ERKNet
The European
Rare Kidney Disease
Reference Network

WELCOME TO

ERKNet
Advanced Webinars on Rare Kidney Disorders

Date: 25 Feb 2020

Topic: Molecular Genetics of Joubert Syndrome

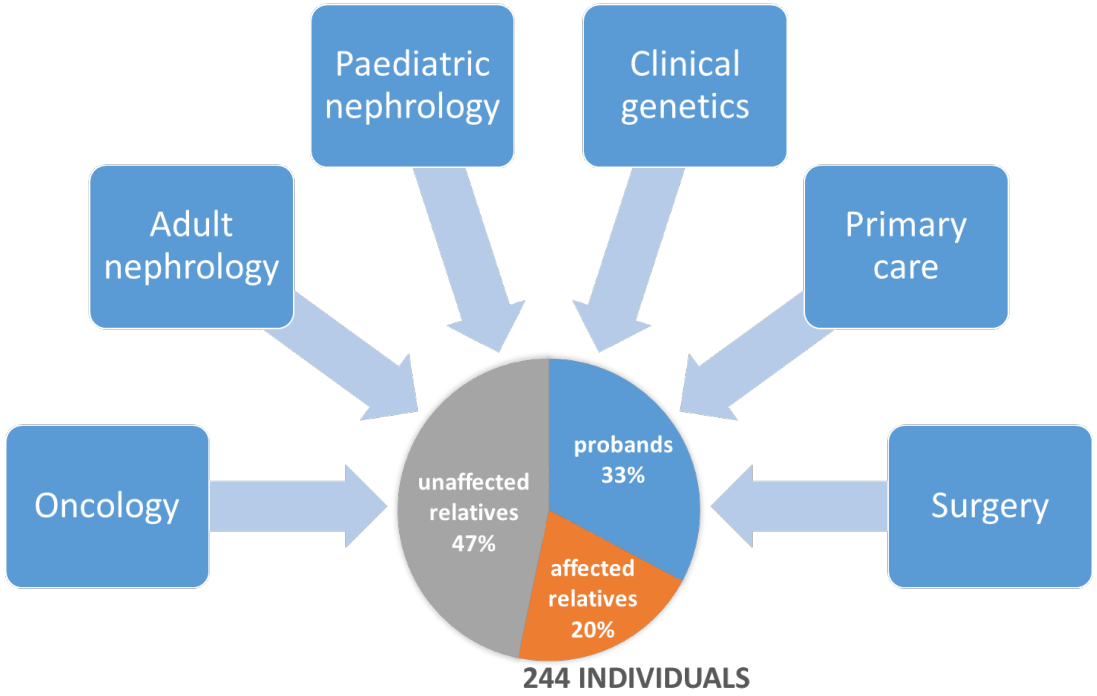
Speaker: John A Sayer, Newcastle University

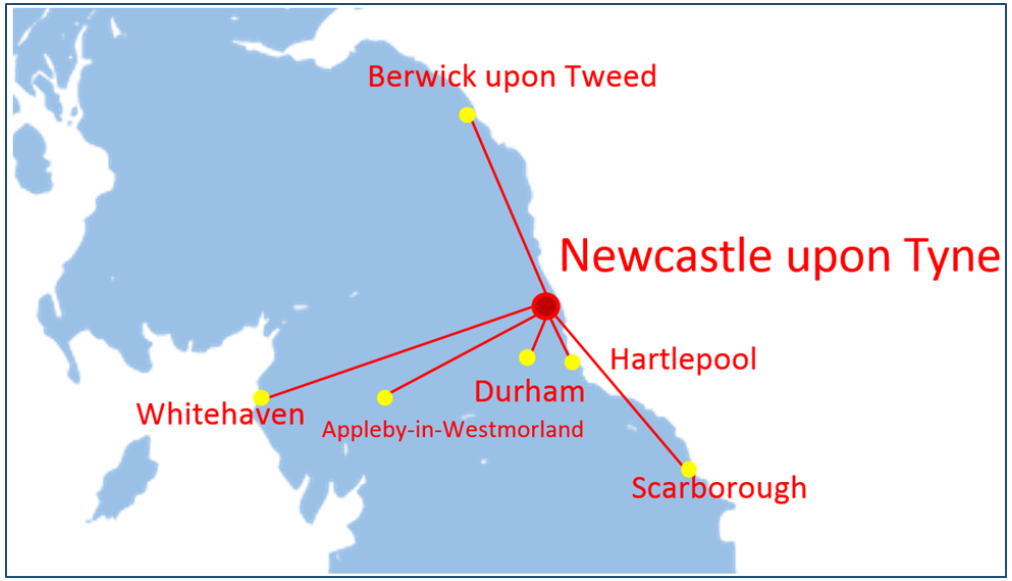
Moderator: Franz Schaefer, University of Heidelberg

Combined Family Renal Genetic Clinic

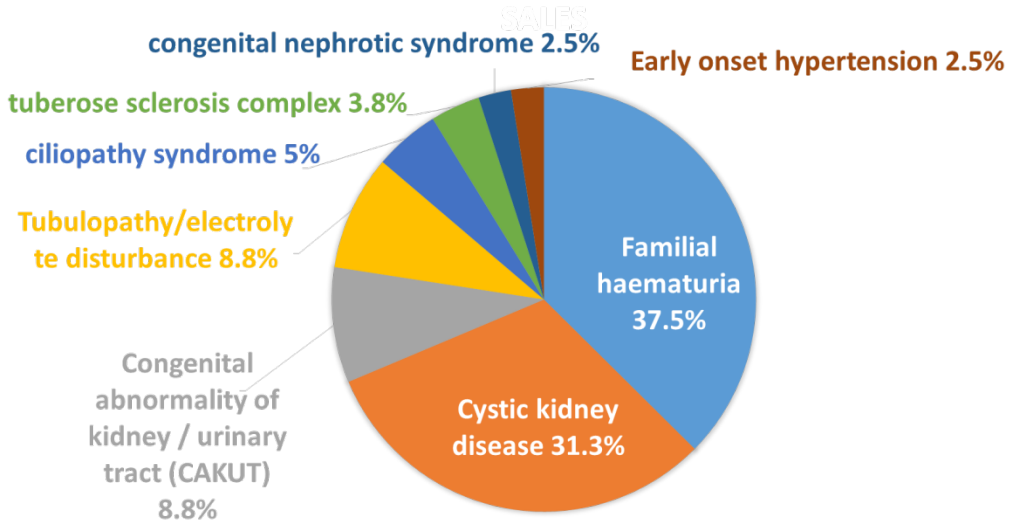
- Tertiary referral clinic
- Multidisciplinary approach
- Hosted every 3 months by pediatric nephrologist, adult nephrologist and clinical geneticist
- Patients and their family
- Aim:
  - Provide genetic diagnosis to guide management
  - Screening and counselling to families with inherited renal disorders in response to the clinical need

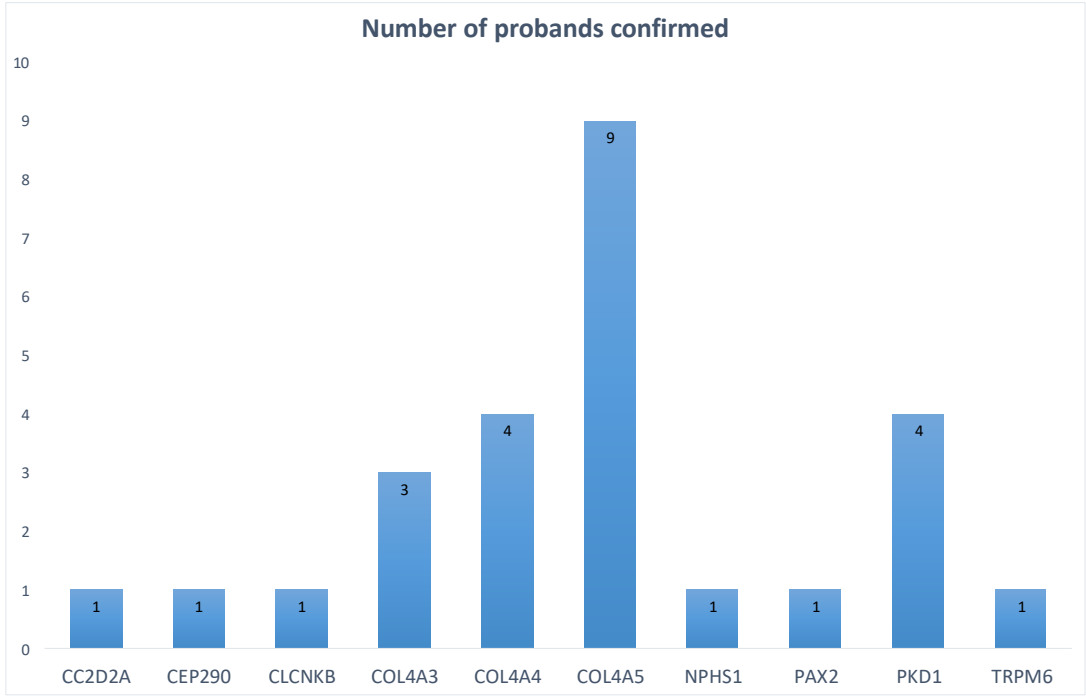
Combined Family Renal Genetic Clinic





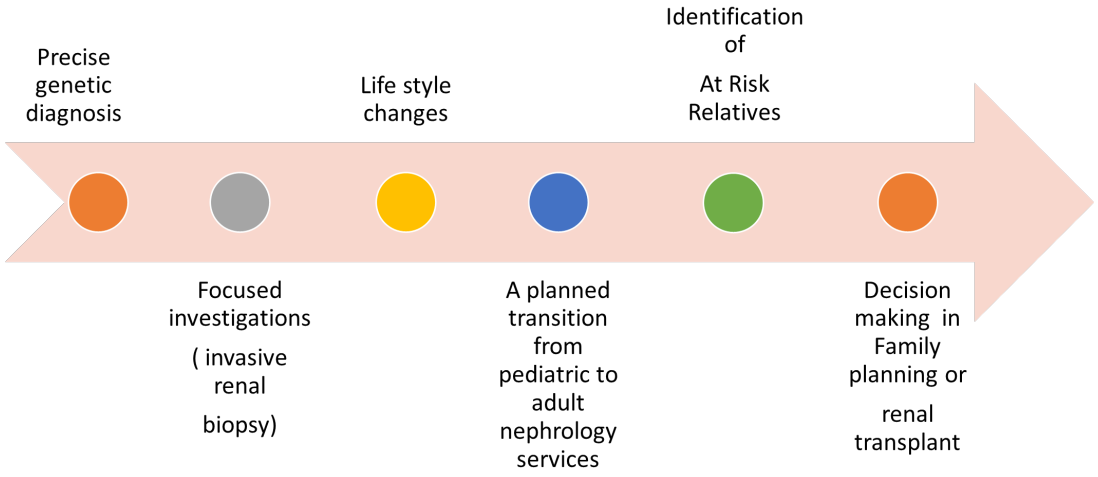
Combined Family Renal Genetic Clinic

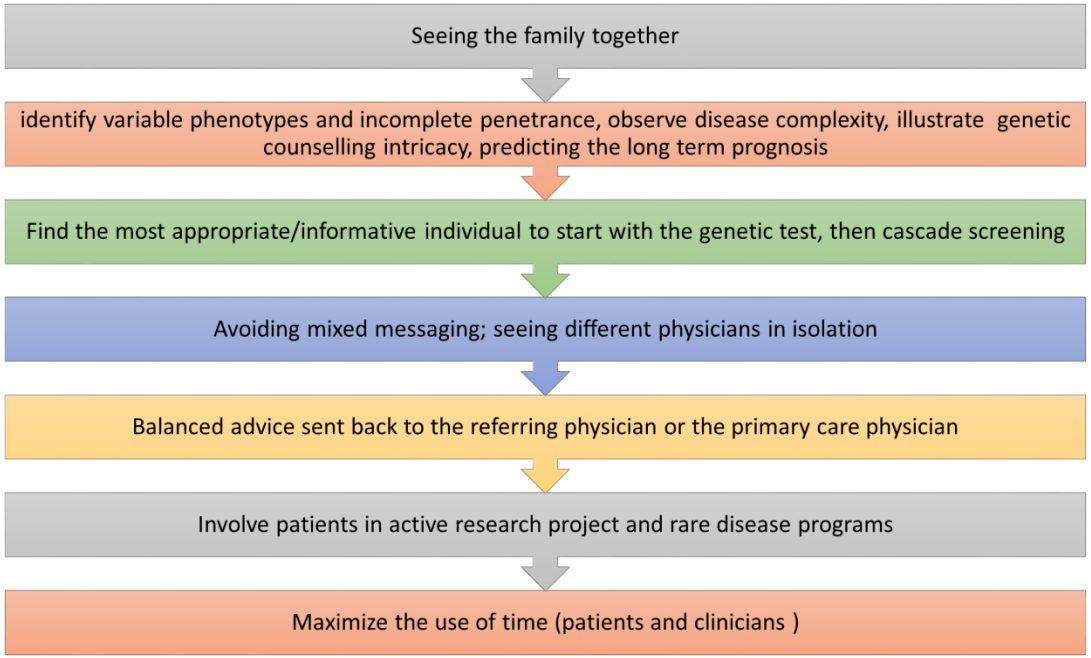




Familial haematuria

Benefits of a Combined Family Renal Genetic Clinic





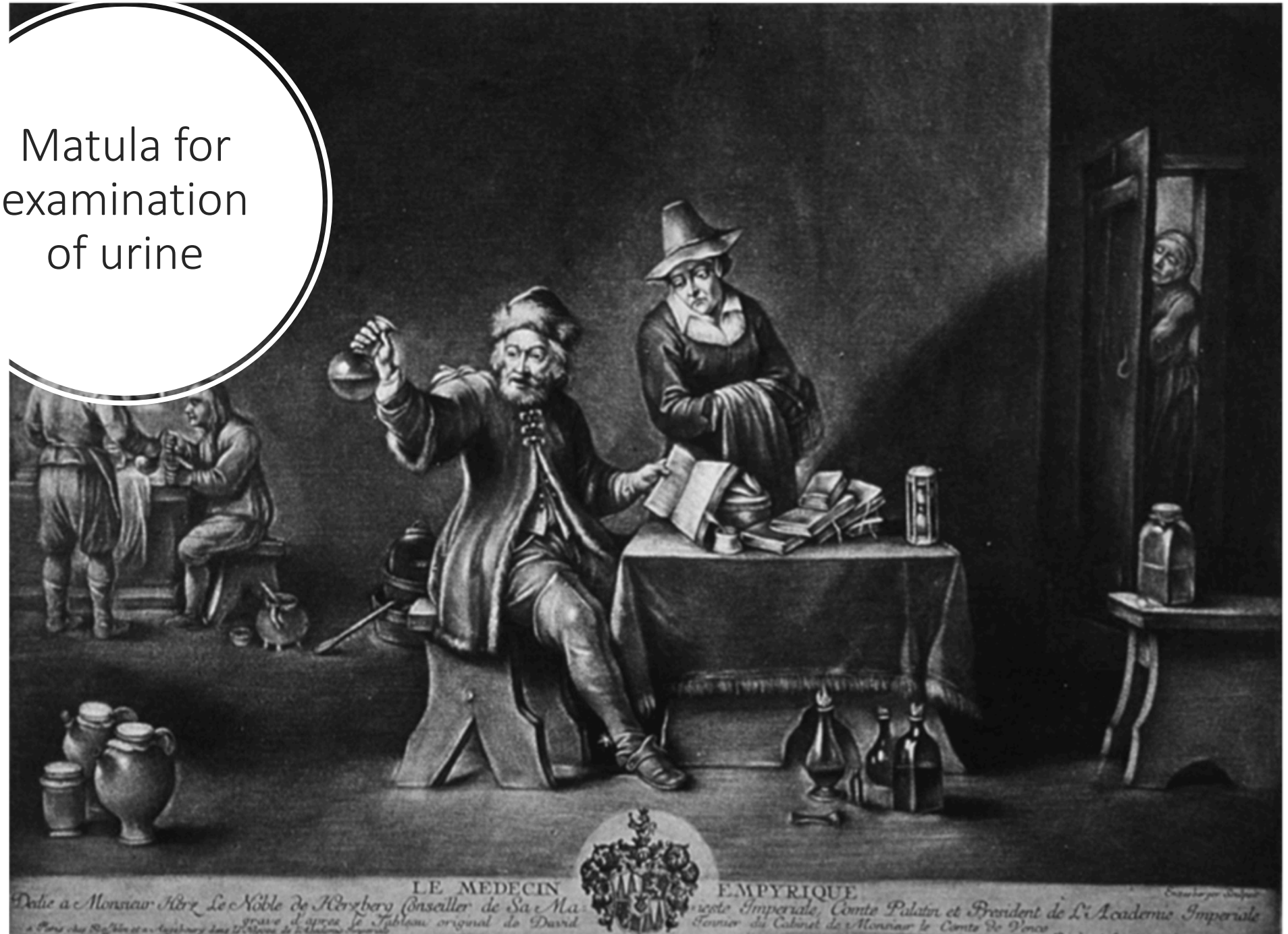
Research within the Renal Genetics Clinic

