Istituto C.S.S. Mendel

Fondazione Casa Sollievo della Sofferenza

Italian Society of Pediatric Nephrology
Milan, October 24, 2012

Joubert syndrome and related disorders: a paradigm to understand the complexity of ciliopathies

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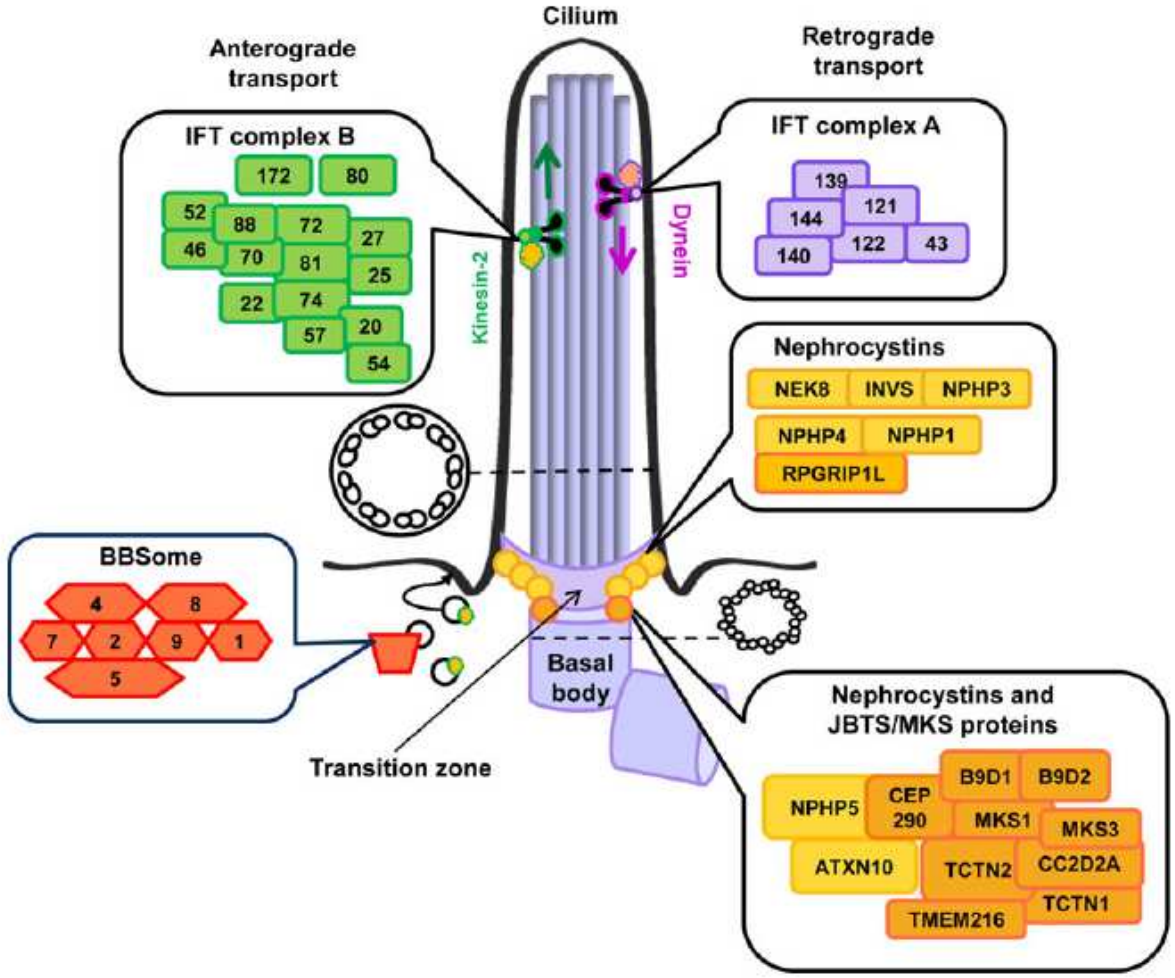
University of Salerno

The primary cilium

membrane-enclosed antenna-like structure with a ring-shaped skeleton (9+0 doublets of mt), a basal body (triplets of mt) and a transition zone

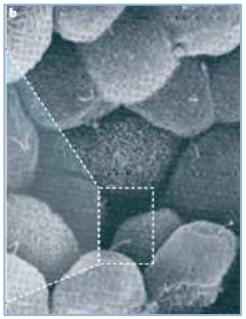


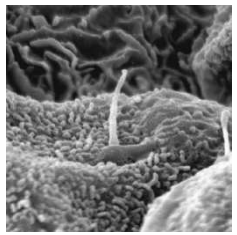
- up to 1000 proteins involved
- mutations identified in over 50 disease-genes
- about 100 disorders may be driven by cilia abnormalities
- minimal estimated collective incidence: 1/1000 conceptuses

Arts & Knoers, Ped Nephrol 2012

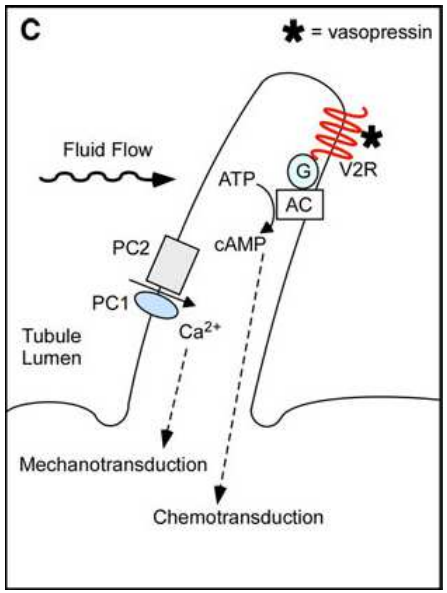
A truly multitasking organelle

Kidney and bile ducts epithelial cells



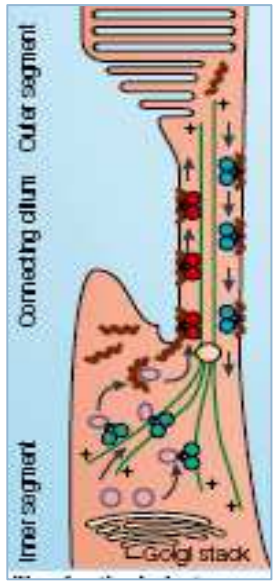


Primary cilia are present on the surface of nearly all cell types, both pre- and post-natally

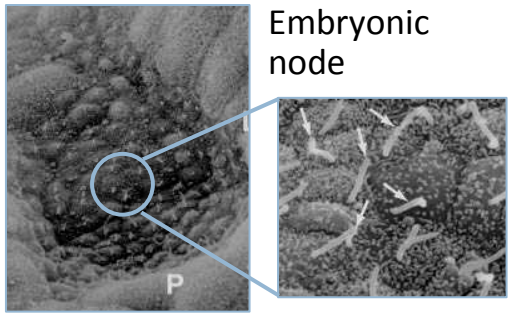


In many tissues, primary cilia link mechanosensory, visual and osmotic stimuli to cell-cycle control and epithelial cell polarity.

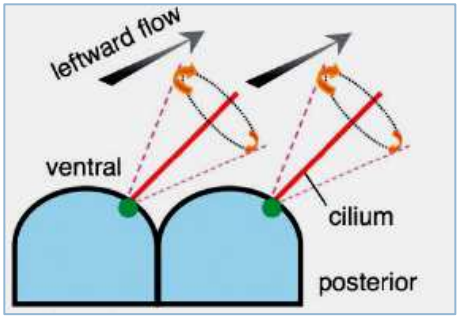
Retinal photoreceptors



In the embryonic node (a transient structure during gastrulation), motile nodal cilia generate a leftward nodal flow that is essential for L-R axis determination.



