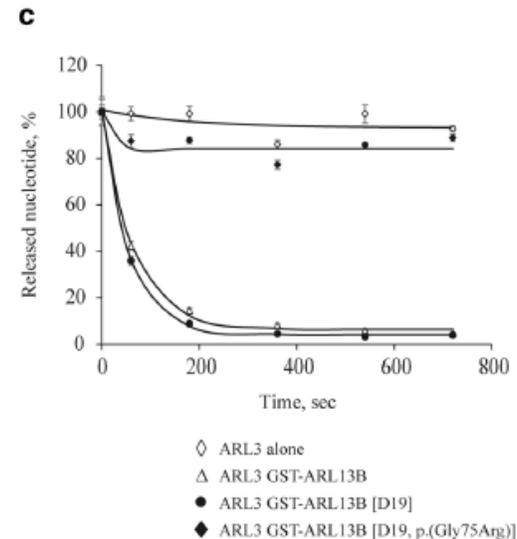
V:4



V:4

Rafiullah 2017 PMID 29255182

- The p.Gly75Arg variant rescued the cilia formation defect in *hennin* MEFs
- However, ARL13B with the variant displayed a displayed a marked loss of ARL3 guanine nucleotide-exchange factor activity when transfected into HEK cells



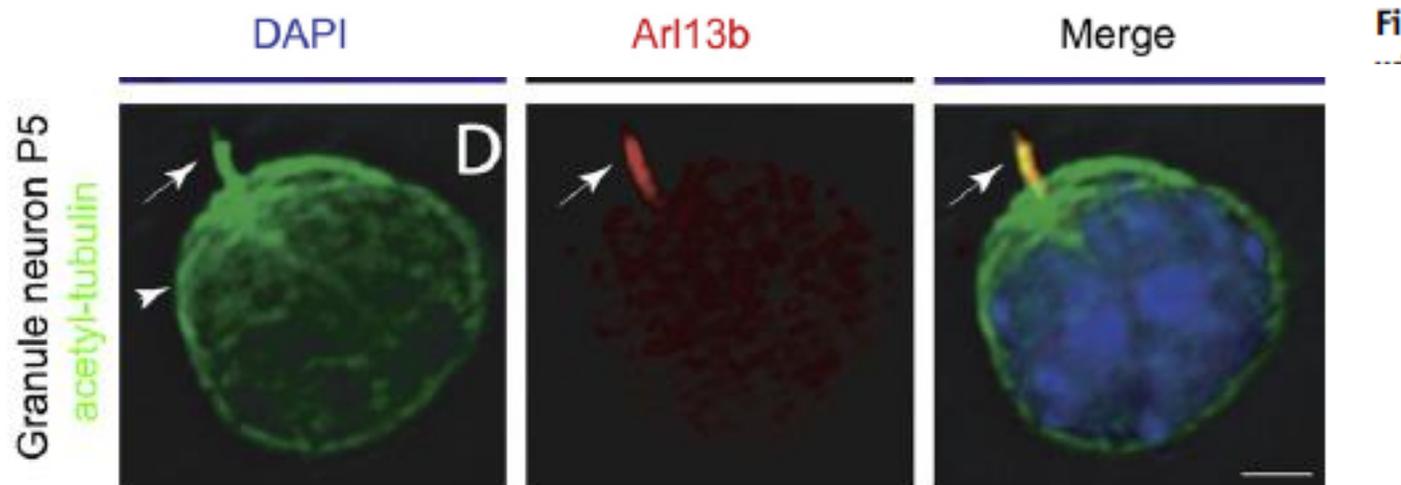
Otto 2011 PMID:21068128

- 120 individuals with severe Nephronophthisis-associated ciliopathies were tested for variants in 18 associated genes.
- The p.R390L in ARL13B was identified in the heterozygous state in one consanguineous individual from Pakistan, but a second variant was not identified. Further phenotype information was not provided.
- **Did not score this variant.**

Experimental Evidence

Cantagrel 2008 PMID 18674751

- Expression (**standard 0.5pts**):
 - ARL13B is expressed in all tissues affected by JS and localized to cilia in kidney, retina, and developing cerebellar granule neurons
 - Measured expression in mouse organ sections with IF
 - This was also demonstrated in Caspary 2007 PMID 17488627



Thomas 2015 PMID 25138100

- Expression: ISH was used to investigate ARL13B in human embryo
- ARL13B was detected in the alar and basal plate of the myelencephalon, as well as in the mesencephalon and metencephalon at CS16
- At CS19, ARL13B is expressed in the ventricular layer of the diencephalon and myelencephalon as well as the tegmentum of the pons and the cerebellar rhombic lips
- ARL13B transcript is also found in dorsal root ganglia, the vestibular ganglion and within the neuronal epithelium surrounding the otic vesicle

0.5 additional points because studies done in human

Cantagrel 2008 PMID 18674751

Scorpion zebrafish animal model (genes ~60% identical)

- Caused by a retroviral insertion in the first exon of ARL13B, which inactivates the gene
- Curved tail and cystic kidneys
- None of these patients have cystic kidneys, but it is a possible feature of JSRD

1 point?