- DNA sample consent and storage
- WES/WGS via 100,000 G project
- Urine for culture of renal epithelial cells

Examination of urine

- Fascinated people since beginning of recorded history
- Oldest medical test
- Marks beginning of laboratory medicine
- Described 6000 years ago in Sumerian and Babylonian clay tablets

Matula for examination of urine



THE

PISSE-PROPHET

OR,

CERTAIN PISSE-POT *LECTURES.*

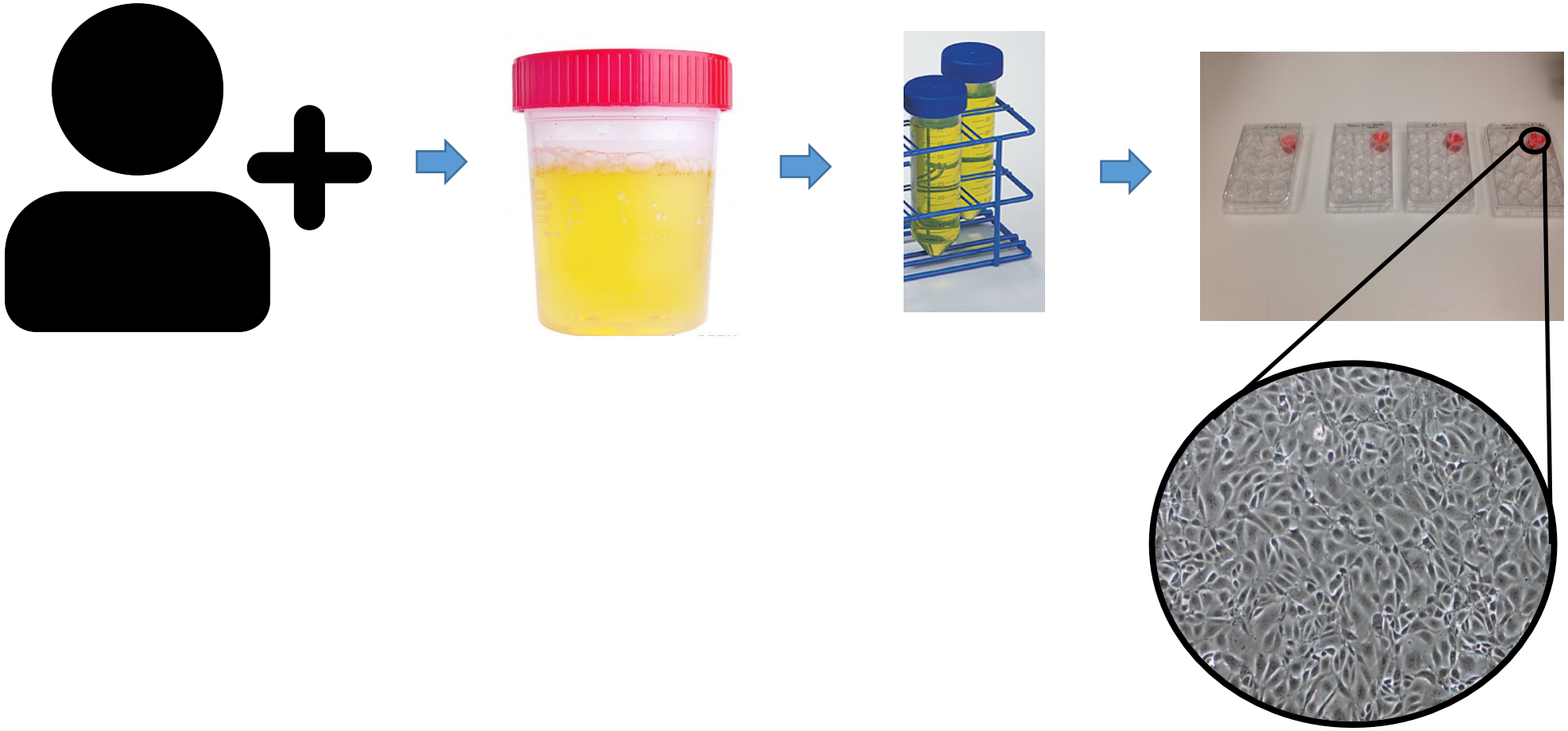
Wherein are newly discovered the old fallacies, deceit, and jugling of the Piss-pot *Science*, *used by all those* (*whether Quacks*, and Empiricks, or other methodical Physicians) who pretend knowledg of Diseases, by the Urine, in giving judgement of the same.

. P. lately in the City
now in *Colchester*,
SSEX.

olished by any man in
h Tongue.

Learning point:
Phenotype is key to understanding underlying disease

URECs= Urine-derived Renal Epithelial Cells



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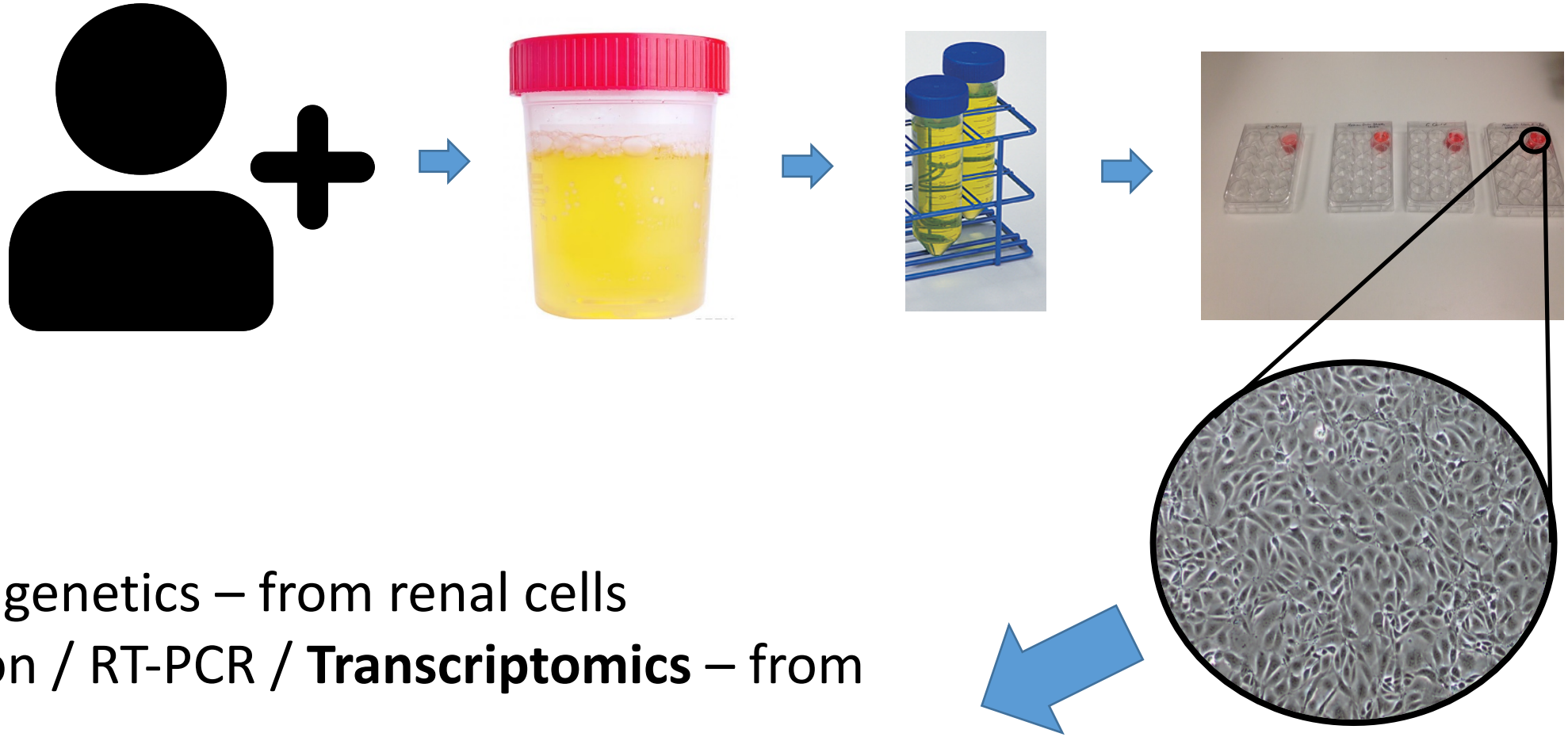


Genomics / genetics – from renal cells

RNA isolation / RT-PCR / **Transcriptomics** – from renal cells

Proteomics

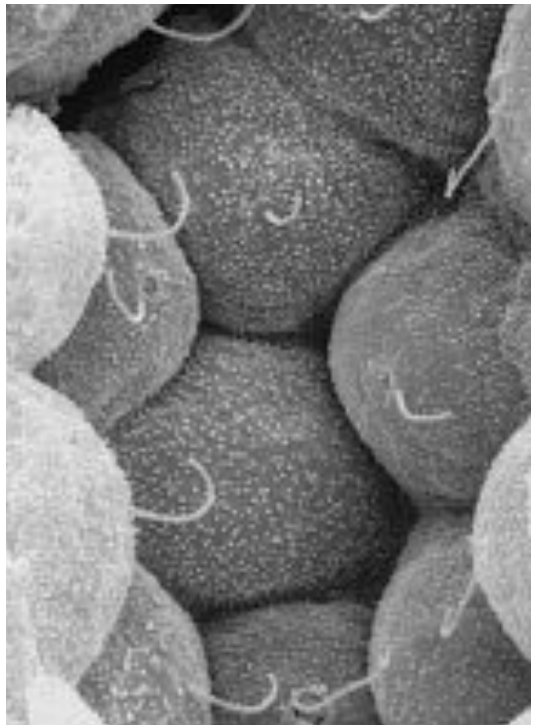
Immunofluorescence studies

Electron microscopy studies

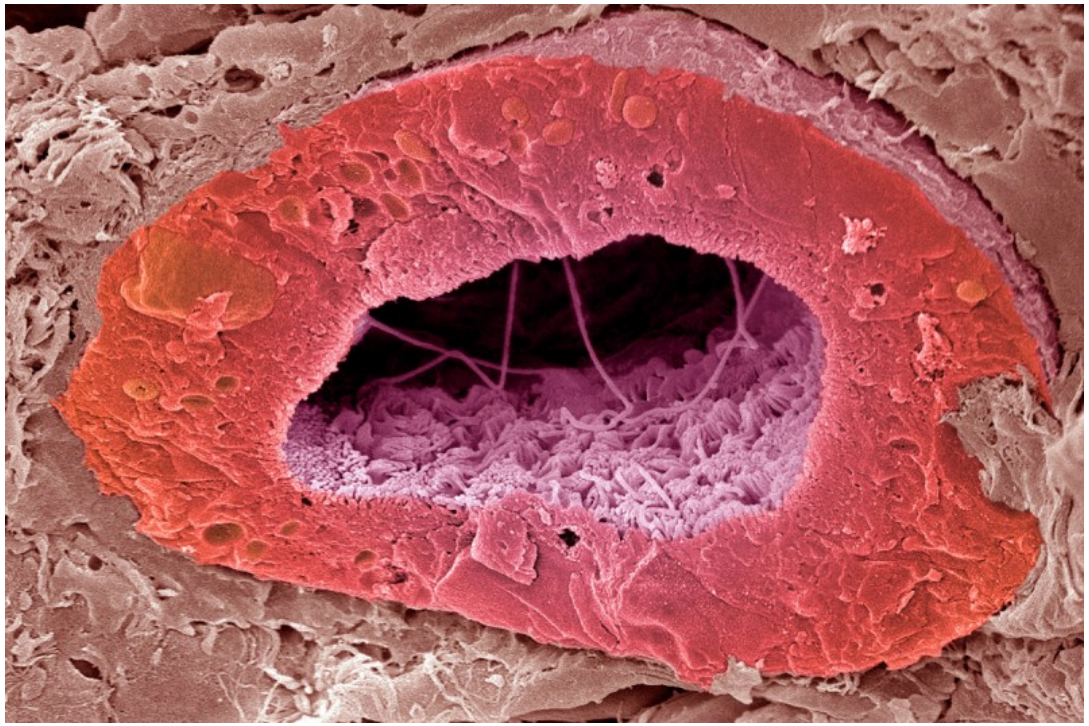
hUREC to iPSC to eye/kidney/brain - organoids

3D spheroids / tubuloids

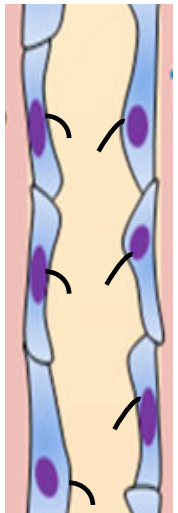
Cilia and cystic kidney disease – renal ciliopathies

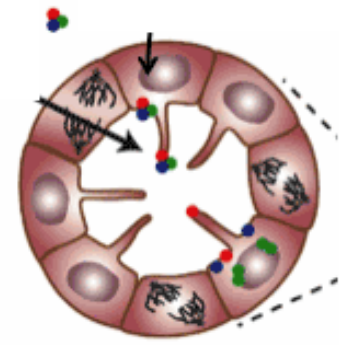


(from Somlo & Igarashi *JASN* 13:2384, 2002)

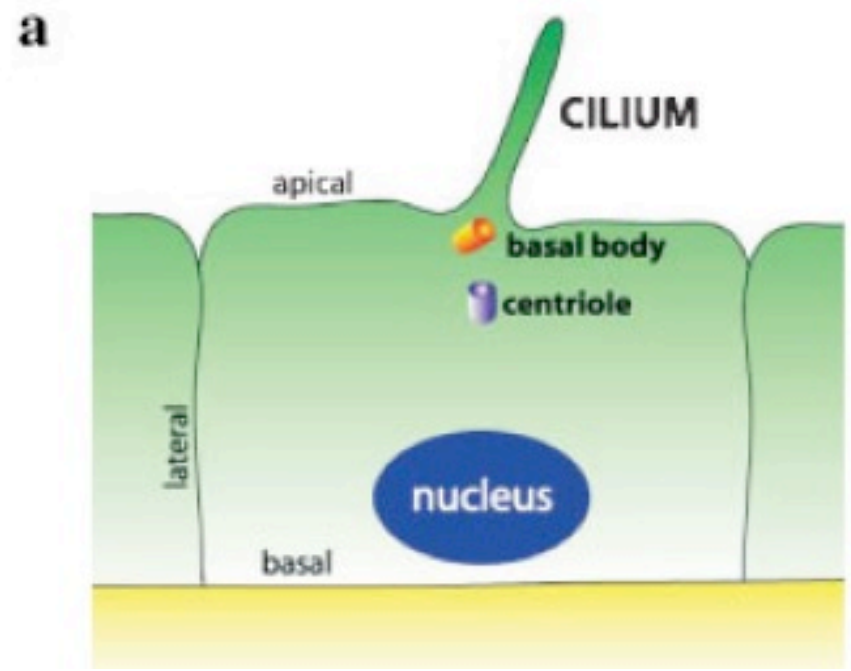


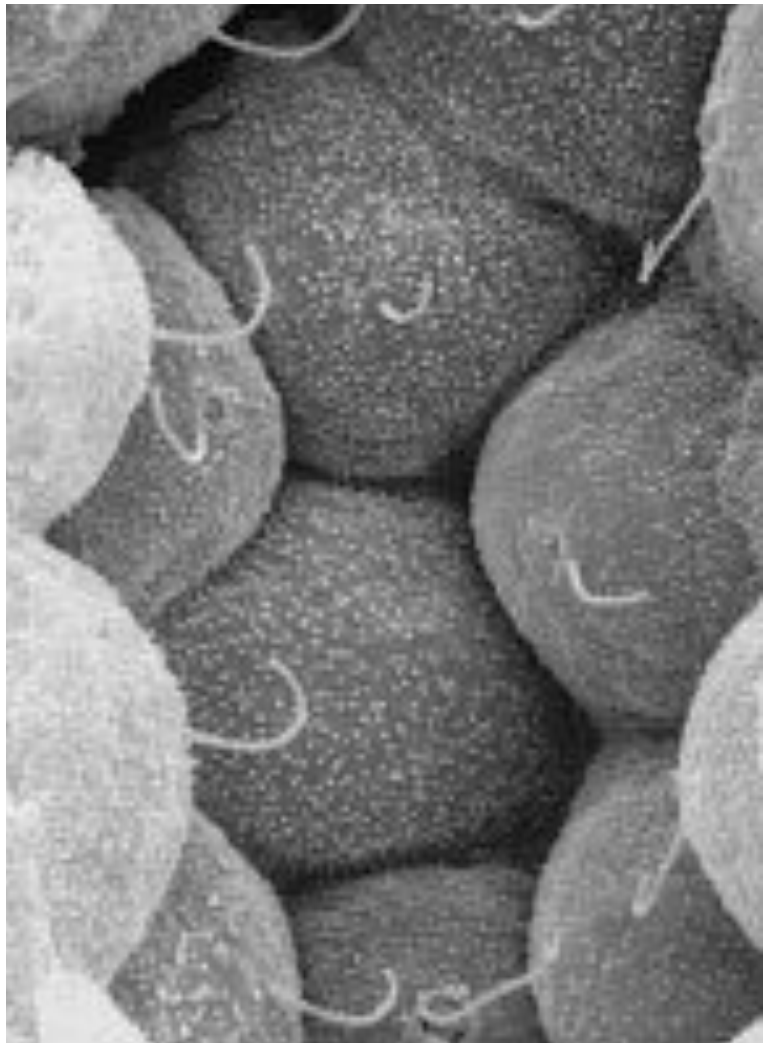
(from Eva Kielser)