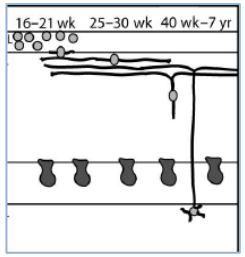
Embryonic node

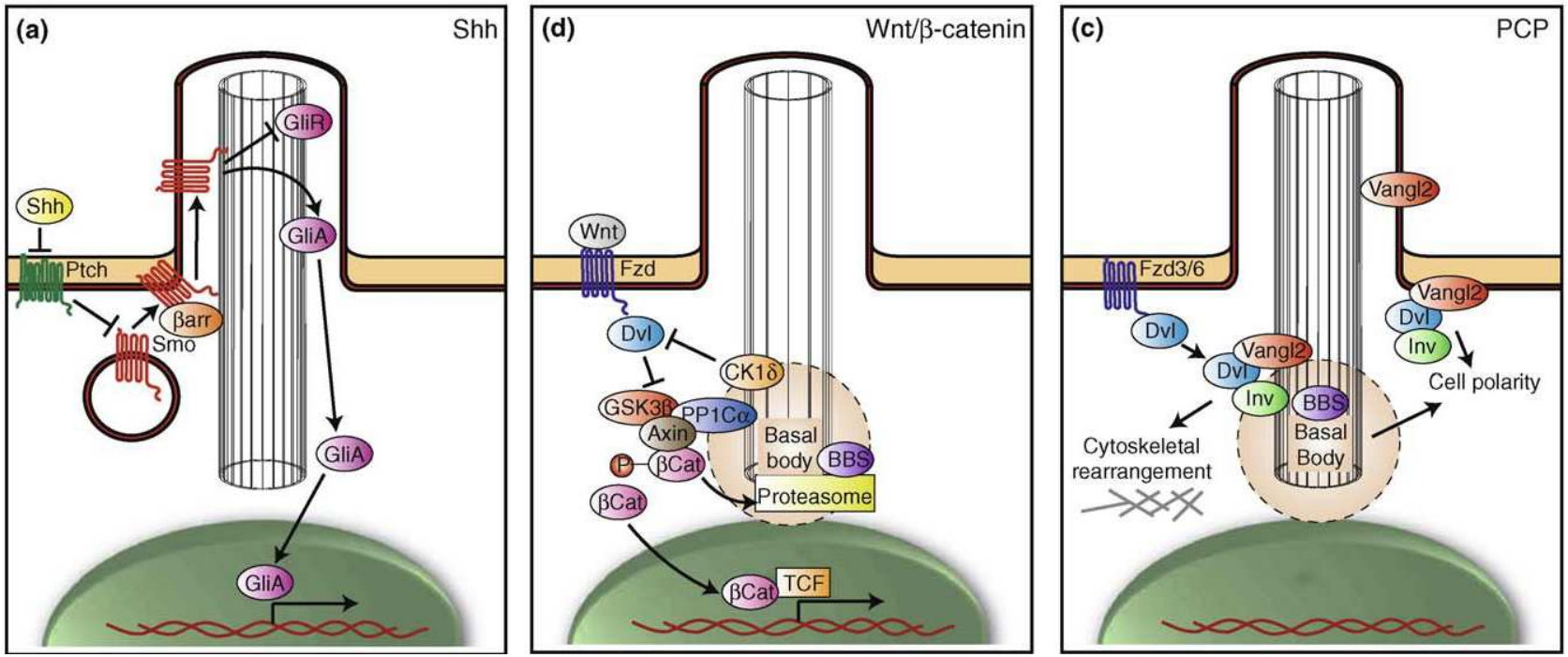


Primary cilia play a key role during development

Primary cilia control neural and limb patterning, by modulating:

- Sonic Hedgehog pathway
- Wnt / beta-catenin pathway
- planar cell polarity pathway





Goetz et al, Nat Rev Genet 2010

Common features of ciliopathies

- Disorders caused by genes encoding for proteins of the primary cilium and its apparatus (basal body, centrosome)
- Variable severity and multiorgan involvement
- Clinical and genetic overlap among distinct conditions

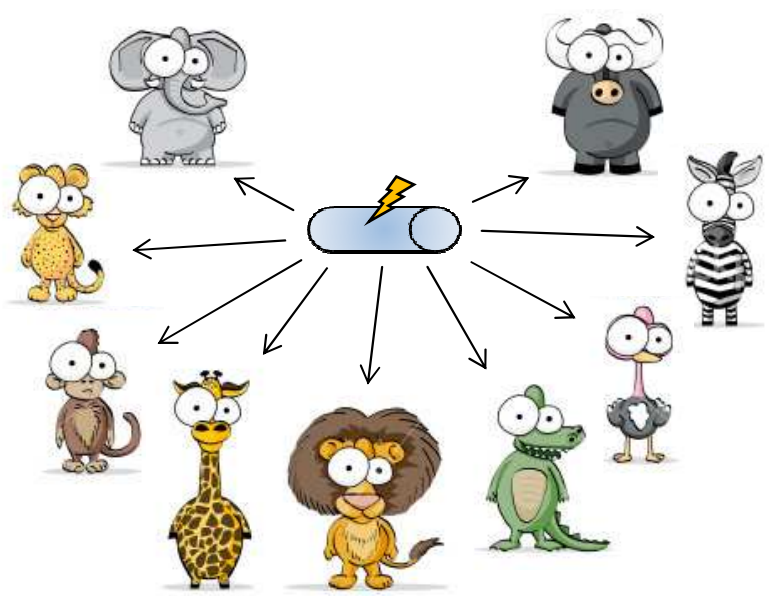
	BBS	MKS	JBTS	NPHP	SLSN	OFD1	CED	ATD	SRP
Cystic kidneys	□	□	□	□	□	□	□	□	□
Hepatobiliary disease	□	□	□	□		□	□	□	□
Retinal degeneration	□	□	□		□	□	□	□	
Laterality defects	□	□		□	□			□	□
Intellectual disability	□	□	□			□		□	
Cerebellar vermis hypoplasia			□			□	□	□	□
Encephalocele		□	□						
Polydactyly	□	□	□			□	□	□	□
Obesity	□								
Shortening/bowing of bones		□					□	□	□
Ectodermal dysplasia						□	□		□

Arts & Knoers, Ped Nephrol 2012

Ciliopathies: the concept of «splitting and lumping»

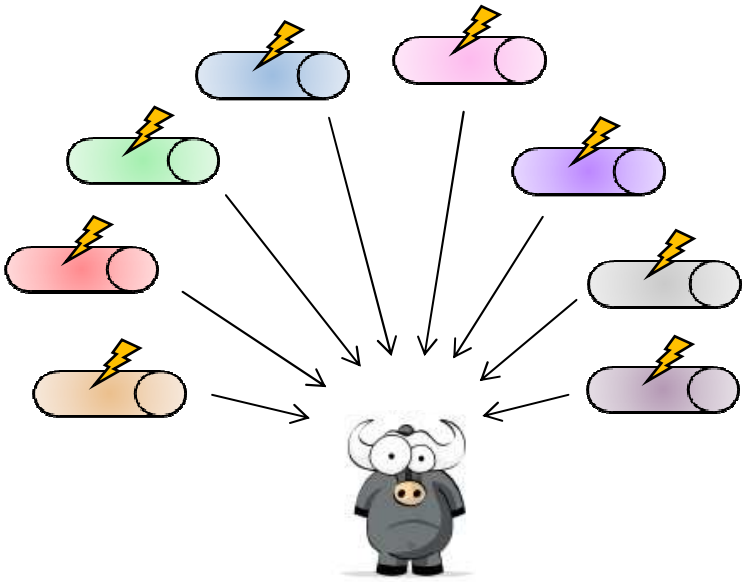
SPLITTING

same gene → distinct phenotypes



LUMPING

distinct genes → same phenotype



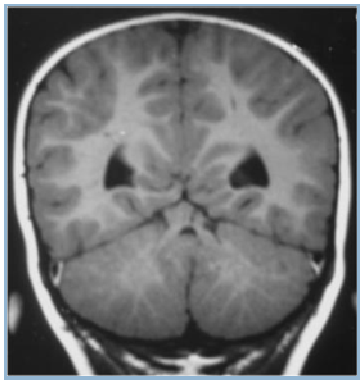
Joubert syndrome

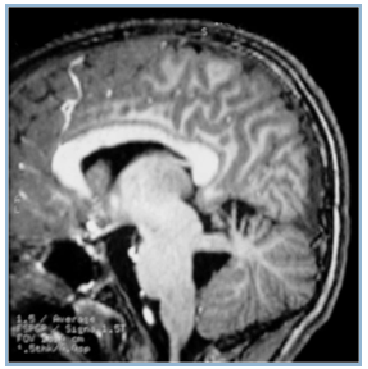
Reprinted with permission from *Neurology*, 1969;19:813–825.