Downgraded because it is a zebrafish and doesn't
Exactly fit the human phenotype



WT



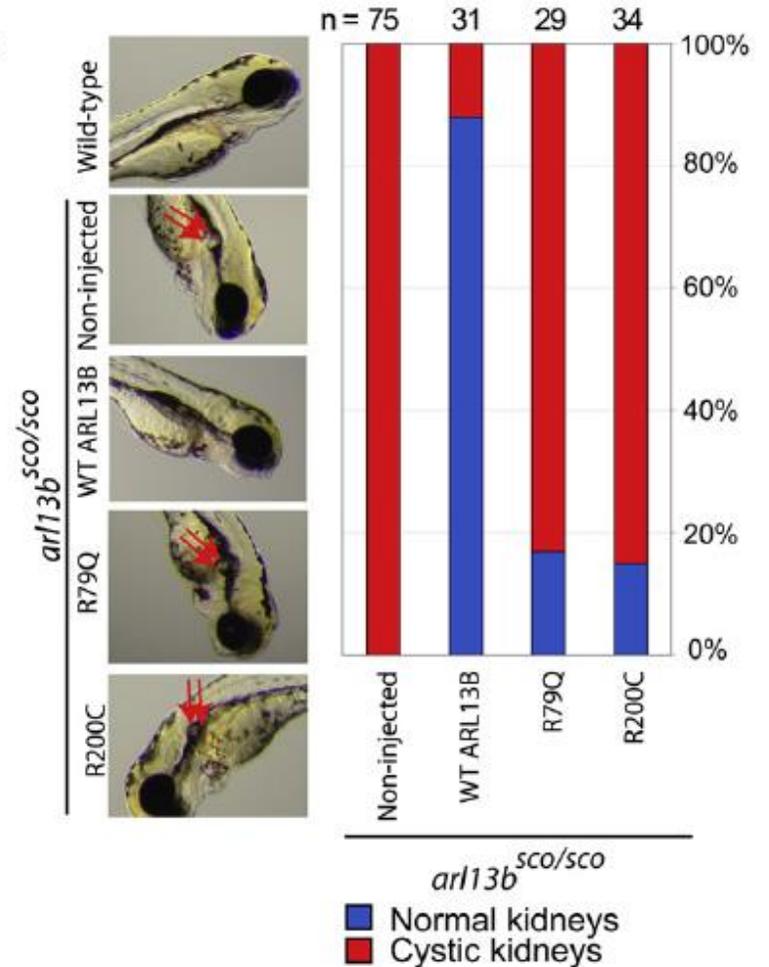
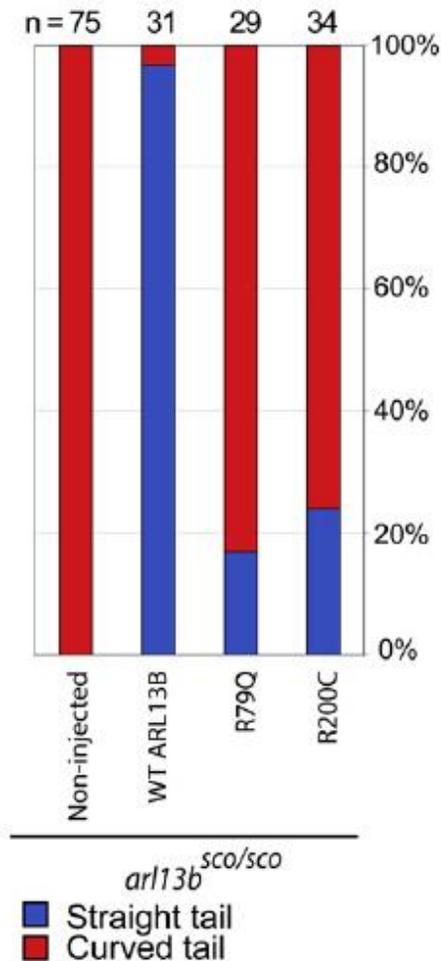
Scorpion