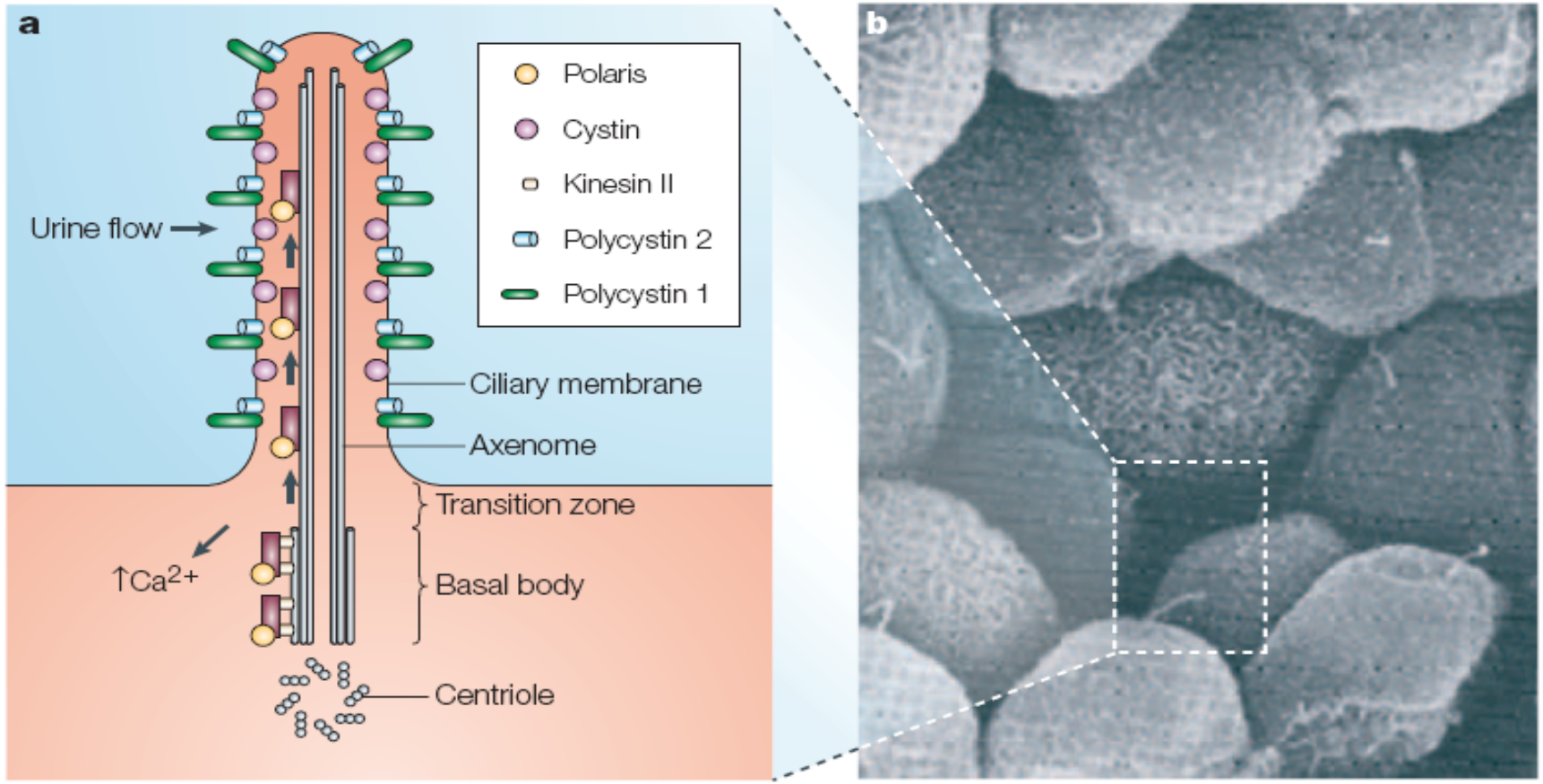
The primary cilium





(from Somlo & Igarashi *JASN* 13:2384, 2002)

Renal primary cilia

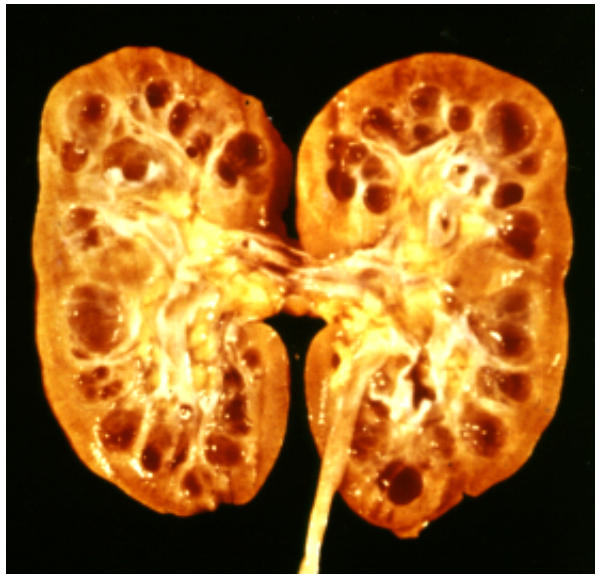


(from Somlo & Igarashi *JASN* 13:2384, 2002)

Genes involved in cystic kidney disease expressed in primary cilium

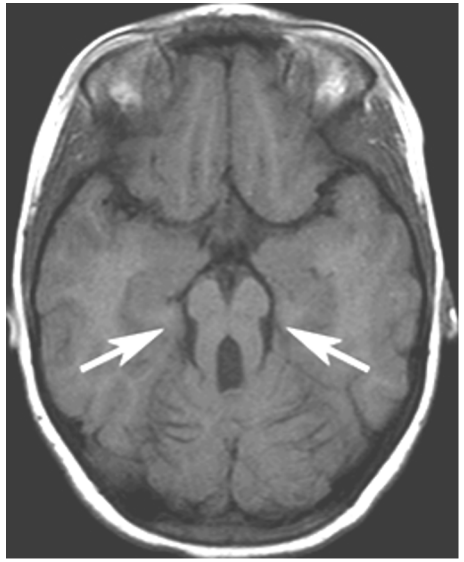
Cilia involved in key signaling pathways including Hedgehog signalling

Nephronophthisis and the link with cilia dysfunction



Inheritance:	autosomal recessive
Symptoms:	ESRD age 1 - 25 years polyuria, polydipsia, anaemia, growth retardation,
Pathology:	cortico-medullary cysts
Histology:	tubular basement membrane disruption, tubular atrophy and cysts, interstitial fibrosis
Frequency:	most frequent genetic cause for ESRD age 1- 30 yrs
Onset of ESRD:	infantile juvenile adolescent
Associations:	retinitis pigmentosa (Senior-Loken syndrome) oculo-motor apraxia type Cogan, Joubert syndrome

Nephronophthisis and extrarenal manifestations

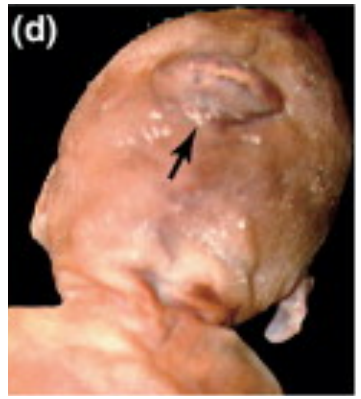


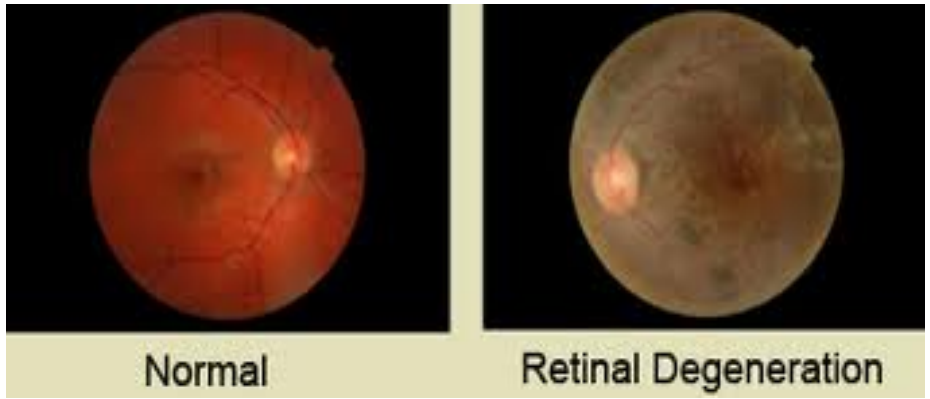
Cerebellar vermis aplasia

Molar tooth sign (Joubert Syndrome)

Gaze palsy (oculomotor apraxia)

Encephaloceole

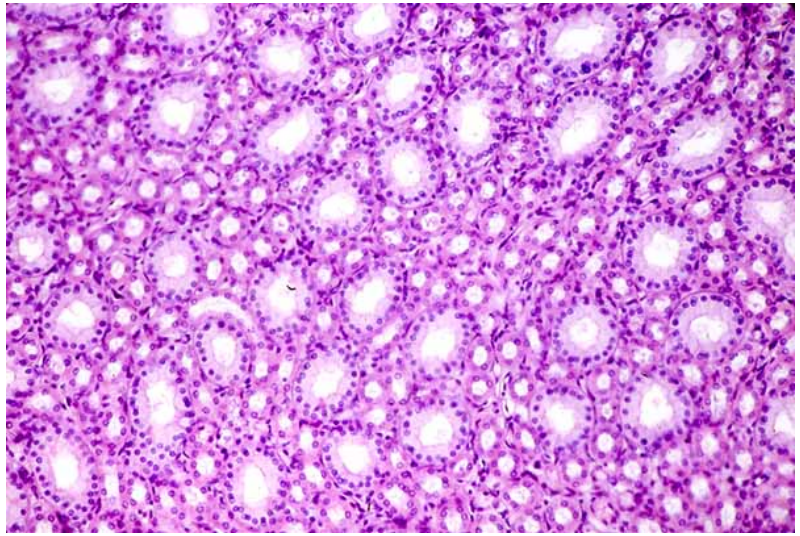


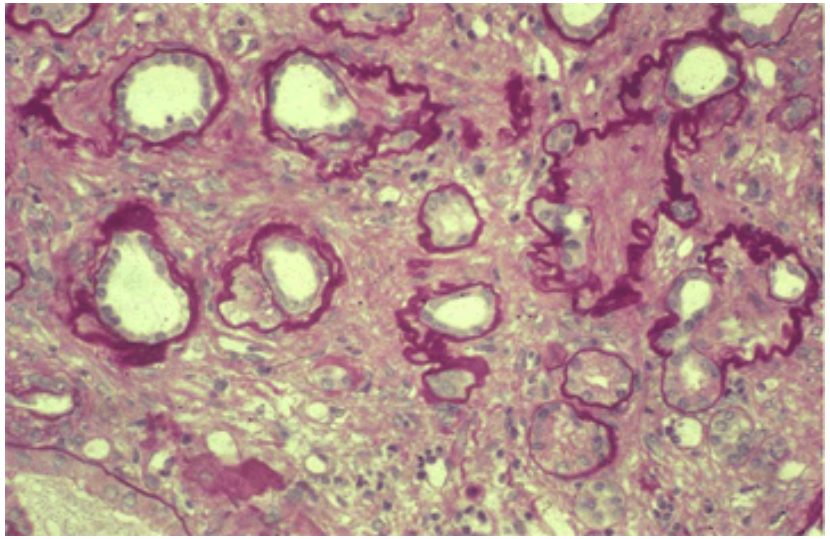


Retinitis Pigmentosa / Lebers congenital amuarosis

Situs inversus

Liver fibrosis





Nephronophthisis:

Literally means disappearance of nephrons

Tubular basement thickening in certain areas

Tubular atrophy and interstitial fibrosis

Corticomedullary cyst formation

Nephronophthisis Genes		
NPHP1	*RPGRIP1L*	*CEP164*
INVS	*NEK8*	*ANKS6*
NPHP3	*SDCCAG8*	*IFT172*
NPHP4	*TMEM67*	*CEP83*
NPHP5	*TTC21B*	*DCDC2*
CEP290	*WDR19*	*MAPKBP1*
GLIS2	*ZNF423*	

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NPHP

- https://omim.org/phenotypicSeries/PS256100

Download As

Phenotypic Series – PS256100

Nephronophthisis – PS256100 – 17 Entries

View corresponding clinical synopses as a table

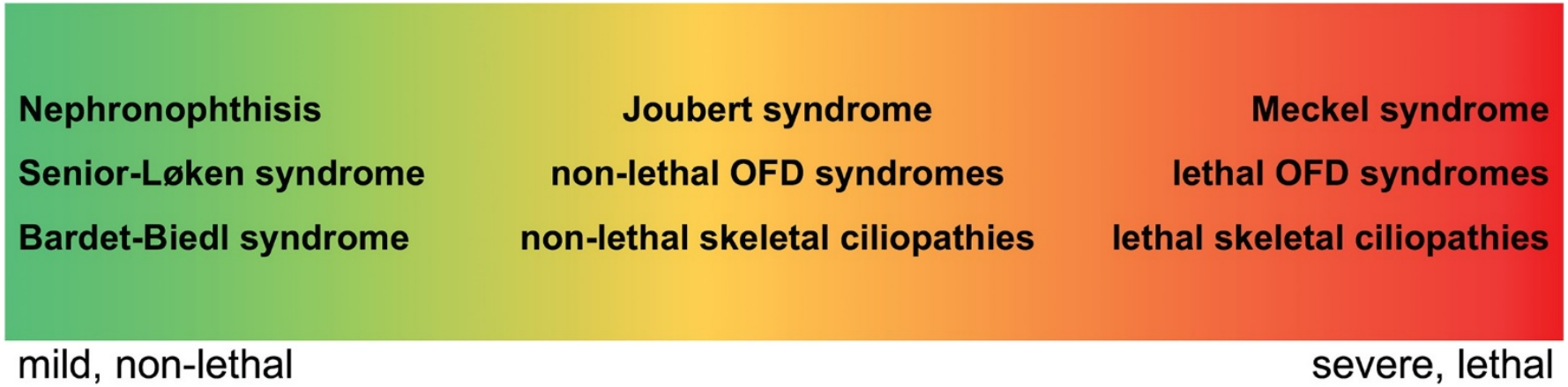
Location	Phenotype	Inheritance	Phenotype mapping key	Phenotype MIM number	Gene/Locus	Gene/Locus MIM number
1p36.31	Nephronophthisis 4	AR	3	606966	NPHP4	607215
2q13	Nephronophthisis 1, juvenile	AR	3	256100	NPHP1	607100
2q24.3	Nephronophthisis 12	AD, AR	3	613820	TTC21B	612014
3q22.1	Nephronophthisis 3	AR	3	604387	NPHP3	608002
4p14	Nephronophthisis 13	AR	3	614377	WDR19	608151
6p22.3	Nephronophthisis 19	AR	3	616217	DCDC2	605755
8q22.1	Nephronophthisis 11	AR	3	613550	TMEM67	609884
9q22.33	Nephronophthisis 16	AR	3	615382	ANKS6	615370
9q31.1	Nephronophthisis 2, infantile	AR	3	602088	INVS	243305
11q23.3	Nephronophthisis 15	AR	3	614845	CEP164	614848
12q22	Nephronophthisis 18	AR	3	615862	CEP83	615847
15q15.1	Nephronophthisis 20	AR	3	617271	MAPKBP1	616786
16p13.3	Nephronophthisis 7		3	611498	GLIS2	608539
16q12.1	Joubert syndrome 19	AD, AR	3	614844	ZNF423	604557
16q12.1	Nephronophthisis 14	AD, AR	3	614844	ZNF423	604557
17q11.2	?Nephronophthisis 9		3	613824	NEK8	609799
22q13.2	Nephronophthisis-like nephropathy 1	AR	3	613159	XPNPEP3	613553