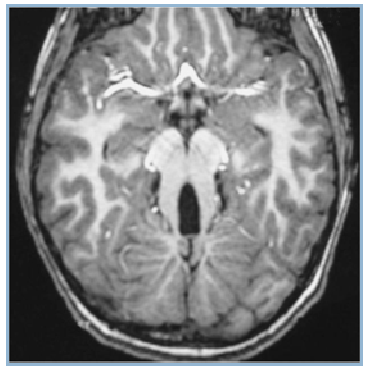
Familial Agenesis of the Cerebellar Vermis: A Syndrome of Episodic Hyperpnea, Abnormal Eye Movements, Ataxia, and Retardation

Marie Joubert, MD; Jean-Jacques Eisenring, MD; J. Preston Robb, MD; Frederick Andermann, MD

- Autosomal recessive condition (three affected siblings)
- Hypotonia and ataxia
- Oculomotor apraxia, other eye movement anomalies
- Developmental delay, mental retardation
- Neonatal breathing abnormalities
- Behavioural problems







Molar Tooth Sign

Starting from the MTS: the expanding group of JSRD

Joubert syndrome (JS)
- neurological features, MTS
- ± *postaxial polydactily*
- ± *encephalocele*
- ± *posterior fossa cyst*

COACH and Gentile syndromes
- neurological features, MTS
- hepatic fibrosis
- ± *coloboma*
- ± *renal disease*

Senior-Loken syndrome
- Leber congenital amaurosis
- nephronophthisis
- ± *neurological features, MTS*

Dekaban-Arima syndrome (DAS)
- neurological features, MTS
- Leber congenital amaurosis
- cystic dysplastic kidneys
- ± *coloboma*
- ± *postaxial polydactily*

Varadi-Papp syndrome (OFD VI)
- neurological features, MTS
- midline orofacial dysplasia
- polydactyly, Y-shaped central metacarp
- ± *hypothalamic hamartoma*
- ± *periventricular nodular heterotopia*
- ± *congenital heart disease*

•MALTA syndrome
- neurological features, MTS
- encephalocele
- hydrocephalus
- renal cystic disease
- ± *coloboma*
- ± *retinal dystrophy*

JS + retinopathy
- neurological features, MTS
- Leber congenital amaurosis or other retinopathy
- ± *postaxial polydactily*
- ± *encephalocele*

Marsh syndrome
- neurological features, MTS
- white matter cysts
- renal cysts

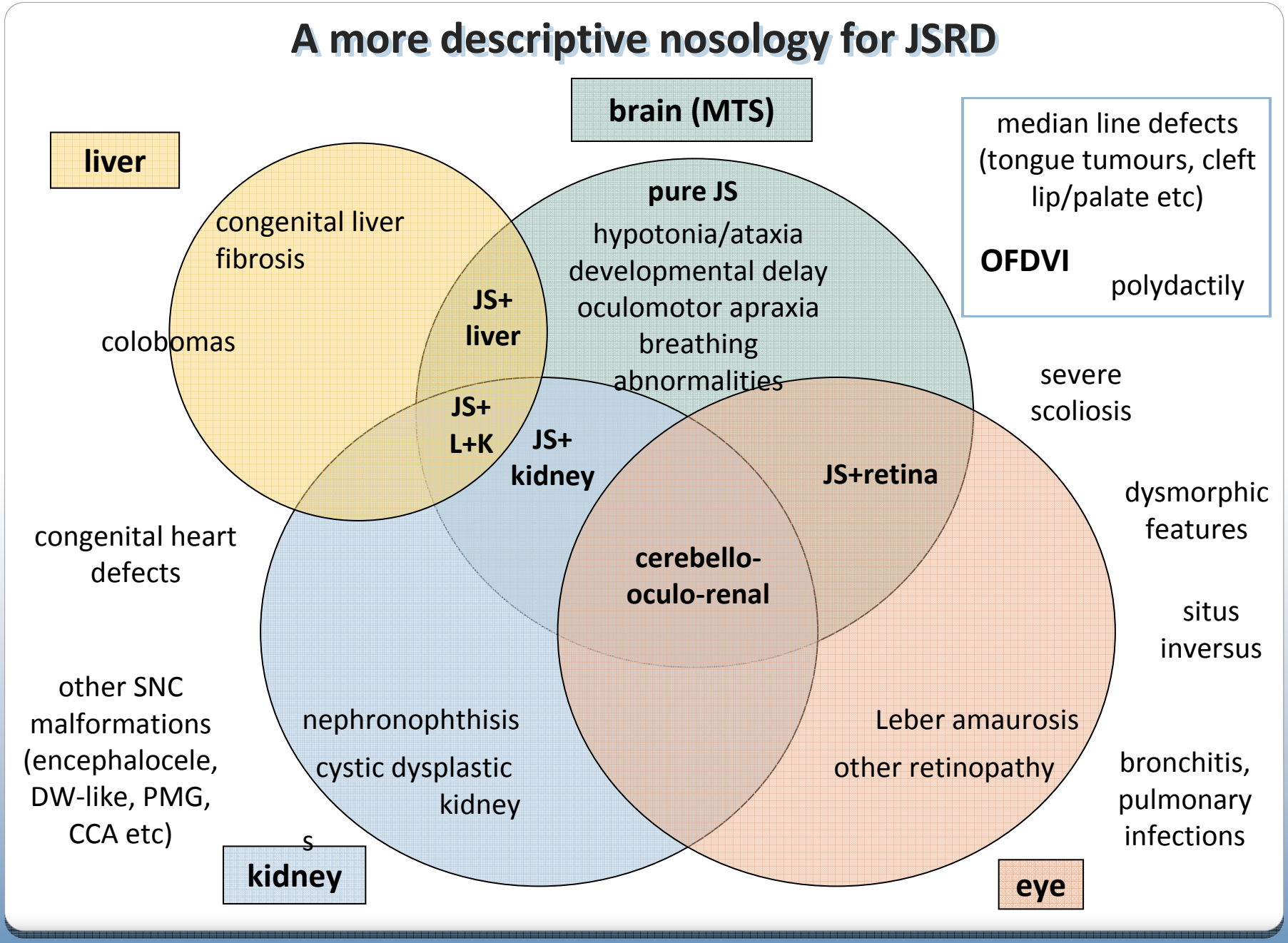
Al Gazali-Sztriha syndrome
- neurological features, MTS
- absent pituitary gland

JB + polymicrogyria
- neurological features, MTS
- cortical polymicrogyria

JS + nephronophthisis
- neurological features, MTS
- nephronophthisis

Gleeson et al, AJMG 2004

A more descriptive nosology for JSRD



Valente et al, EJMG 2008

Genetic heterogeneity in JSRD

	locus	gene/protein	JSRD	MKS	NPH/SLS
2004-2010	9q34	INPP5E	JBTS1		
	11	TMEM216	JBTS2	MKS2	
	6q23	AHI1/Jouberin	JBTS3		
	2q13	Nephrocystin	JBTS4		NPHP1/SLSN1
	12q21	CEP290	JBTS5	MKS4	NPHP6/SLSN6
	8q24	TMEM67/Meckelin	JBTS6	MKS3	NPHP11
	16q	RPGRIP1L	JBTS7	MKS5	NPHP8
	3q11	ARL13B	JBTS8		
	4p15	CC2D2A	JBTS9	MKS6	
	Xp	CXORF5/OFD1	JBTS10		
2011-2012	2q24	*TTC21B*	*JBTS11*	*MKS*	
	15q26	KIF7	JBTS12		
	12q24	TCTN1	JBTS13		
	12q24	TCTN2	JBTS	MKS8	
	2q33	TMEM237	JBTS14		
	7q32	TSGA14/CEP41	JBTS15	MKS	
	11	TMEM138	JBTS16	MKS	
	5p13	C5ORF42	JBTS17		
	10q24	TCTN3/OFD4	JBTS18		
	16q12	ZNF423	JBTS19		NPHP14
	3q22	NPHP3		MKS7	NPHP3