Cantagrel 2008 PMID 18674751

- Injected RNA of human sequence into 1 cell stage embryos

WT ARL13B rescued
Tail and kidney
Phenotype

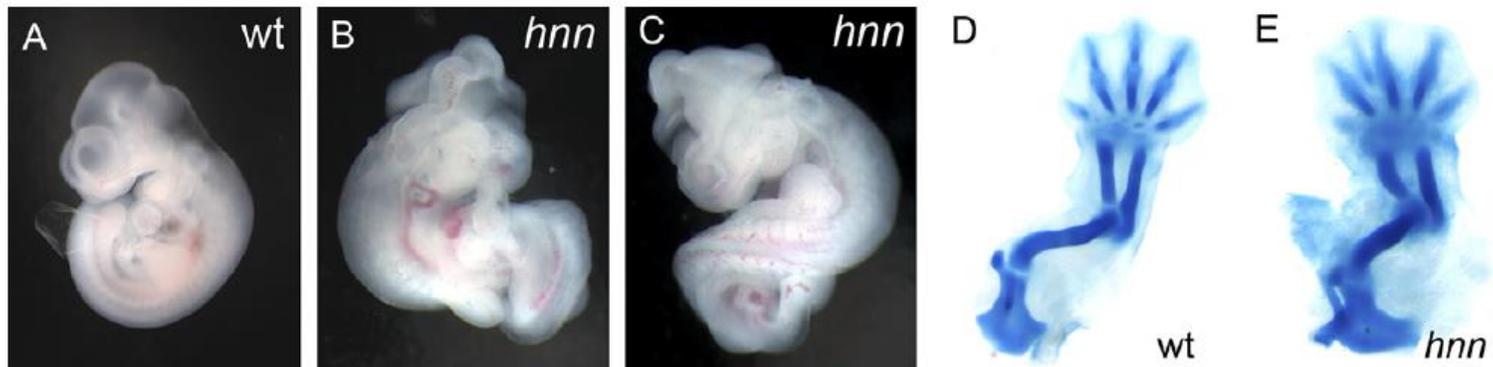
(The variants did not)
2 points, standard rescue



Caspary 2007 PMID 17488627

There is also a retina-specific KO mouse and a KO yeast model

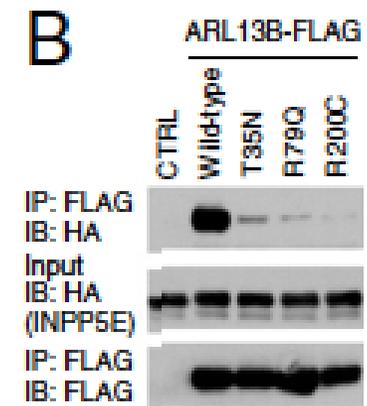
- *Hennin* mouse discovered in ENU mutagenesis screen identifying mutations that disrupted the E9.5 mouse embryo
- Sequencing identified a variant in the splice acceptor of exon 2
- RT-PCR analysis confirmed that transcripts lacked exon 2
- The mouse is characterized by neural tube defects such as exencephaly and spina bifida, polydactyly, and nodal cilia are shortened



1 point because phenotype
Is more severe and doesn't exactly
match

Humbert 2012 PMID 23150559

- INPP5E (not currently curated by our group, but over 50 variants identified in individuals with JS) physically interacts with ARL13B
- (Co-immunoprecipitation in transfected HEK cells)
- The variants we've scored (R79Q, R200C) disrupt this interaction



- **0.5 points** (standard points, but we could downgrade or not score if we would want to curate INPP5E first)

Total Score

- 6.5 points genetic evidence (5.5 of case level and 1 of segregation)
- 5.5 points experimental evidence
- 12.0 points total
- Replicated over time
- DEFINITIVE
- If we would like more genetic evidence, there is one LoF variant from *Invitae* we might be able to score

Evidence Type				Count	Total Points	Points Counted		
Genetic Evidence	Case-Level	Variant	Autosomal Dominant OR X-linked Disorder	Proband with other variant type with some evidence of gene impact		0	0	0
				Proband with predicted or proven null variant		0	0	0
				Variant is <i>de novo</i>		0	0	0
		Autosomal Recessive Disorder		Two variants (not predicted/proven null) with some evidence of gene impact in <i>trans</i>		7	3.5	5.5
				Two variants in <i>trans</i> and at least one <i>de novo</i> or a predicted/proven null variant		1	2	
		Segregation				Summed LOD	Family Count	1
	Candidate gene sequencing			0	0			
	Exome/genome or all genes sequenced in linkage region			2.91	1			
	Total Summed LOD Score			2.91				
	Case-Control				0	0	0	
	Genetic Evidence Total						6.5	
	Experimental Evidence	Functional		Biochemical Functions		0	0	1.5
Protein Interactions				1	0.5			
Expression				2	1			
Functional Alteration		Patient cells		0	0	0		
		Non-patient cells		0	0			
Models		Non-human model organism		2	2	4		
		Cell culture model		0	0			
Rescue		Rescue in human		0	0			
		Rescue in non-human model organism		1	2			
		Rescue in cell culture model		0	0			
		Rescue in patient cells		0	0			
Experimental Evidence Total						5.5		
Total Points						12		