Joubert syndrome genes

- https://omim.org/phenotypicSeries/PS213300

Location	Phenotype	Inheritance	mapping key	MIM number	Gene/Locus	MIM number
1p36.32	Joubert syndrome 25	AR	3	616781	CEP104	616690
2q13	Joubert syndrome 4	AR	3	609583	NPHP1	607100
2q33.1	Joubert syndrome 14	AR	3	614424	TMEM237	614423
2q37.1	Joubert syndrome 30	AR	3	617622	ARMC9	617612
2q37.1	?Joubert syndrome 22	AR	3	615665	PDE6D	602676
3q11.1-q11.2	Joubert syndrome 8	AR	3	612291	ARL13B	608922
4p15.32	Joubert syndrome 9	AR	3	612285	CC2D2A	612013
5p13.2	Joubert syndrome 17	AR	3	614615	CPLANE1	614571
5q23.2	Joubert syndrome 31	AR	3	617761	CEP120	613446
6q23.3	Joubert syndrome 3	AR	3	608629	AHI1	608894
7q32.2	Joubert syndrome 15	AR	3	614464	CEP41	610523
8q13.1-q13.2	Joubert syndrome 21	AR	3	615636	CSPP1	611654
8q22.1	Joubert syndrome 6	AR	3	610688	TMEM67	609884
9q34.3	Joubert syndrome 1	AR	3	213300	INPP5E	613037
10q22.2	Joubert syndrome 36	AR	3	618763	FAM149B1	618413
10q24.1	Joubert syndrome 18	AR	3	614815	TCTN3	613847
10q24.32	Joubert syndrome 32	AR	3	617757	SUFU	607035
10q24.32	Joubert syndrome 35	AR	3	618161	ARL3	604695
11q12.2	Joubert syndrome 16	AR	3	614465	TMEM138	614459
11q12.2	Joubert syndrome 2	AR	3	608091	TMEM216	613277
12q21.32	Joubert syndrome 5	AR	3	610188	CEP290	610142
12q24.11	Joubert syndrome 13	AR	3	614173	TECT1	609863
12q24.31	Joubert syndrome 24	AR	3	616654	TCTN2	613846
13q21.3-q22.1	Joubert syndrome 33	AR	3	617767	PIBF1	607532
14q23.1	Joubert syndrome 23	AR	3	616490	KIAA0586	610178
15q26.1	Joubert syndrome 12	AR	3	200990	KIF7	611254
15q26.1	Acrocallosal syndrome	AR	3	200990	KIF7	611254
Joubert syndrome 26	AR	3	616784	KATNIP	616650
Joubert syndrome 19	AD, AR	3	614844	ZNF423	604557
Nephronophthisis 14	AD, AR	3	614844	ZNF423	604557
Joubert syndrome 7	AR	3	611560	RPGRIP1L	610937
Joubert syndrome 20	AR	3	614970	TMEM231	614949
?Joubert syndrome 29	AR	3	617562	TMEM107	616183
Meckel syndrome 13	AR	3	617562	TMEM107	616183
Joubert syndrome 27	AR	3	617120	B9D1	614144
Joubert syndrome 28	AR	3	617121	MKS1	609883
?Meckel syndrome 10	AR	3	614175	B9D2	611951
Joubert syndrome 34	AR	3	614175	B9D2	611951
Joubert syndrome 10	XLR	3	300804	OFD1	300170

Motile and non-motile cilia in human pathology: from function to phenotypes



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frontiers in Pediatrics

REVIEW
published: 05 January 2018
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Many Genes—One Disease? Genetics of Nephronophthisis (NPHP) and NPHP-Associated Disorders

*Shalabh Srivastava[1,2], Elisa Molinari[1], Shreya Raman[3] and John A. Sayer[1,4]**

[1] Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom, [2] Renal Unit, City Hospitals Sunderland and South Tyneside NHS Foundation Trust, Sunderland, United Kingdom, [3] Department of Histopathology, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom, [4] Renal Services, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom

Nephronophthisis (NPHP) is a renal ciliopathy and an autosomal recessive cause of cystic kidney disease, renal fibrosis, and end-stage renal failure, affecting children and young adults. Molecular genetic studies have identified more than 20 genes underlying this disorder, whose protein products are all related to cilia, centrosome, or mitotic spindle function. In around 15% of cases, there are additional features of a ciliopathy syndrome, including retinal defects, liver fibrosis, skeletal abnormalities, and brain developmental disorders. Alongside, gene identification has arisen molecular mechanistic insights into the disease pathogenesis. The genetic causes of NPHP are discussed in terms of how they help us to define treatable disease pathways including the cyclic adenosine monophosphate pathway, the mTOR pathway, Hedgehog signaling pathways, and DNA damage response pathways. While the underlying pathology of the many types of NPHP remains similar, the defined disease mechanisms are diverse, and a personalized medicine approach for therapy in NPHP patients is likely to be required.

Keywords: ciliopathy, molecular genetics, nephronophthisis, cilia, centrosome, DNA damage, cyclic adenosine monophosphate, Joubert syndrome

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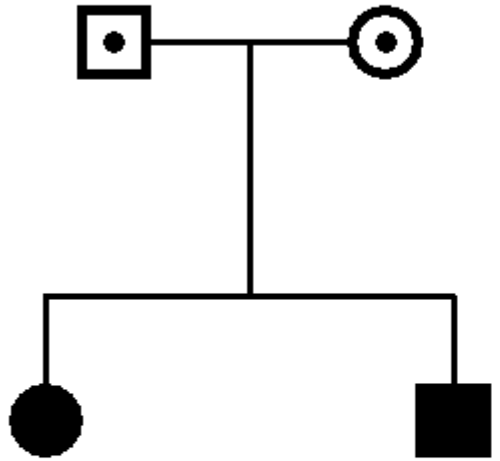
Edited by:
Max Christoph Liebau, Universitätsklinikum Köln, Germany

Reviewed by:
Ruxandra Bachmann-Gagescu, University of Zurich, Switzerland
Katja Höpker, Universitätsklinikum Köln, Germany

Family BB



II:1

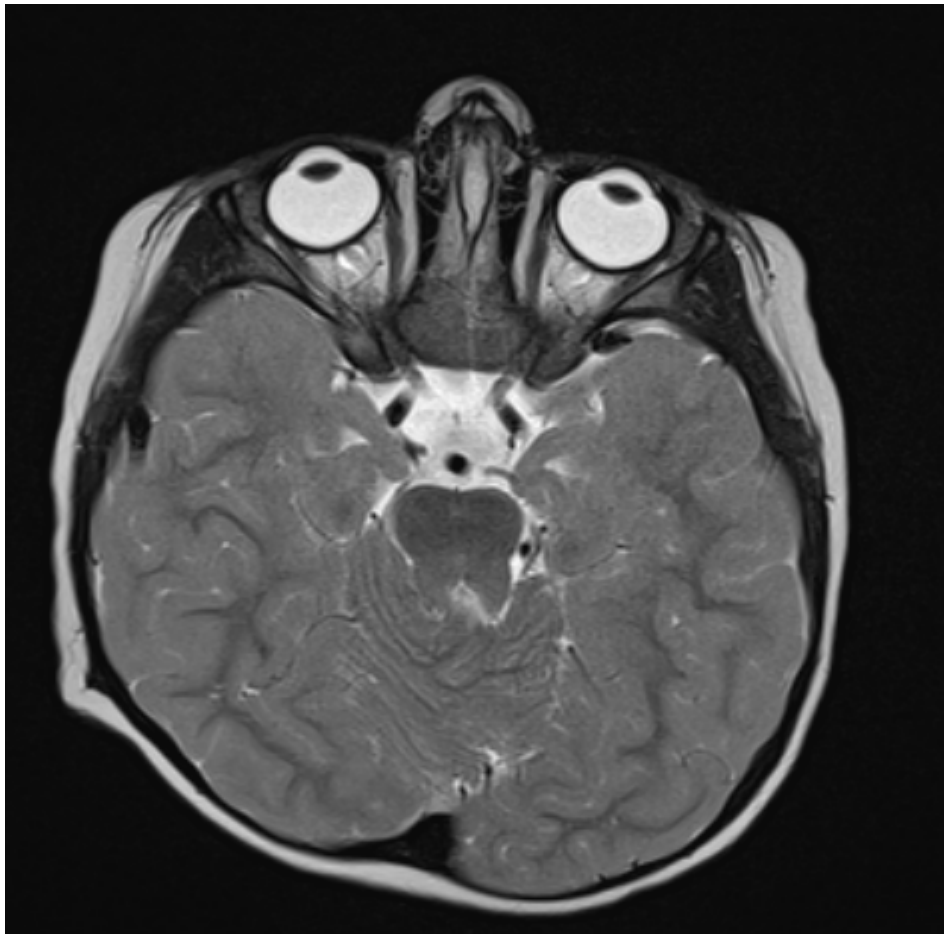
6 years

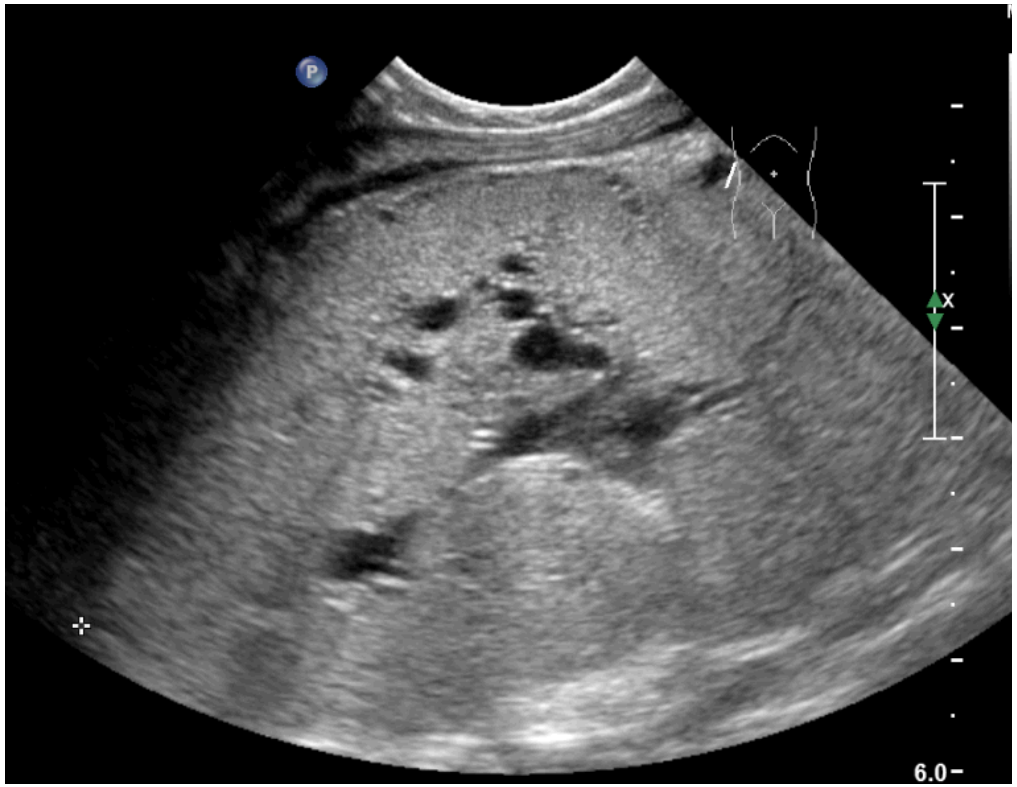
II:2

4 years

Emma

- Nystagmus, LCA – blindness by 6 months
- Seizures, developmental delay, MTS
- ESRD 1yr 11 m – Peritoneal Dialysis

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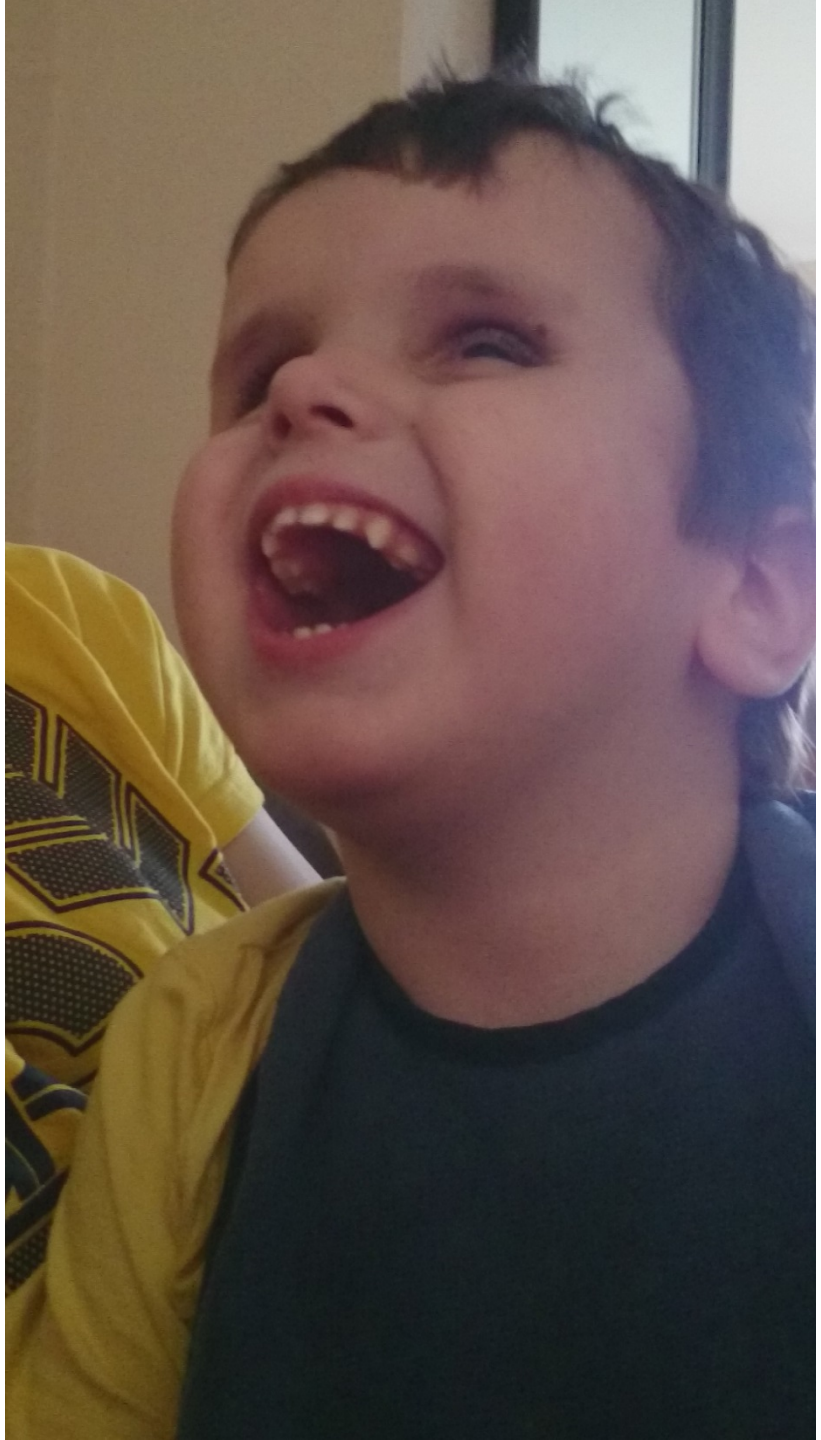
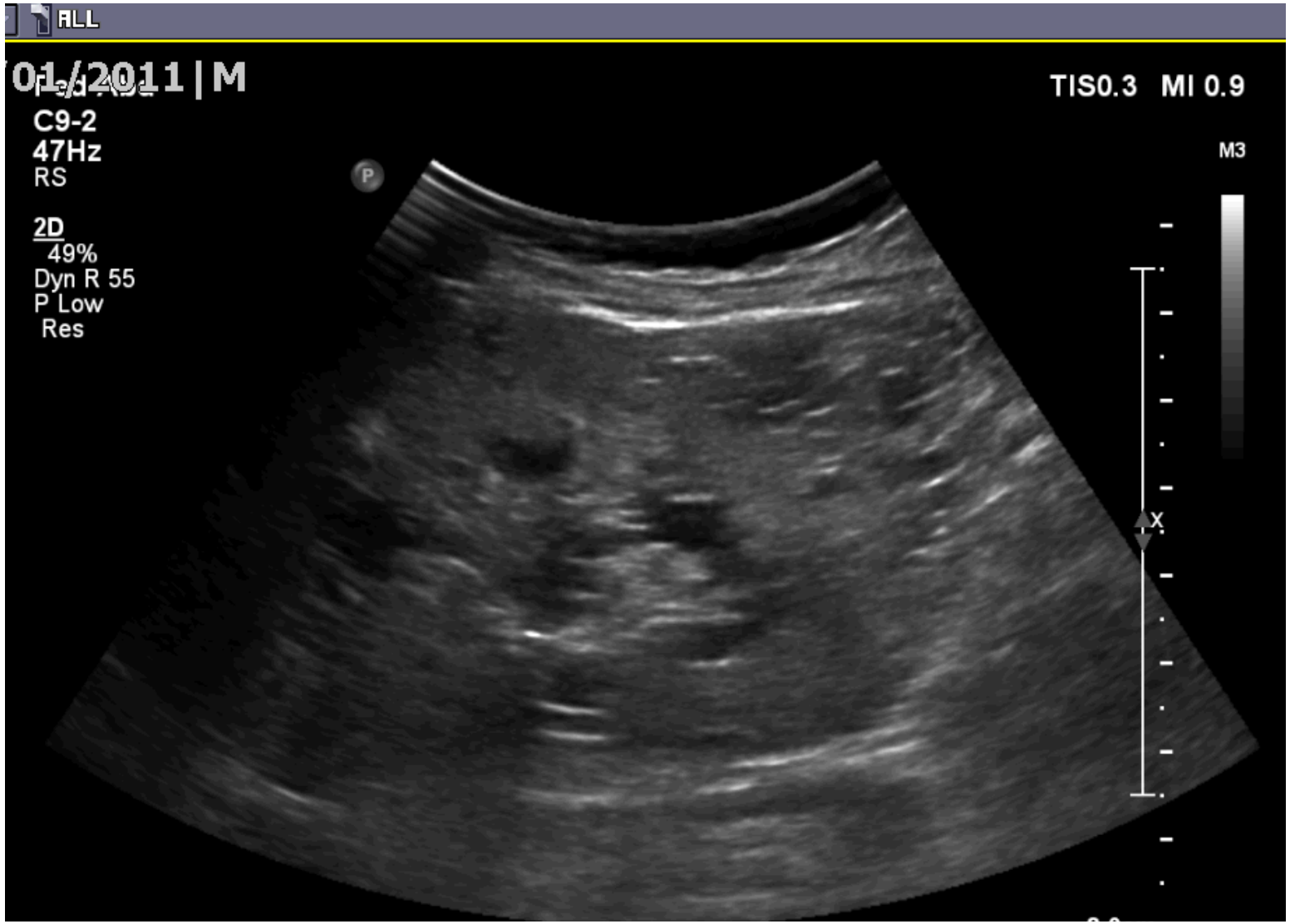