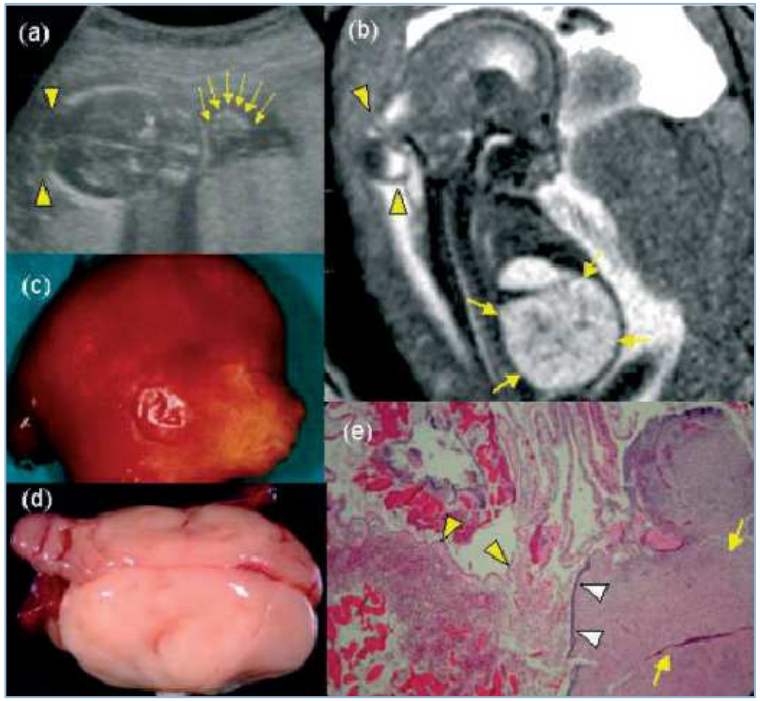
the currently known genes are responsible for only ~50% cases

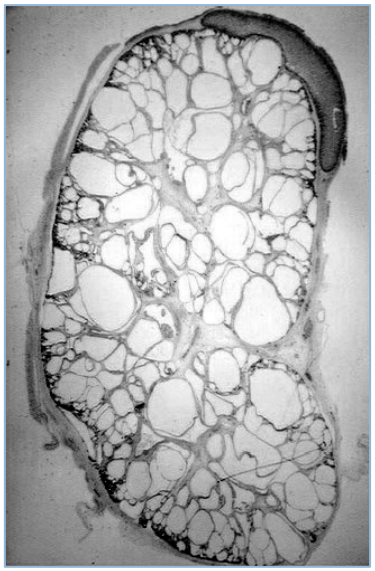
all JSRD genes encode for proteins of the primary cilium

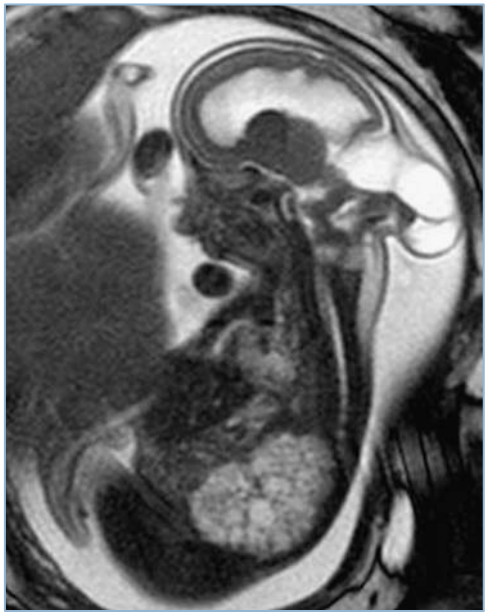
Overlap with other ciliopathies: Meckel syndrome

- cystic dysplastic kidneys
- occipital encephalocele, other posterior fossa abn
- liver fibrosis (ductal plate malformation)
- postaxial polydactyly

- other: ocular/retinal abn, CHD, genital abn







9 genes shared with JSRD

- in utero / early letalithy
- autosomal recessive inheritance

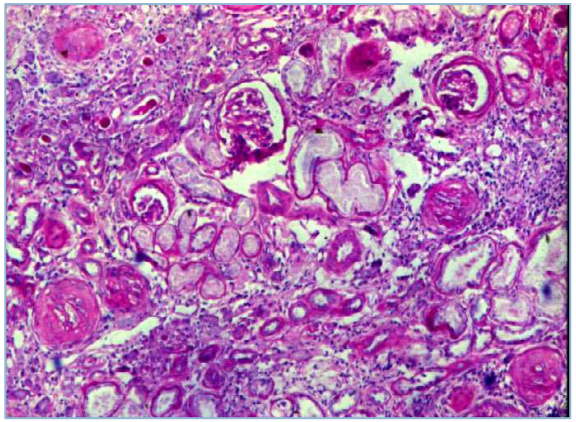
Isolated Nephronophthisis and Senior-Loken syndrome

Isolated juvenile NPH is the most common genetic cause of ESRF in childhood

Asymptomatic in the first decade of life

Symptoms at onset (late first decade):

- polyuria, polydypsia
- anemia, growth retardation
- urinary concetration defect
- acute renal failure!!!!



Kidney ultrasound (variable): small kidneys, cortico-medullary hyper-echogenicity, isolated small cysts

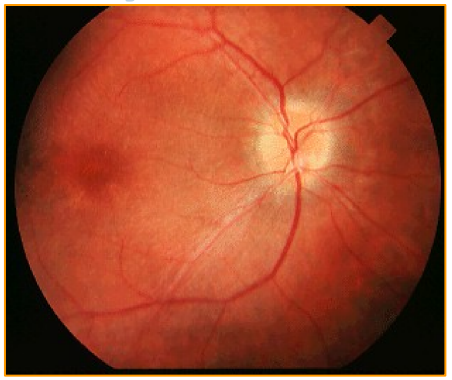
Kidney biopsy: thickening of the tubular basal membrane, interstitial fibrosis

DDAVP test: deficit of urinary concentration ability after Desmopressin stimulation (positive from 3-4 years of age!!)

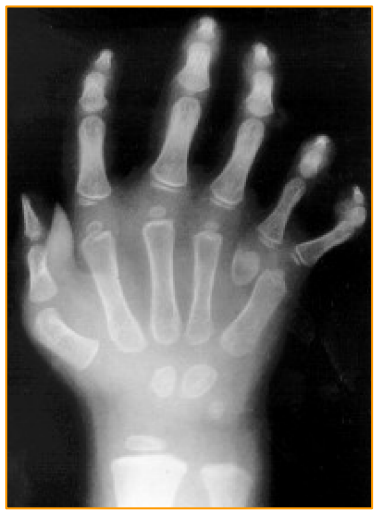
5 genes shared with JSRD

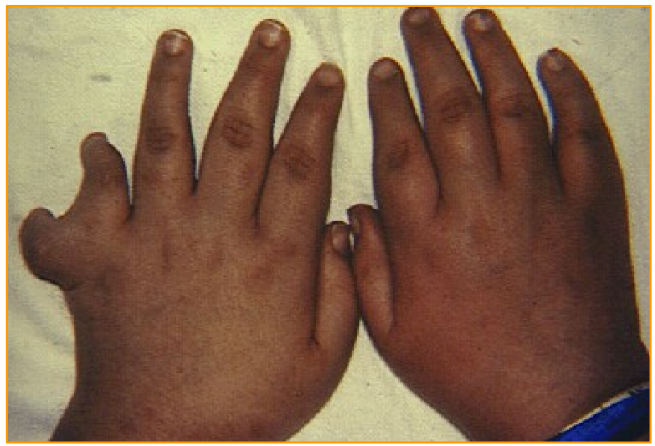
Overlap with other ciliopathies: Bardet-Biedl syndrome

- obesity, hypogenitalism
- retinal dystrophy
- renal dysplasia (including cysts)
- polydactyly
- congenital heart defects
- hepatic fibrosis
- (cognitive impairment, ataxia, deafness, neural tube defects)

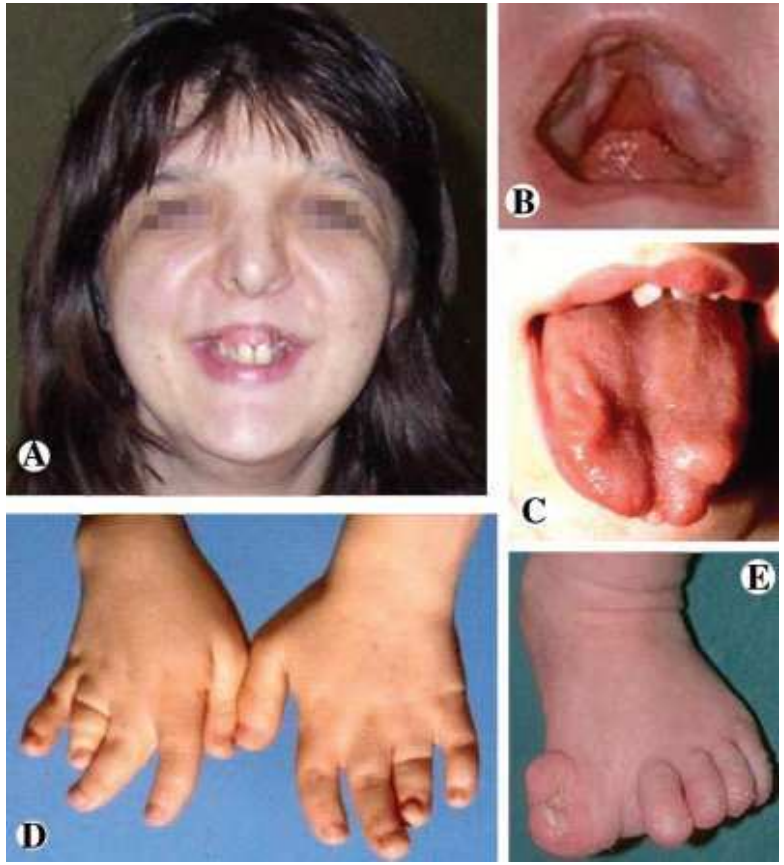


3 genes shared with JSRD, more with MKS





Overlap with other ciliopathies: OFD1 syndrome

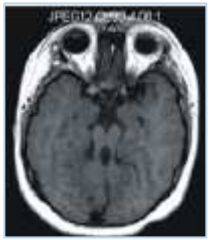


X-linked dominant, male lethality

Facial and oral abnormalities
- tongue anomalies, frenula
- cleft palate/lip
- abnormal teeth and hair
- dysmorphic features

Skeletal abnormalities
- brachydactyly, polydactyly, other

Other organs
- cystic kidneys
- CNS malformations



Joubert patients:
- MTS or CVA
- PMG, hydrocephalus
- retinitis pigmentosa
- postaxial polydactyly
- polycystic kidneys

OFD1 Is Mutated in X-Linked Joubert Syndrome and Interacts with *LCA5*-Encoded Lebercilin

Karlien L.M. Coene,[1,2,9] Ronald Roepman,[1,2,9,*] Dan Doherty,[4] Bushra Afroze,[5] Hester Y. Kroes,[6] Stef J.F. Letteboer,[1] Lock H. Ngu,[5] Bartlomiej Budny,[7] Erwin van Wijk,[3] Nicholas T. Gorden,[4] Malika Azhimi,[1] Christel Thauvin-Robinet,[8] Joris A. Veltman,[1,2] Mireille Boink,[1] Tjitske Kleefstra,[1] Frans P.M. Cremers,[1,2] Hans van Bokhoven,[1,2] and Arjan P.M. de Brouwer[1,2]

AJHG 2009

Macca and Franco, AJMG 2009

Intrafamilial variability of ciliopathies

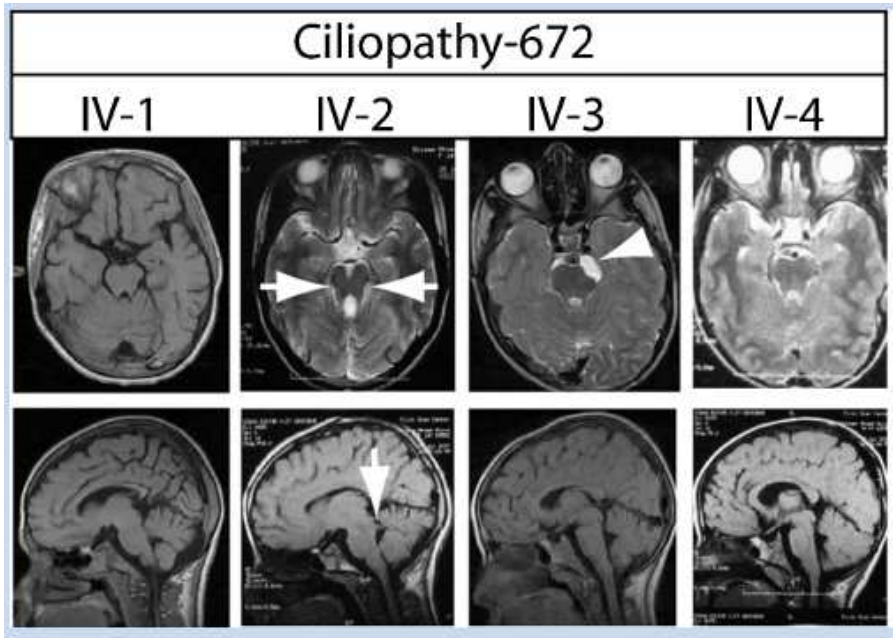
Co-Occurrence of Distinct Ciliopathy Diseases in Single Families Suggests Genetic Modifiers

Maha S. Zaki,[1]* Shifteh Sattar,[2] Rustin A. Massoudi,[2] and Joseph G. Gleeson[2]