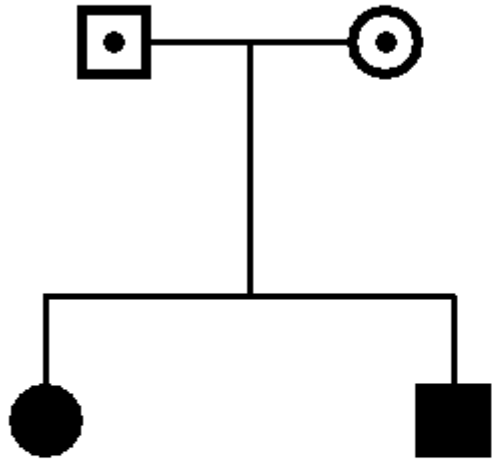
Ben

- Nystagmus, LCA – blindness by 2 months
- Developmental delay, scoliosis
- 4 years – polyuria, CKD 4



USS renal
Ben

Family BB – molecular genetic diagnosis *CEP290* mutations – compound het



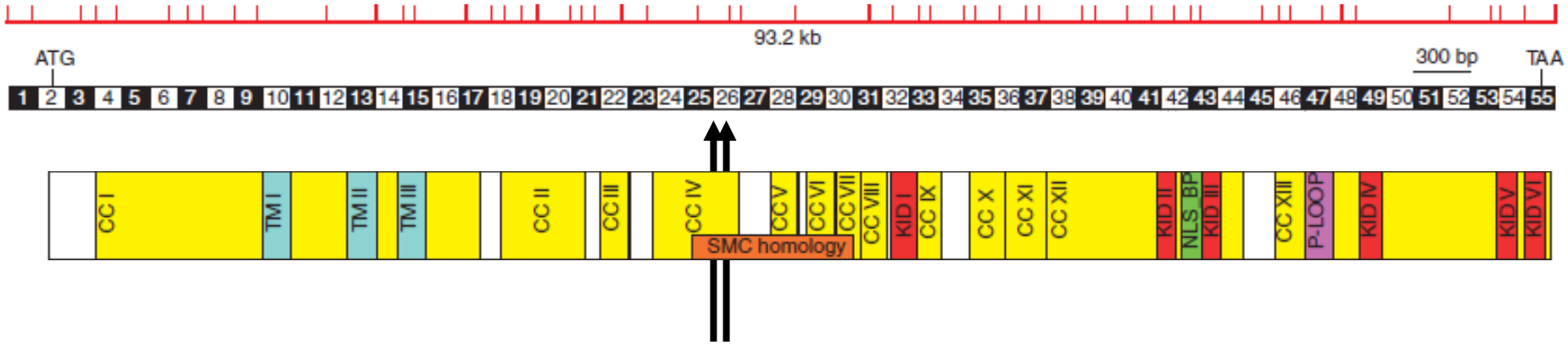
II:1

6 years

II:2

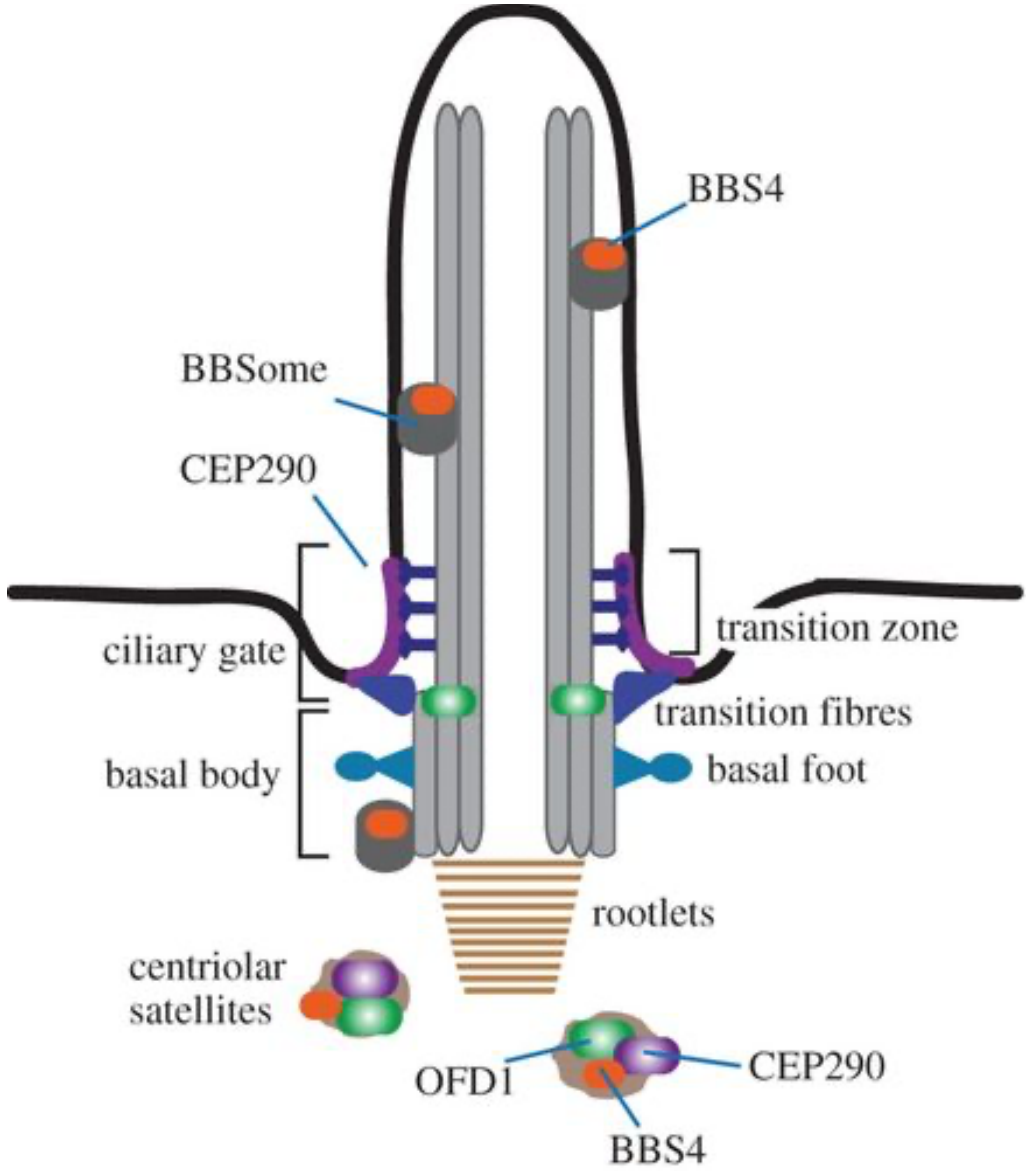
4 years

CEP290



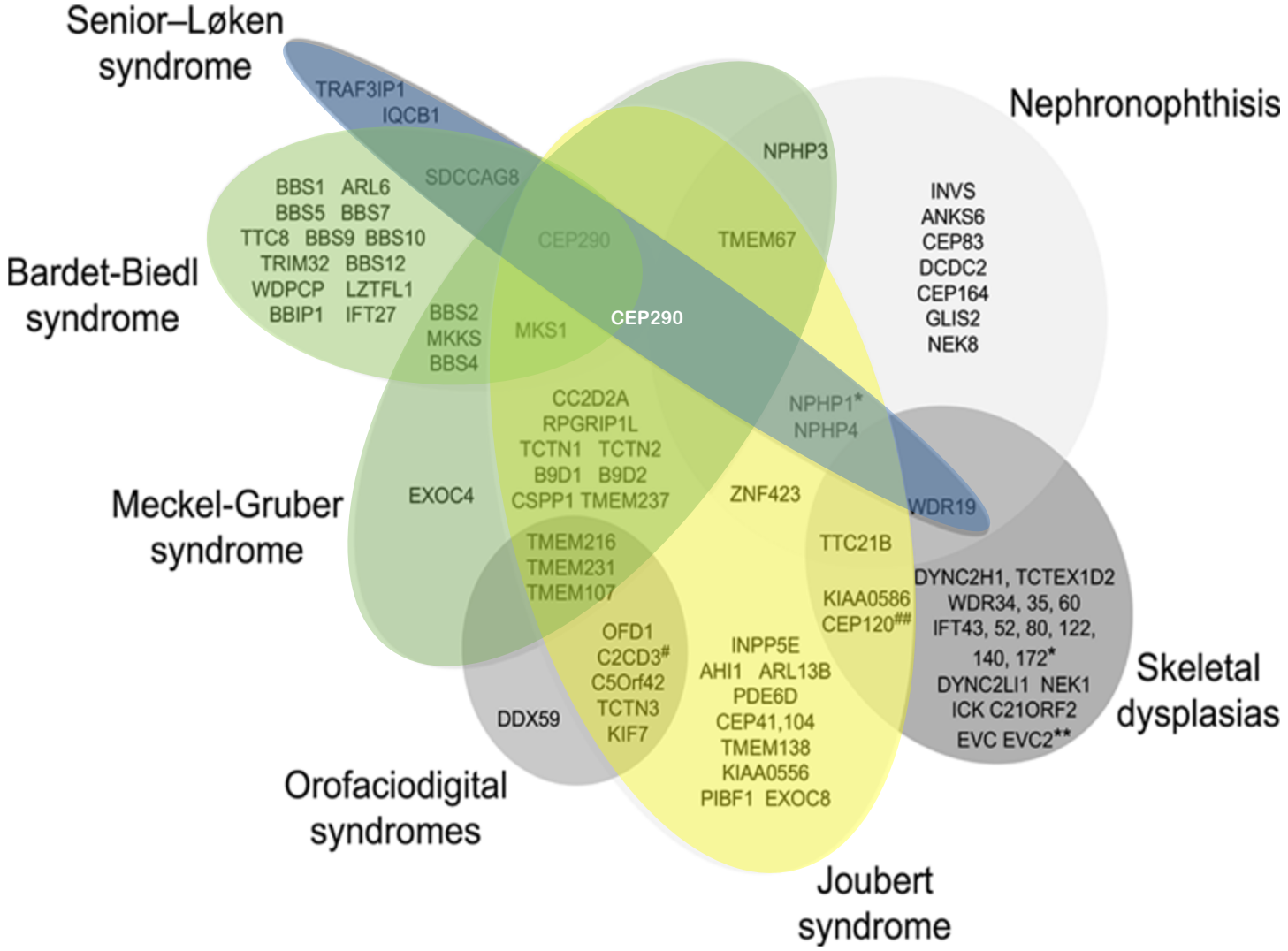
Het c.2817G>T
Het p.K939N
Splice site lost

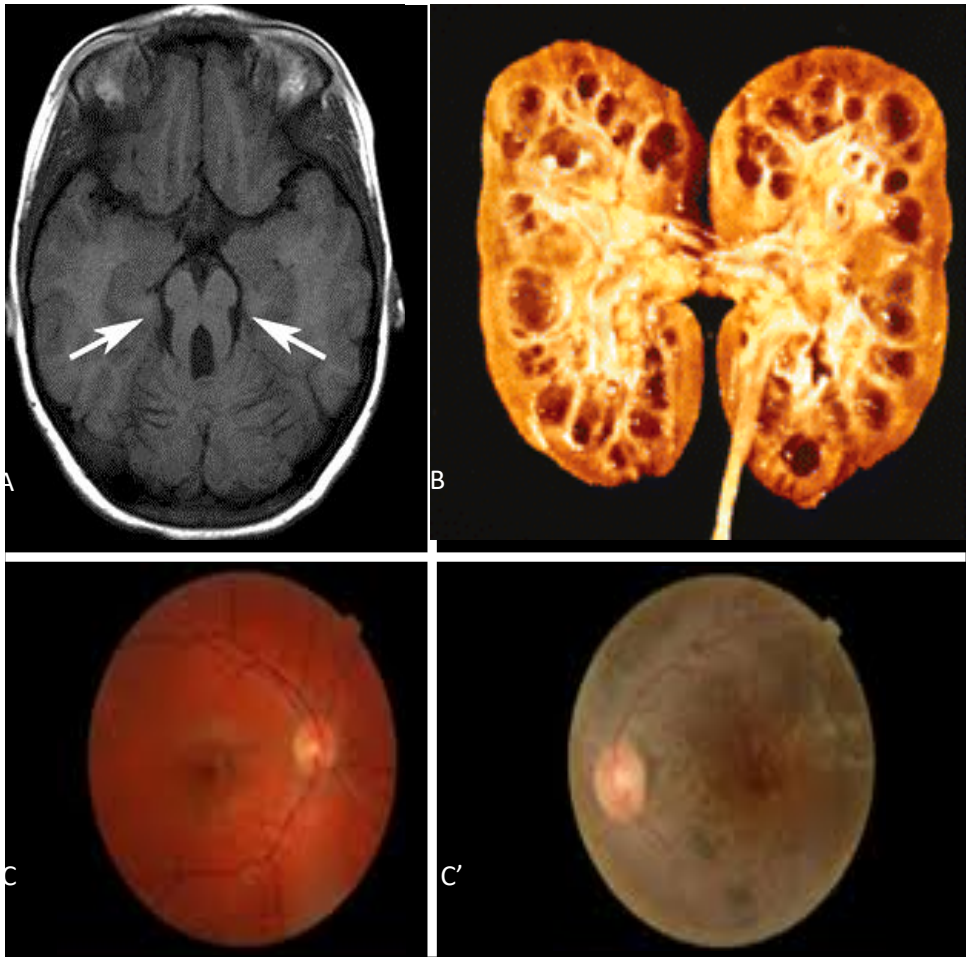
Het c.2848insC
Het p.Q950Pfs*6
frameshift



Chavali et al 2014

- CEP290 is a transition zone protein that regulates protein entry and exit to the primary cilium
- Wide disease spectrum of *CEP290* mutations
  - LCS, Senior-Loken, Joubert, MKS, BBS