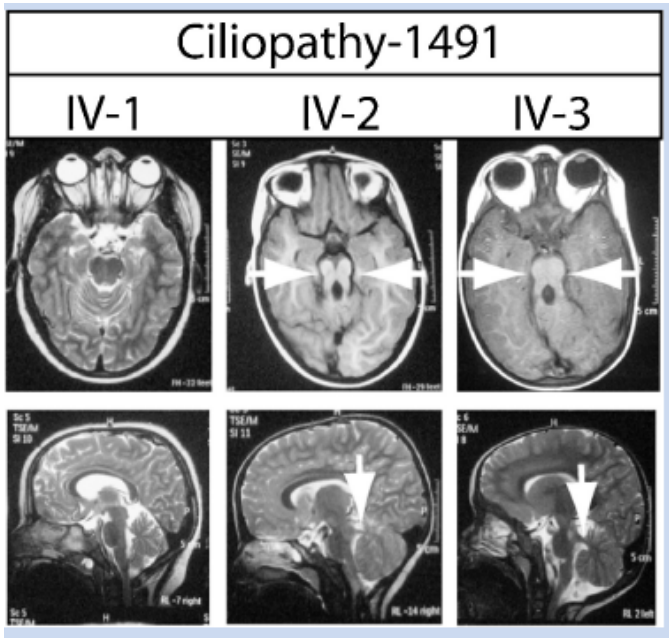
AJMG 2012

JSRD + MKS
- TMEM67 mut
- TMEM216 mut

JSRD + ACLS
- KIF7 mut

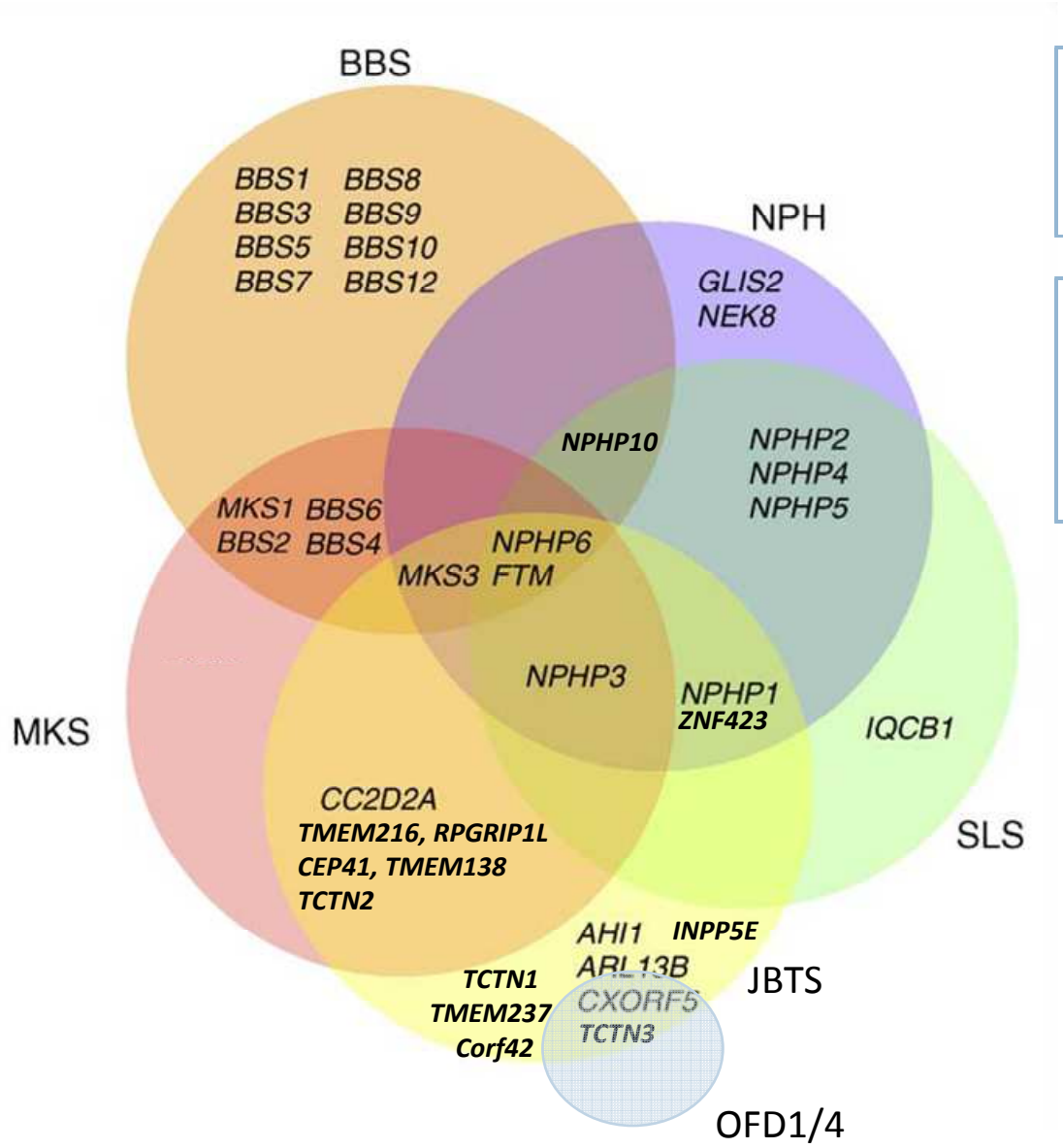


NPH polydactyly mild CVA | pure JS | NPH | NPH MCI



BBS mild CVA | pure JS | JS polydactyly corneal opacity

Genetic overlap between JSRD and other ciliopathies



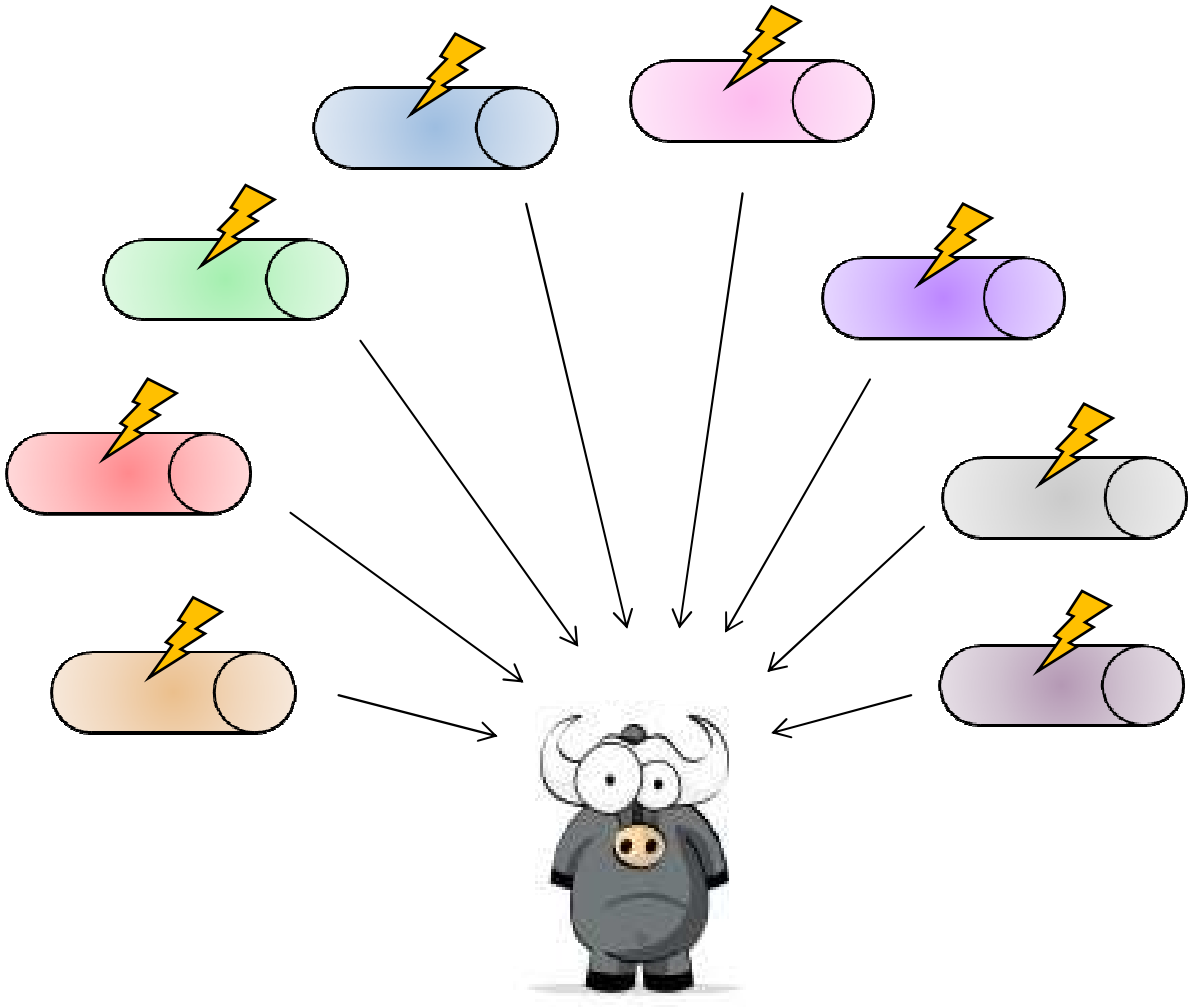
several genes cause distinct ciliopathies with variable clinical overlap

not all genes have been tested for all phenotypes → further associations to come soon