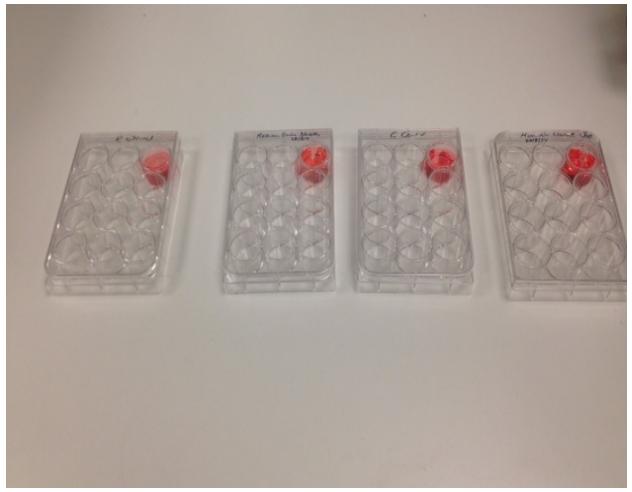


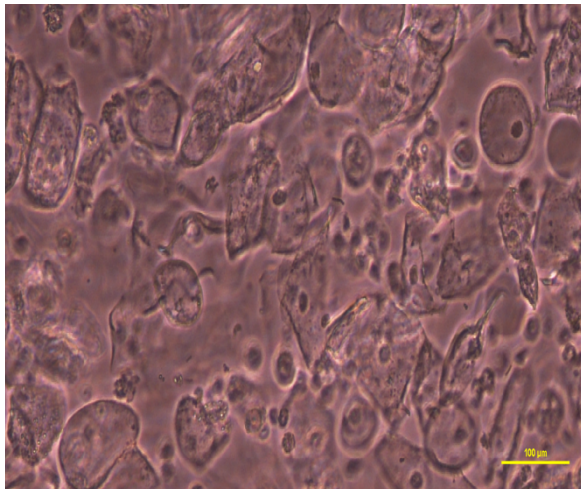


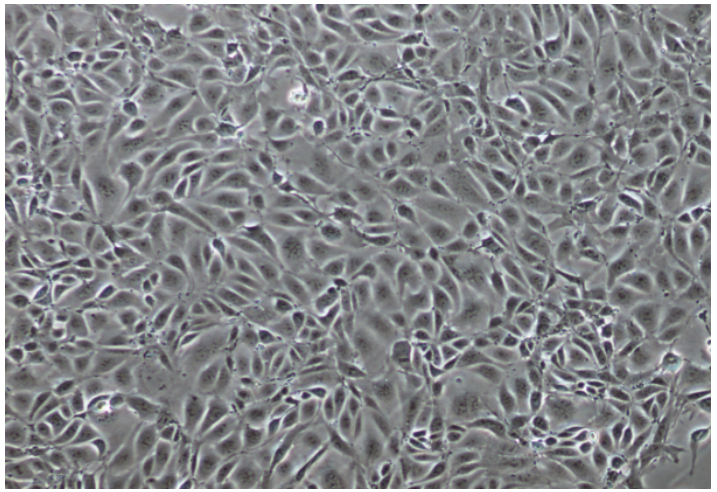
JASN

The Journal of Pathology, 241, 2, 294-309, 2016

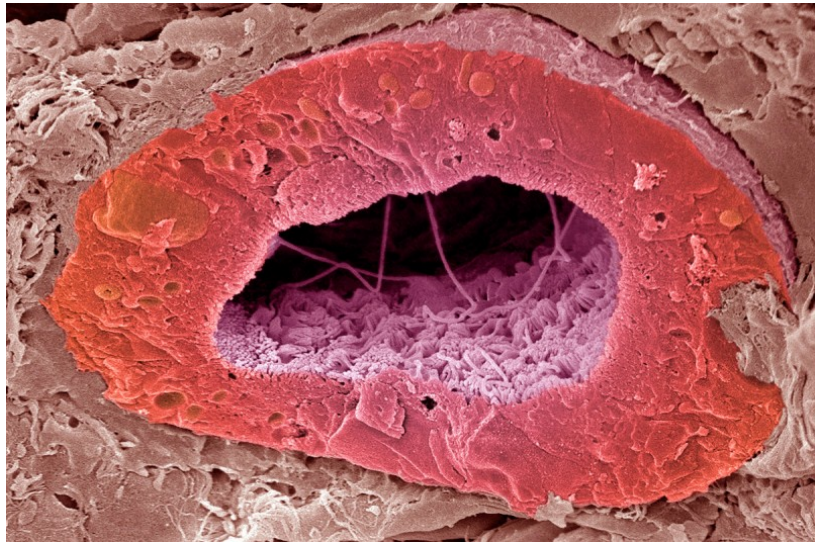
Human Urine derived Renal Epithelial Cells (HuRECS)