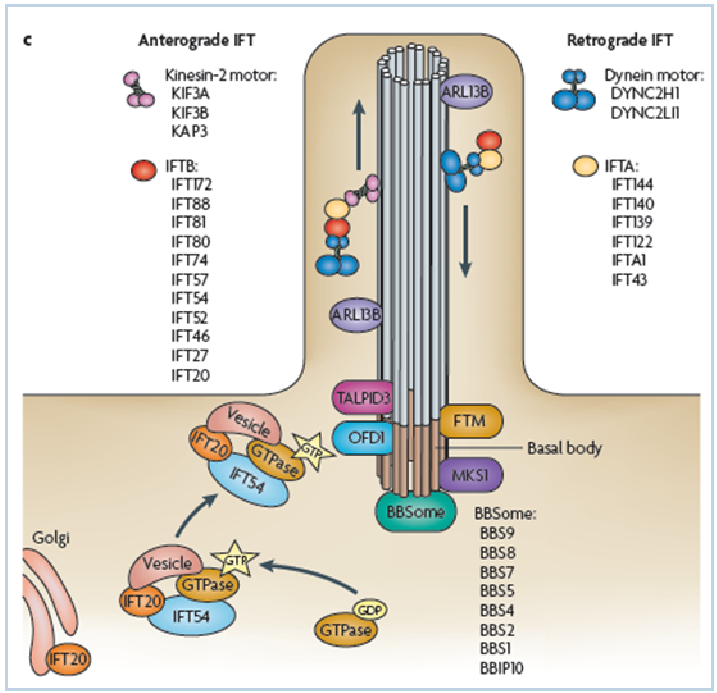
Zaghloul & Katsanis, Trend in Genet 2010

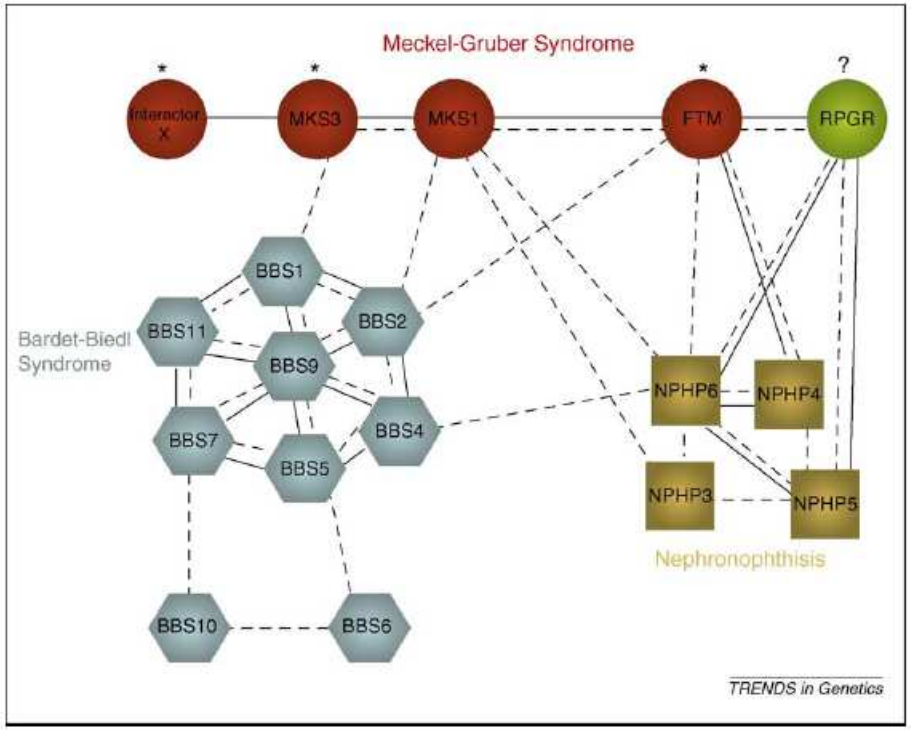
How can we explain the splitting and lumping?

Lumping…



Ciliary proteins interact in complex, integrated networks



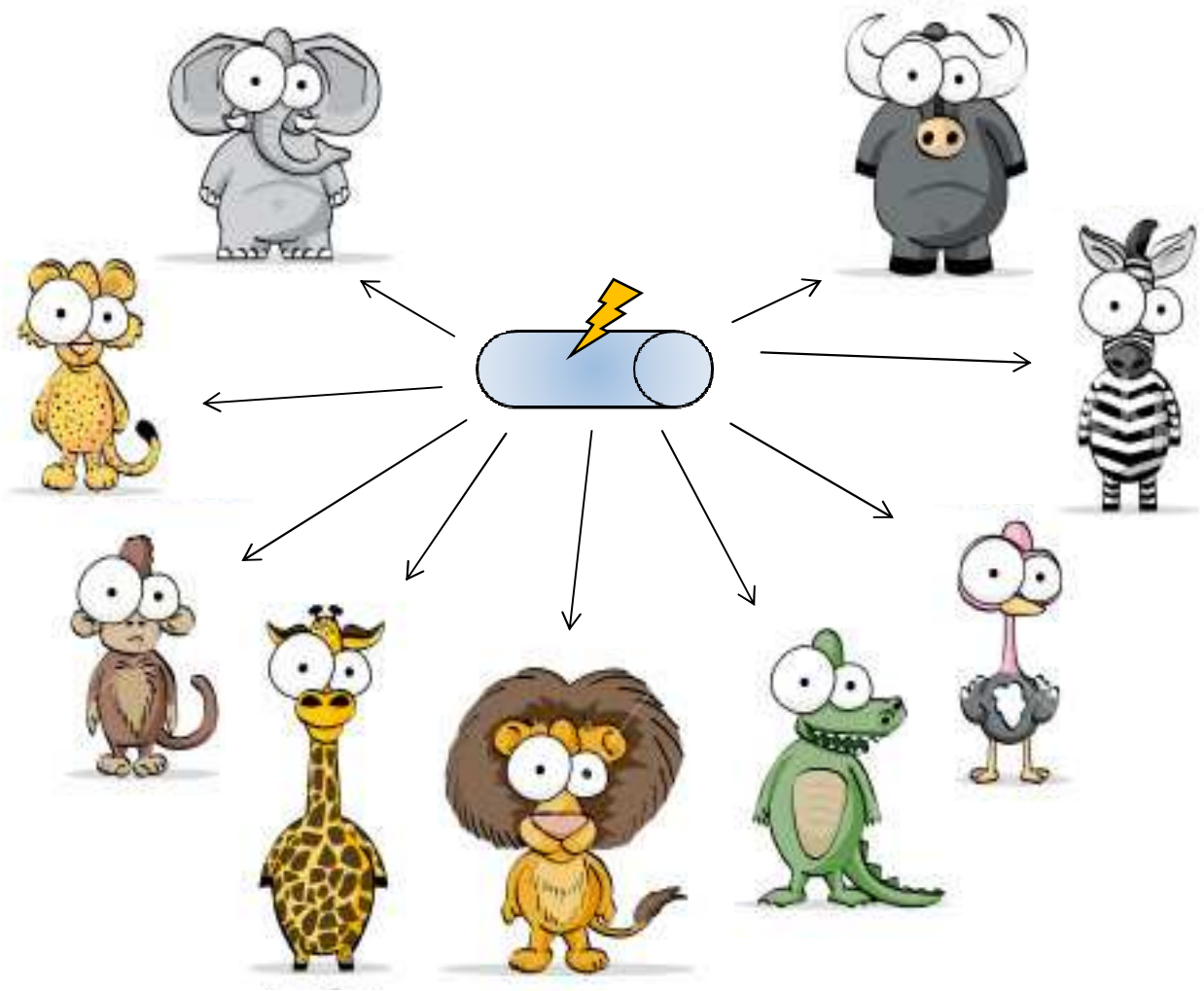


Families of ciliary proteins with distinct functions may associate with specific phenotypes:

- BBS→BBSome
- Skeletal dysplasias → IFT complex
- NPH → NPH complex at the transition zone
- JSRD/MKS →Tectonic complex at the transition zone

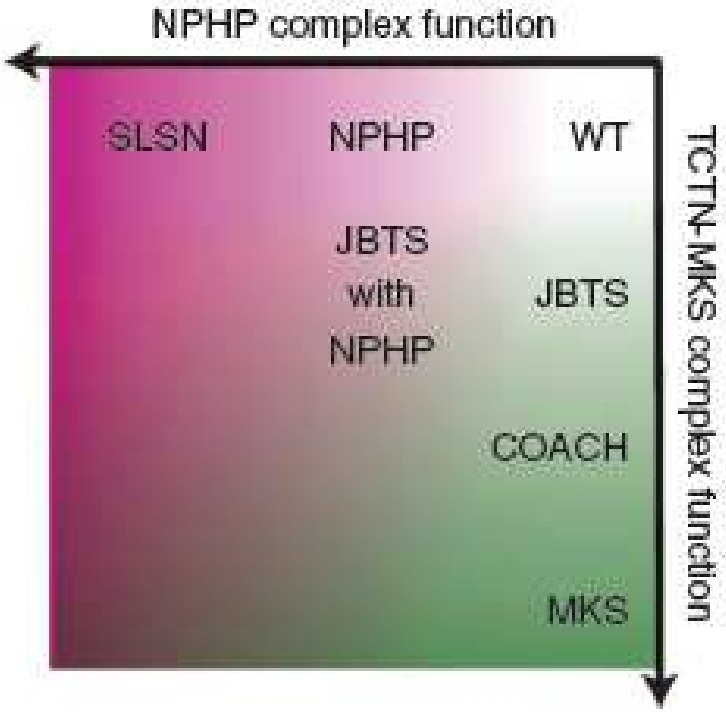
Zaghloul & Katsanis, Trend in Genet 2010; Garcia-Gonzalo et al, Nat Genet 2011

… and splitting



Genotype-phenotype correlates

	RPGRIP1L – TMEM67 – CC2D2A	MKS1	NPHP3
2 truncating mutations	MKS	MKS	MKS
at least 1 missense mutation	JSRD	BBS	NPH



CEP290

wide phenotypic spectrum:
LCA – NPH – SLS – JSRD – MKS
founder hypomorphic mutation → isolated LCA; otherwise, no obvious correlation between mutation type and phenotype

NPHP1

95% cases: same homozygous 250kb deletion encompassing the gene → variable phenotypes (NPH – SLS – JSRD)

Garcia-Gonzalo et al, Nat Genet 2011

Oligogenic inheritance and mutational load in ciliopathies

The oligogenic properties of Bardet–Biedl syndrome

Nicholas Katsanis*

Human Molecular Genetics, 2004, Vol. 13, Review Issue 1
DOI: 10.1093/hmg/ddh092
Advance Access published on February 19, 2004

Evidence of Oligogenic Inheritance in Nephronophthisis

Julia Hoefele,*† Matthias T.F. Wolf,* John F. O'Toole,* Edgar A. Otto,* Ulla Schultheiss,* Georges Dêschenes,‡ Massimo Attanasio,* Boris Utsch,* Corinne Antignac,§ and Friedhelm Hildebrandt*‖

J Am Soc Nephrol 18: 2789–2795, 2007.

JASN Express. Published on April 4, 2007 as doi: 10.1681/ASN.2006101164

High *NPHP1* and *NPHP6* Mutation Rate in Patients with Joubert Syndrome and Nephronophthisis: Potential Epistatic Effect of *NPHP6* and *AHI1* Mutations in Patients with *NPHP1* Mutations

Kálmán Tory,*† Tiphanie Lacoste,*† Lydie Burglen,‡ Vincent Morinière,*† Nathalie Boddaert,§ Marie-Alice Macher,‖ Brigitte Llanas,¶ Hubert Nivet,** Albert Bensman,†† Patrick Niaudet‡‡ Corinne Antignac,*†§§ Rémi Salomon,*‡‡ and Sophie Saunier*†

TTC21B contributes both causal and modifying alleles across the ciliopathy spectrum

IFT139

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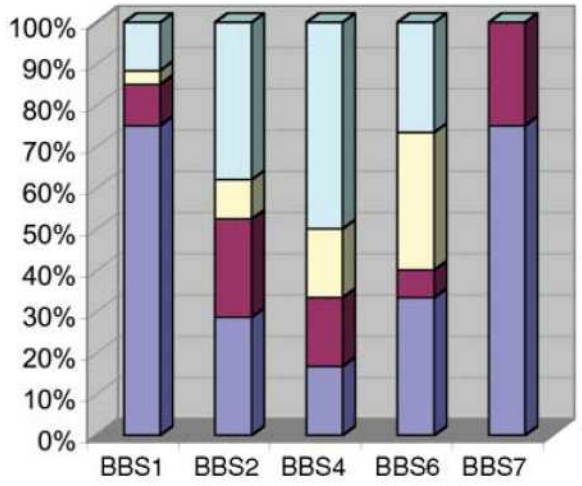
Nat Genet 2011



in several patients, only one heterozygous mutation is identified instead of the expected two

(e.g. het TSGA14 mut + het mut in other genes in half mutated pts)

TTC21B recessive mutations:
- isolated NPH / NPH plus / JATD

TTC21B heterozygous mutations:
-2.5% pts with ciliopathies (some mutated in other genes) vs 0.06% controls

… and common variants acting as genetic modifiers

A common allele in *RPGRIP1L* is a modifier of retinal degeneration in ciliopathies

Hemant Khanna[1,22], Erica E Davis[2,22], Carlos A Murga-Zamalloa[1], Alejandro Estrada-Cuzcano[1], Irma Lopez[3], Anneke I den Hollander[4], Marijke N Zonneveld[4], Mohammad I Othman[1], Naushin Waseem[5], Christina F Chakarova[5], Cecilia Maubaret[5], Anna Diaz-Font[6], Ian MacDonald[7], Donna M Muzny[8], David A Wheeler[8], Margaret Morgan[8], Lora R Lewis[8], Clare V Logan[9], Perciliz L Tan[2], Michael A Beer[2,10], Chris F Inglehearn[9], Richard A Lewis[11–14], Samuel G Jacobson[15], Carsten Bergmann[16], Philip L Beales[6], Tania Attié-Bitach[17], Colin A Johnson[9], Edgar A Otto[18], Shomi S Bhattacharya[5], Friedhelm Hildebrandt[18,19], Richard A Gibbs[8], Robert K Koenekoop[3], Anand Swaroop[1,18,20] & Nicholas Katsanis[2,21]

Nat Genet 2009

RPGRIP1L p.A229T
- controls: 2.8%
- ciliop. non RP: 0%
- ciliop + RP: 4.5% (p<0.001)

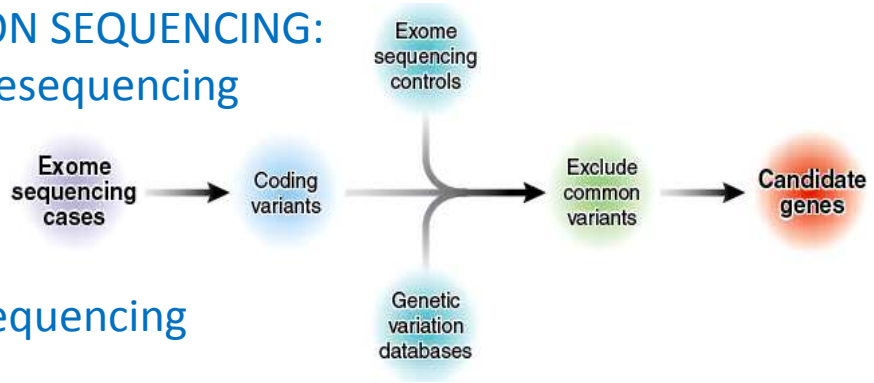
AHI1 is required for photoreceptor outer segment development and is a modifier for retinal degeneration in nephronophthisis

Nat Genet 2010

Carrie M Louie[1], Gianluca Caridi[2], Vanda S Lopes[3,4], Francesco Brancati[5,6], Andreas Kispert[7], Madeline A Lancaster[1], Andrew M Schlossman[1], Edgar A Otto[8,9], Michael Leitges[10], Hermann-Josef Gröne[11], Irma Lopez[12], Harini V Gudiseva[13], John F O'Toole[8,9], Elena Vallespin[14], Radha Ayyagari[13], Carmen Ayuso[14], Frans P M Cremers[15], Anneke I den Hollander[16], Robert K Koenekoop[12], Bruno Dallapiccola[17], Gian Marco Ghiggeri[2], Friedhelm Hildebrandt[8,9], Enza Maria Valente[5,18], David S Williams[3,4] & Joseph G Gleeson[1]

AHI1 p.R830W
- controls: 2.8%
- isolated NPH: 1.8%
- SLS: 25% (p<0.001)
- other ciliopathies: ns

NEXT GENERATION SEQUENCING:
- targeted gene resequencing



- whole exome sequencing