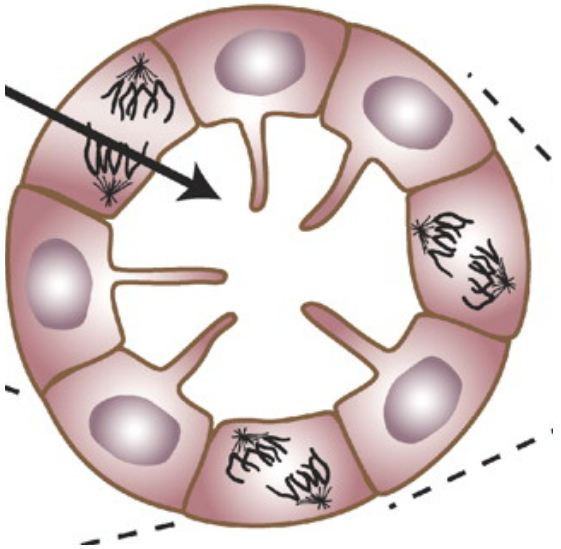


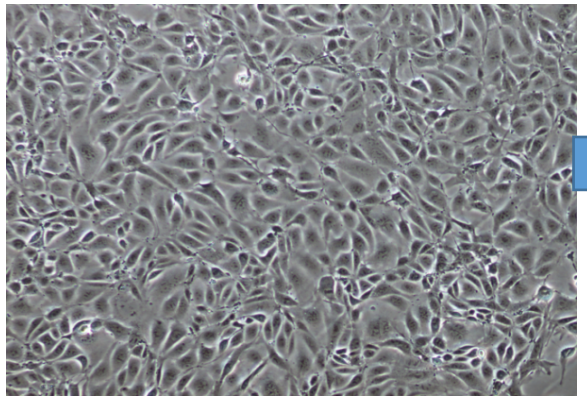




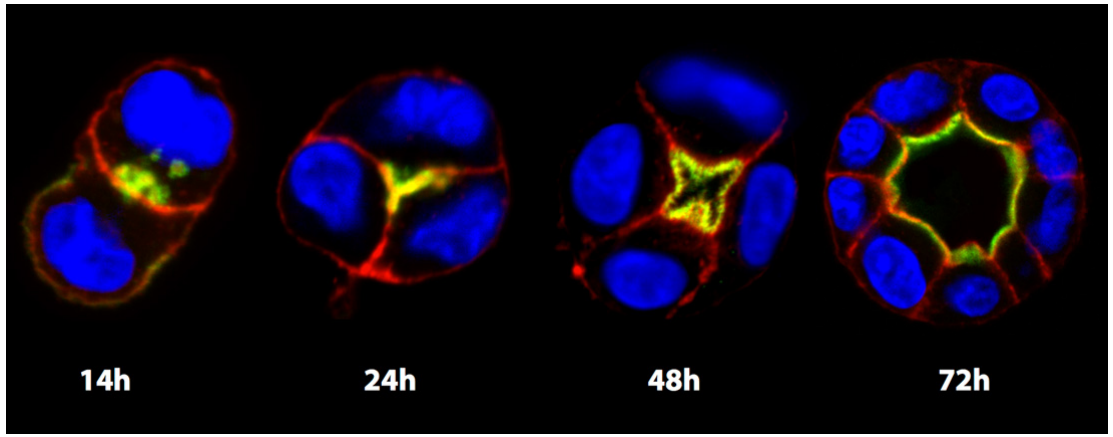
Kidney cells are 3D structures



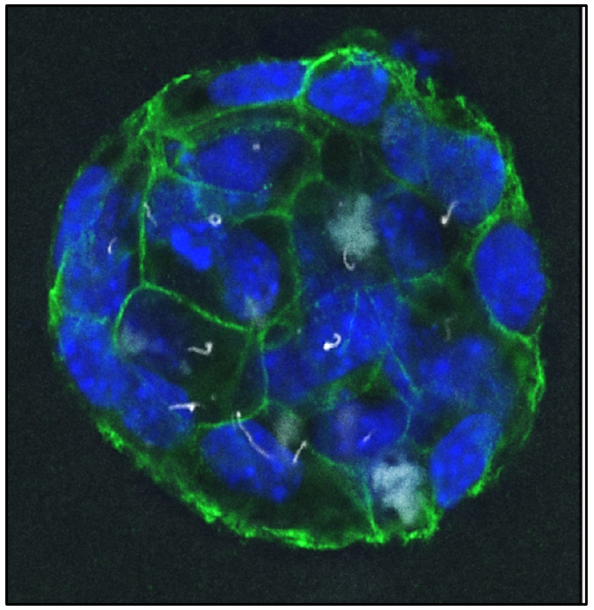




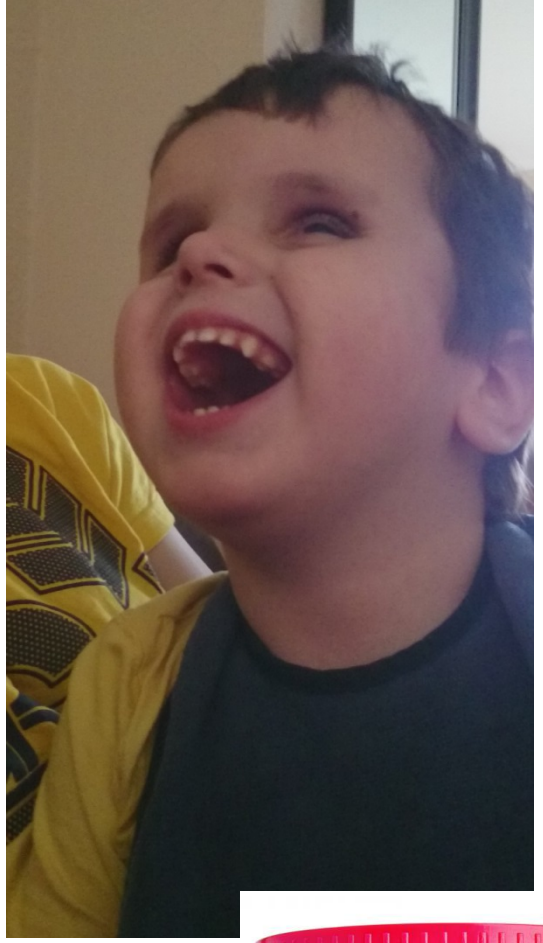
Matrigel



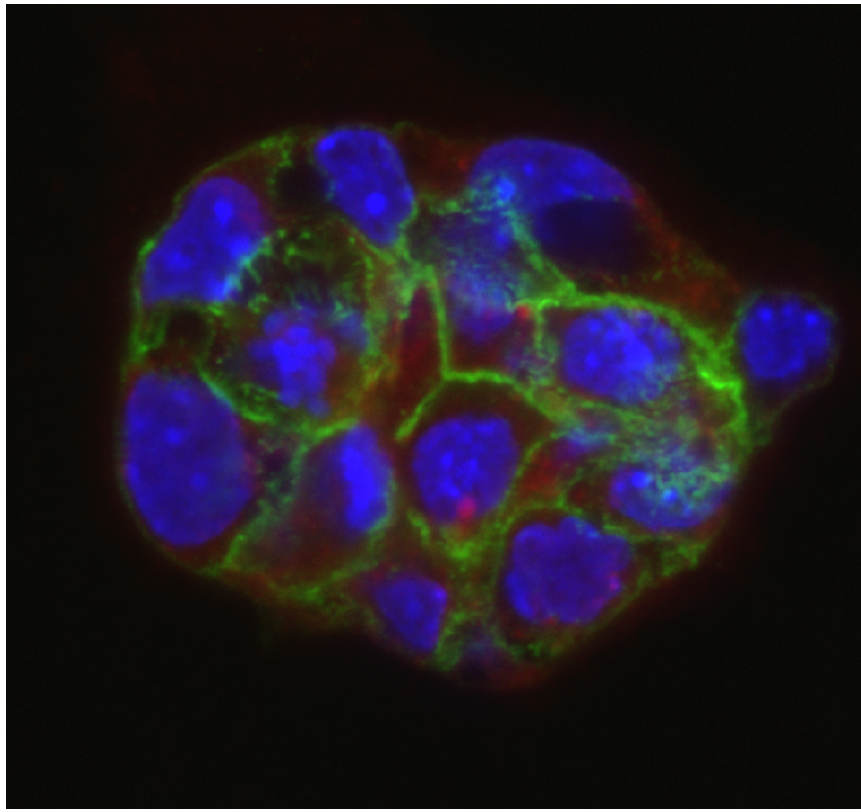
Belmonte et al. 2015

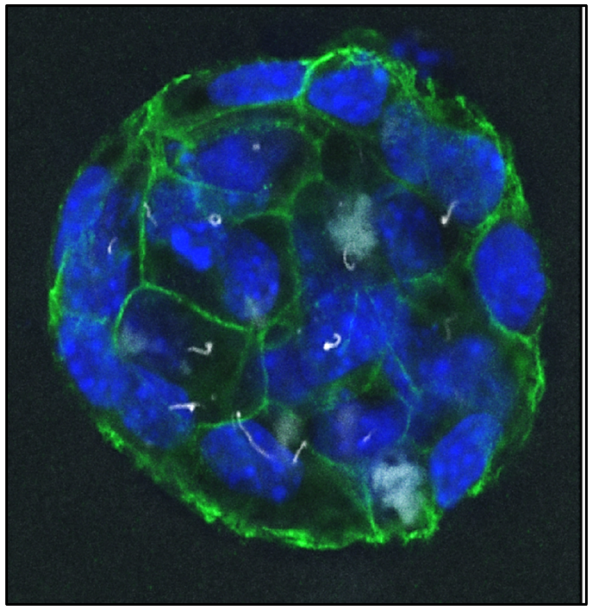


Healthy kidney cells

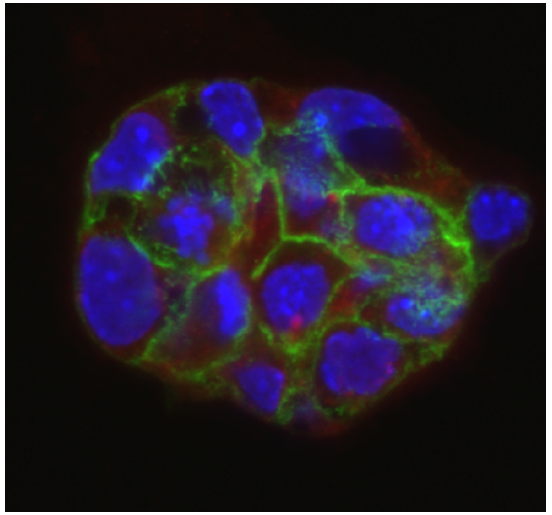


JBTS kidney cells – unable to form 3D Spheroids

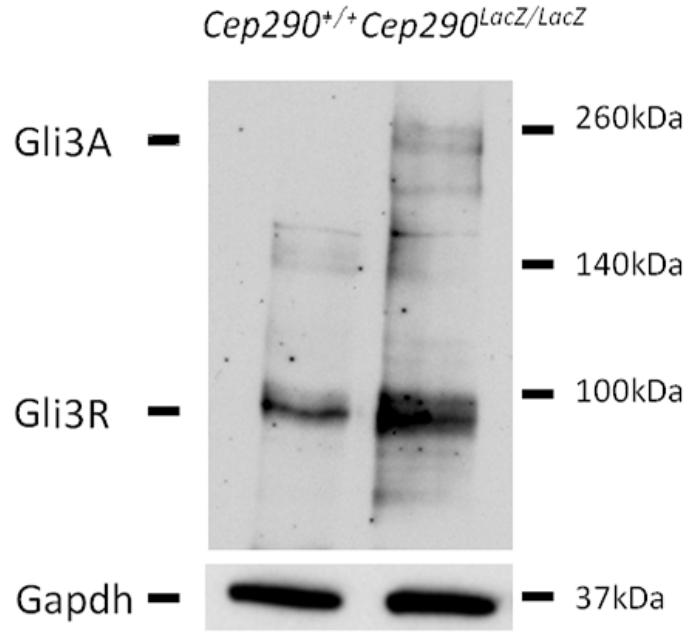




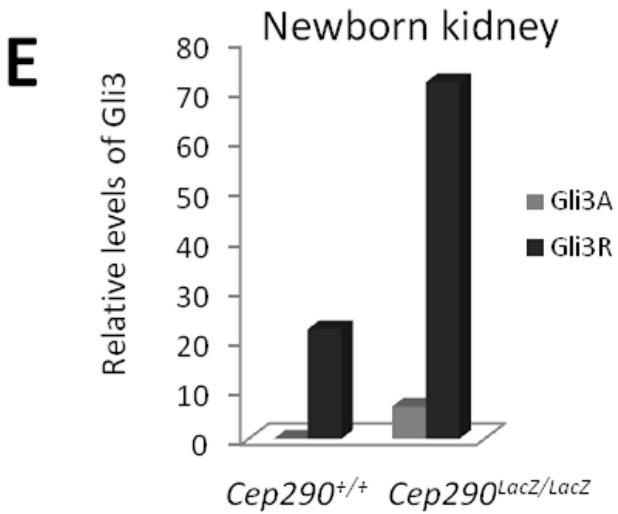
Healthy kidney cells



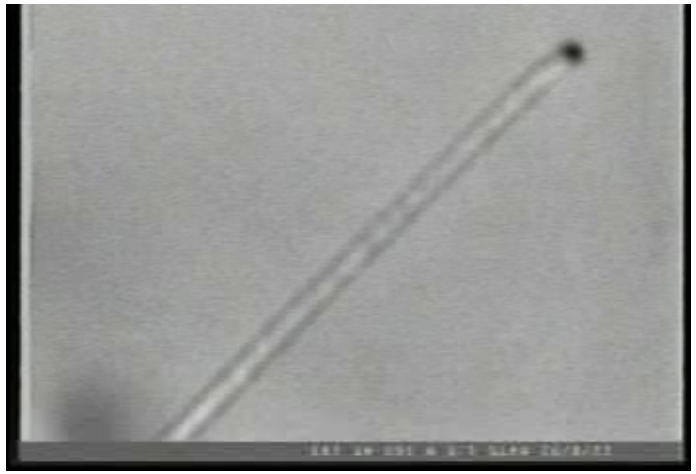
Joubert kidney cells

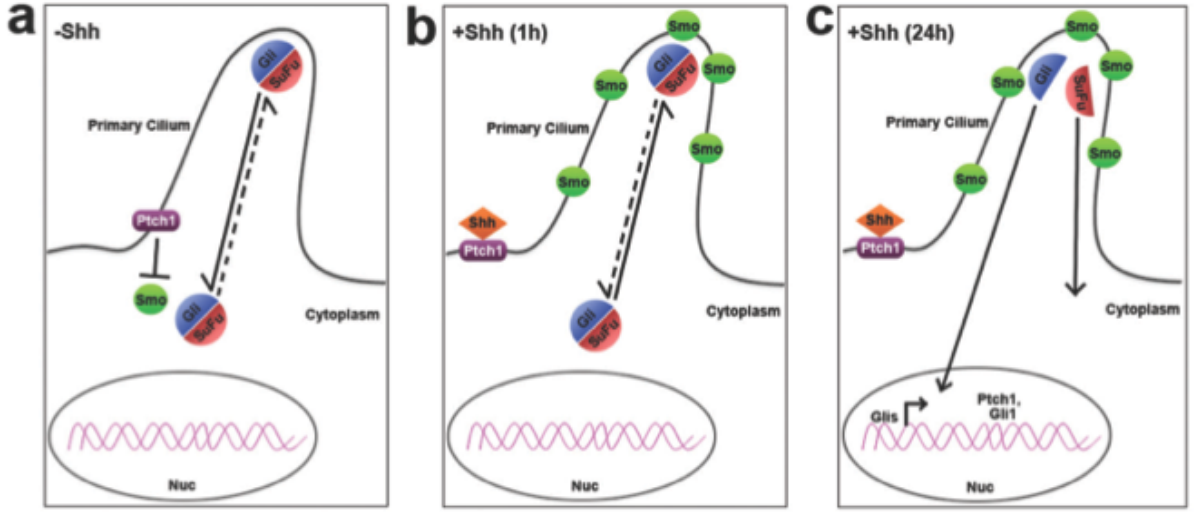


Defective Hh signalling seen in Cep290 mutant kidney

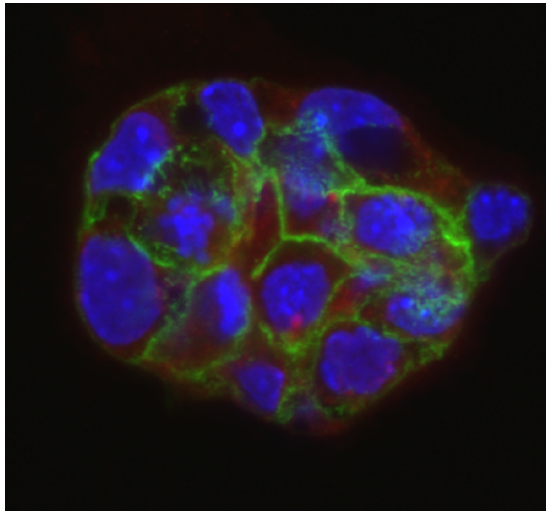


How can we repair these cells?

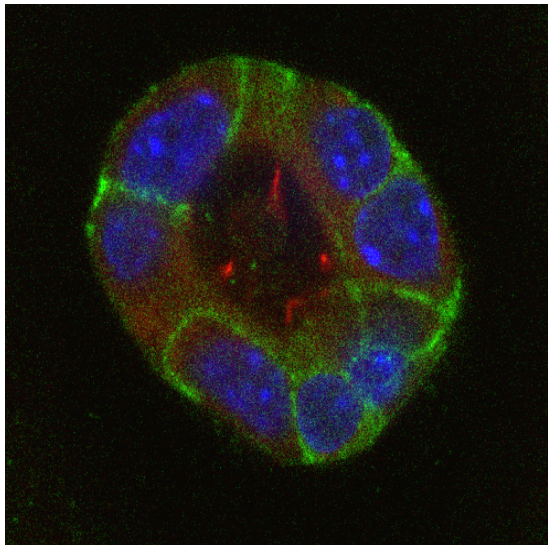


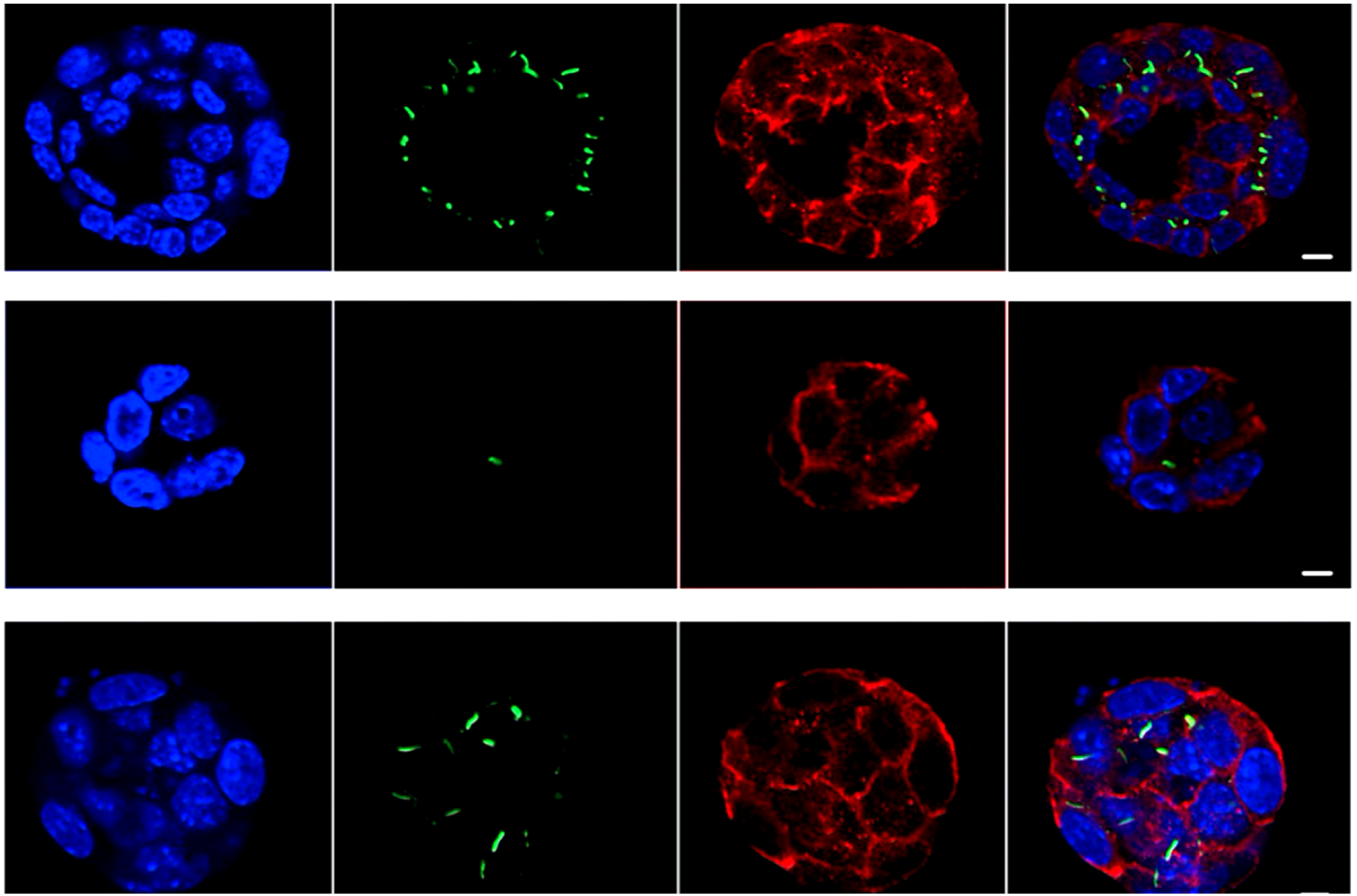


Cilia signaling was turned off in JBTS cells…gatekeeper function of CEP290 lead to abnormal protein signalling
Could we turn it on again?



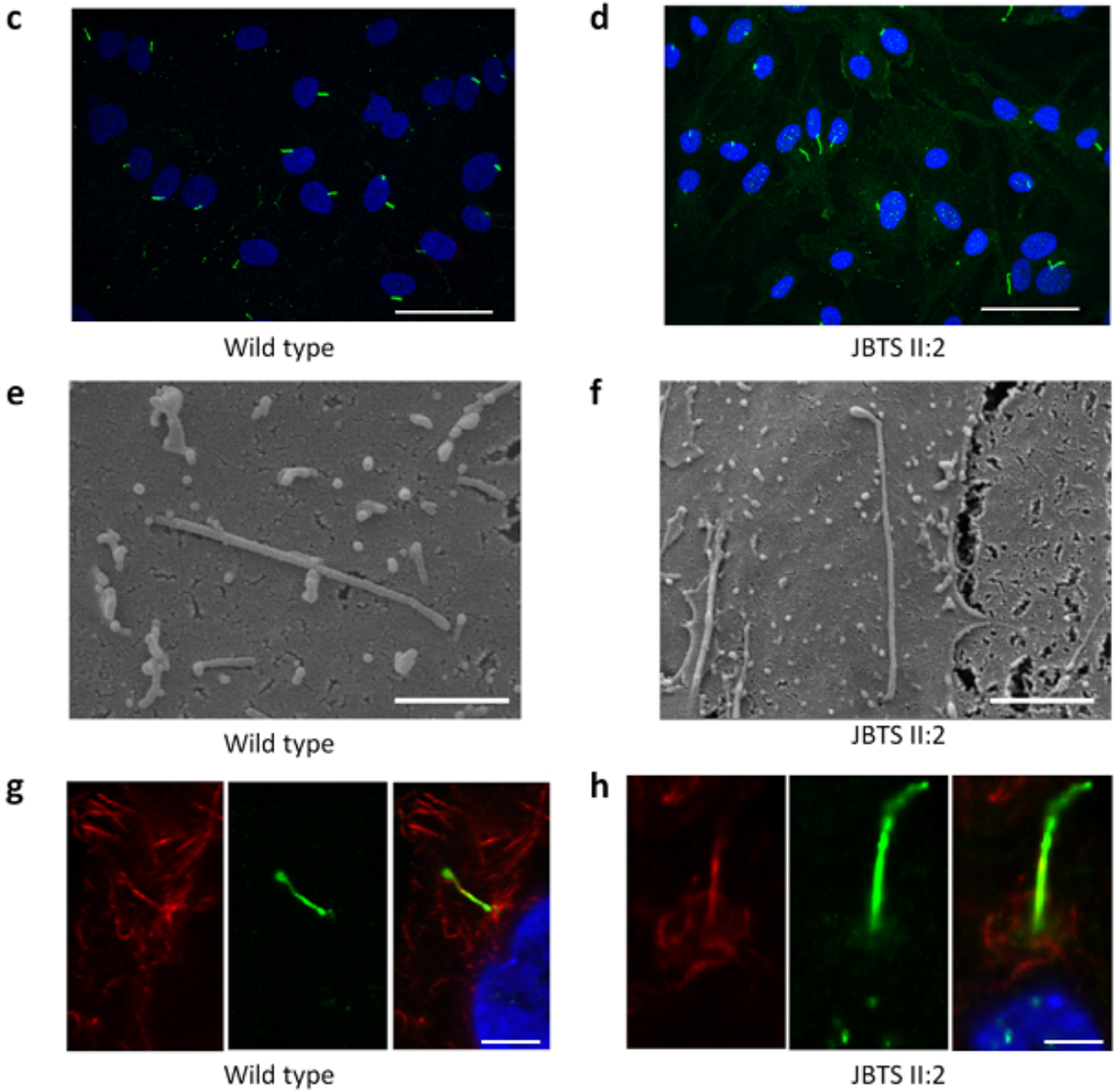
Purmorphamine to switch on cilia signalling





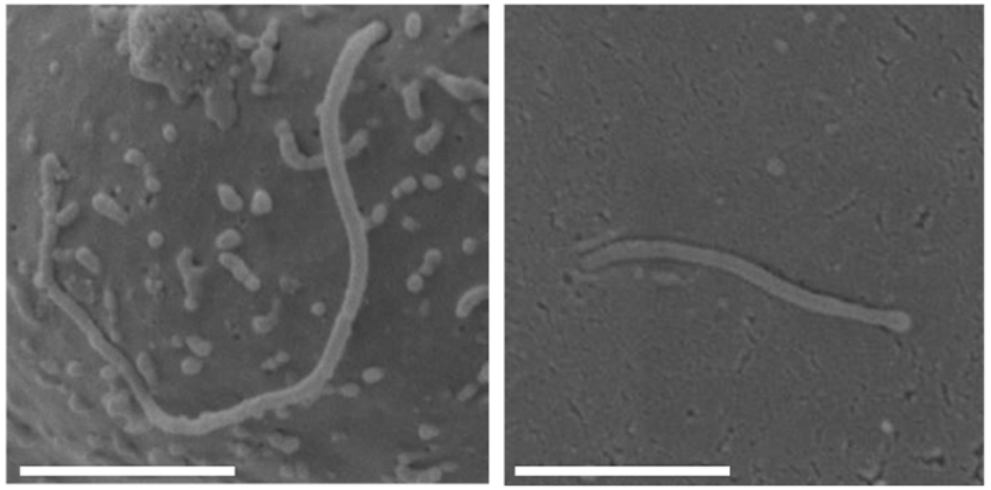
Rescue with purmorphamine treatment

BB extra long cilia



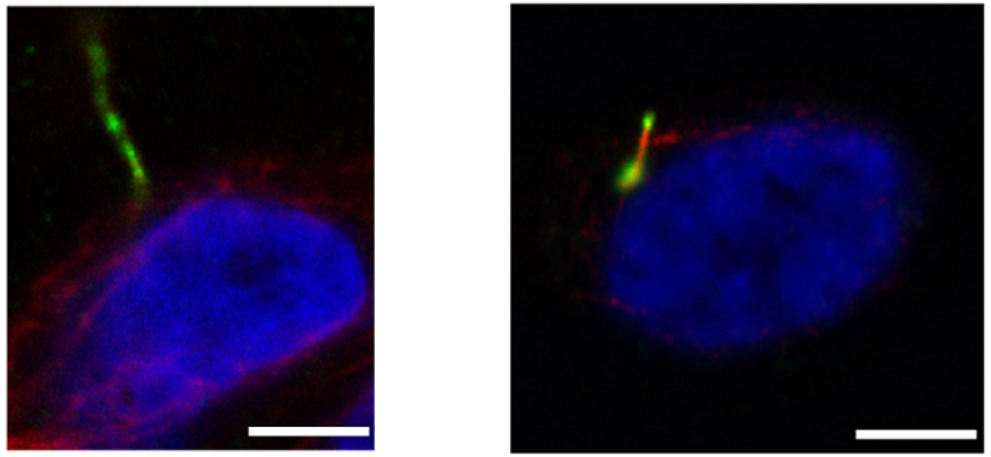
BB long cilia can be rescued

a



JBTS II:2 JBTS II:2 + Pur

c

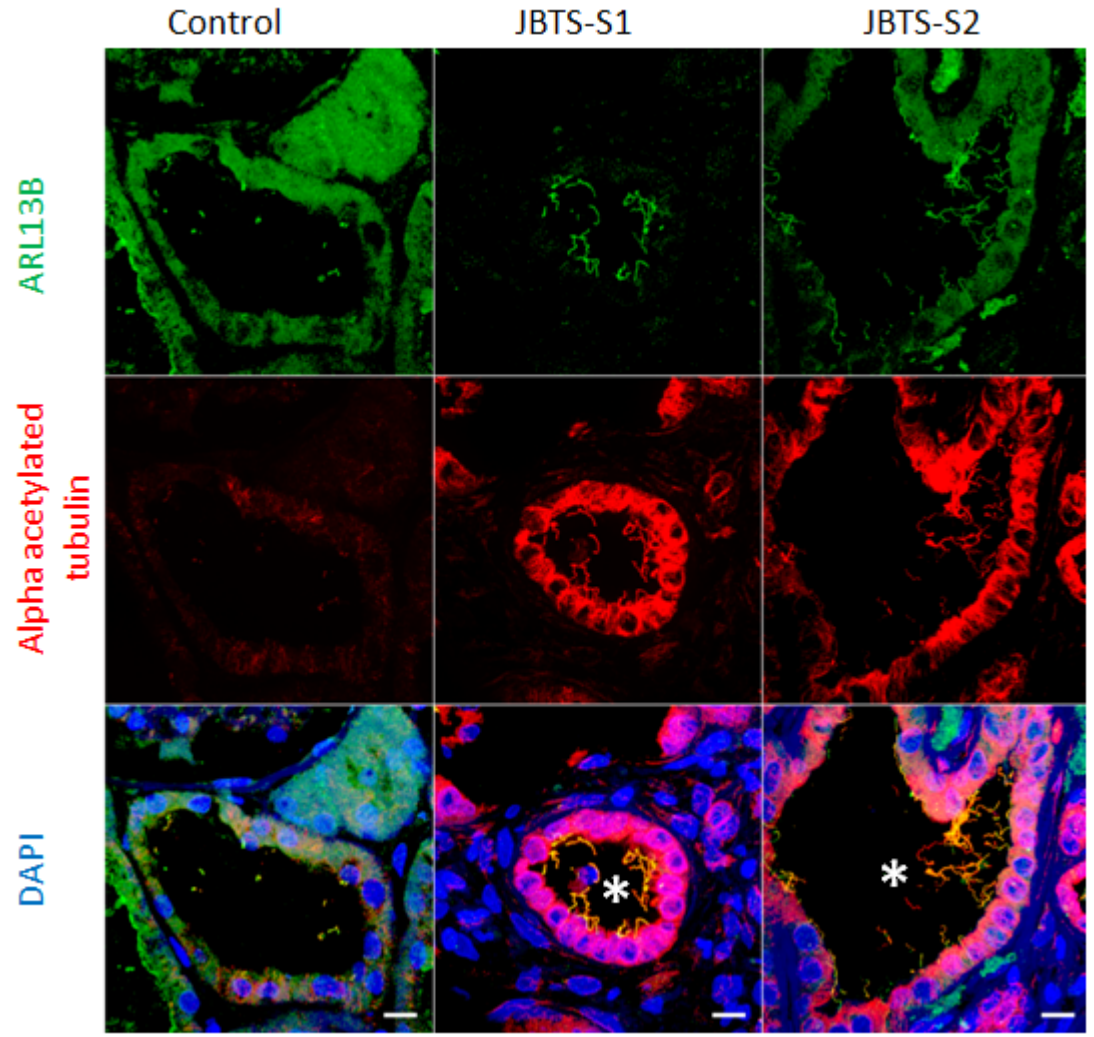


JBTS II:2 JBTS II:2 + Pur

Purmorphamine -toxic

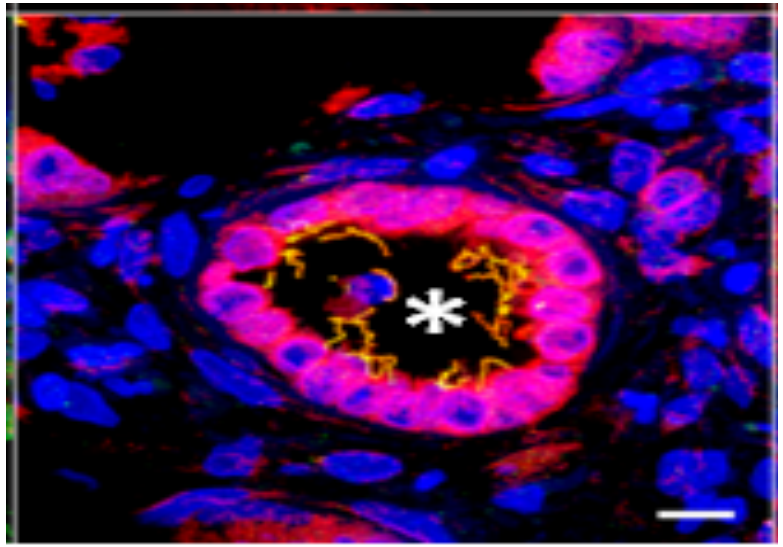
Alternative drugs which have the same affect (and are less toxic)

CEP290 / Joubert syndrome renal biopsies

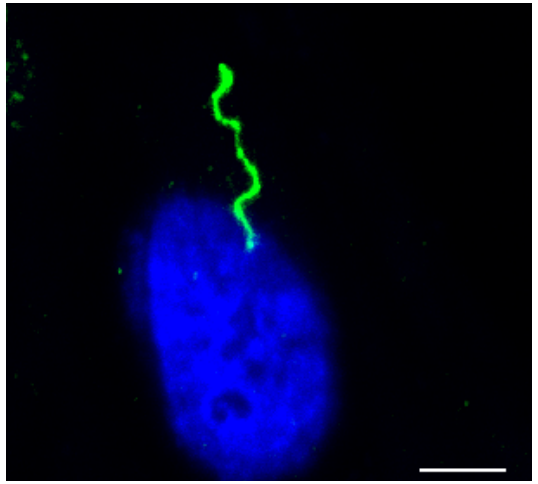


Human Urine Derived renal cells

- A virtual/liquid kidney biopsy

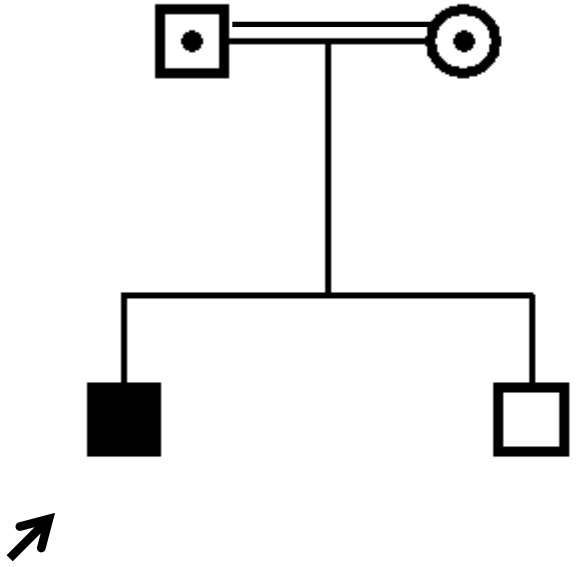


Human Kidney



Human Urine
Derived kidney cell

Family AA



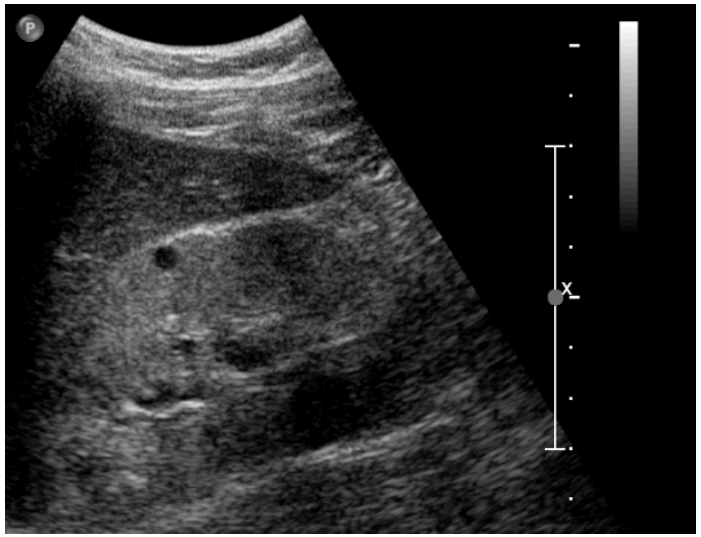
II:1

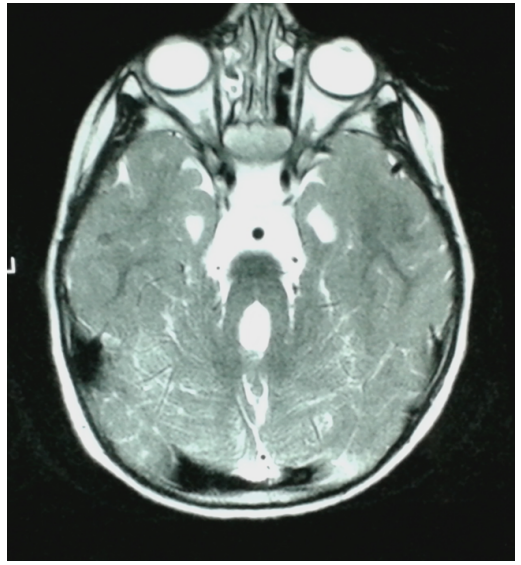
Aged 14

II:2

Aged 11

Family AA

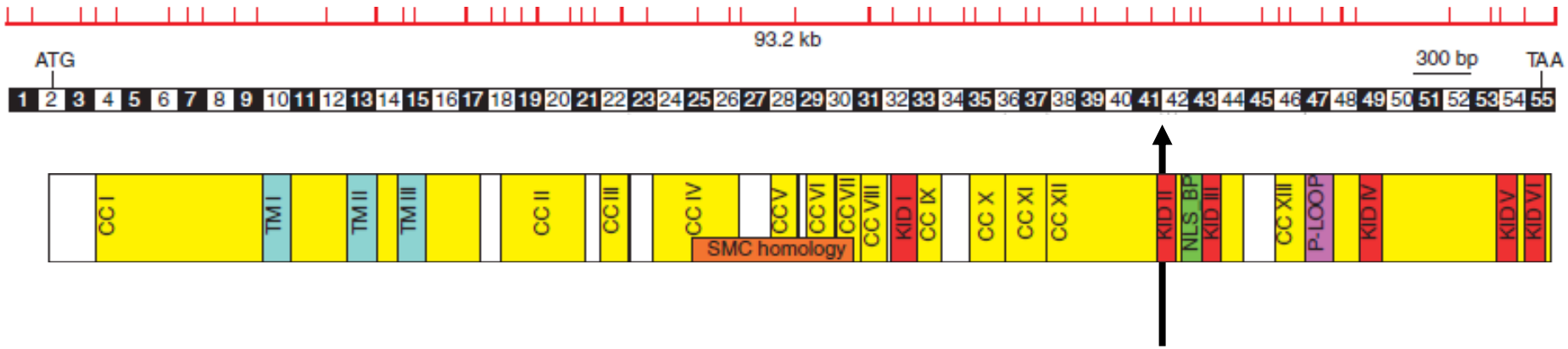




Clinical features

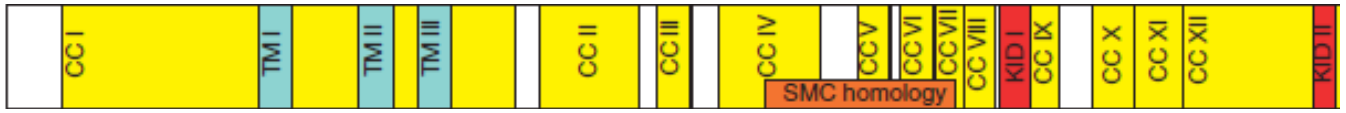
Family (individual)	Origin	Nucleotide alterations	Alteration in coding sequence	Exon (segregation)	Parental Consanguinity	Renal USS	CKD /ESRD (years, months)	Ocular symptoms (age of onset, months)	Central Nervous symptoms (other)
FA (II:1)	Asian	Hom c.5668G>T;	Hom p. Gly1890*	41 (M&P)	Yes	Increased echogenicity, cortical cysts	CDK 3, eGFR 45, Creatinine 169 umol/l (16 y)	Ptosis(1 m) CA (2 m)	Ataxia CVA

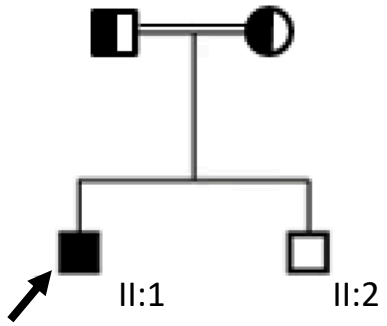
CEP290

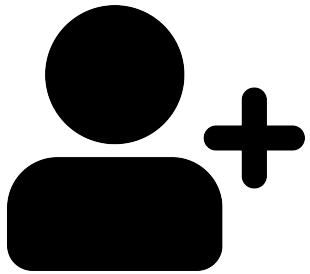


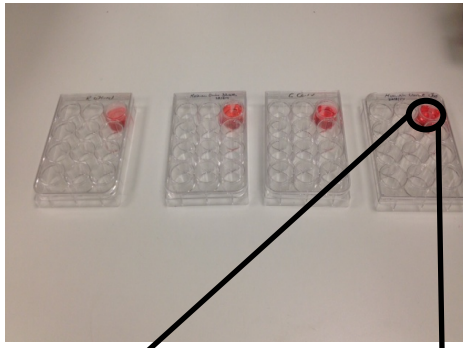
Hom c.5688G>T
Hom p.G1890*

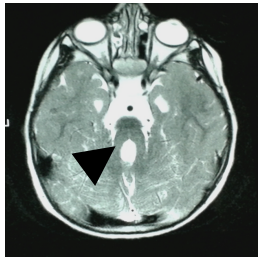
Predicted truncated CEP290 protein

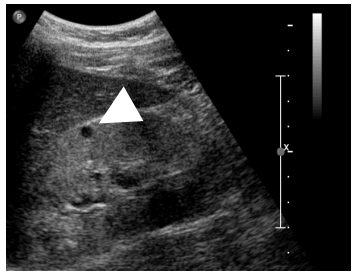








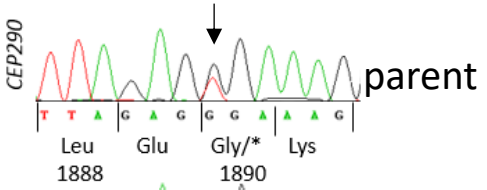


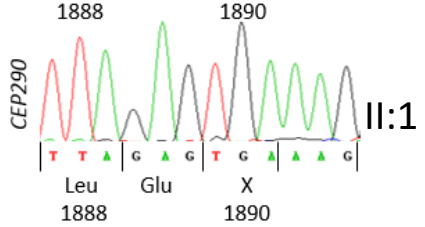


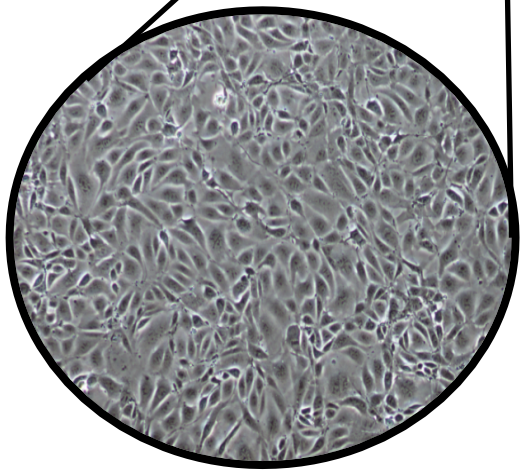
CEP290

Hom c.5668G>T

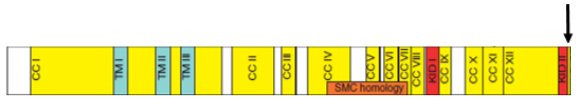
Hom p.G1890X



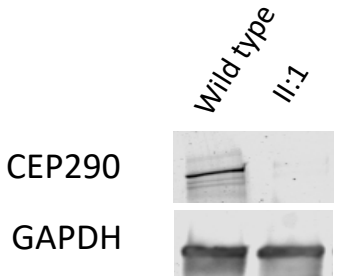


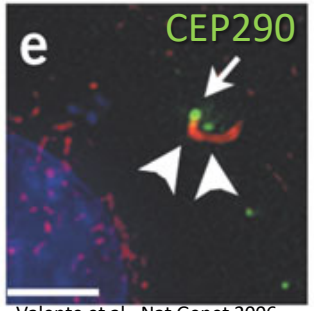


Wild type CEP290 protein

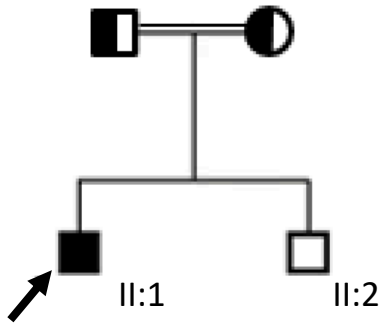


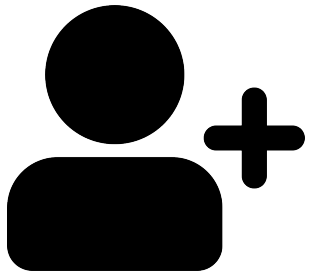
Mutated CEP290 protein

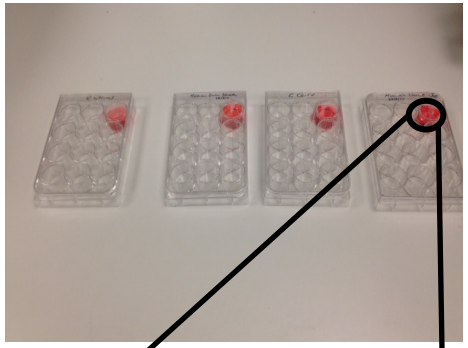


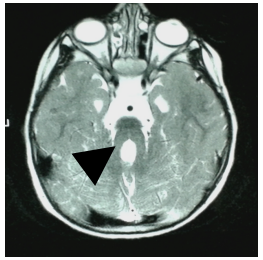


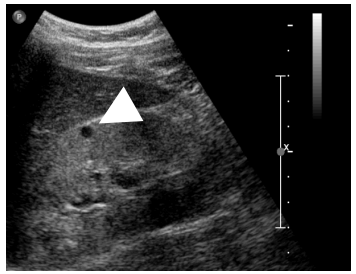
Valente et al., Nat Genet 2006



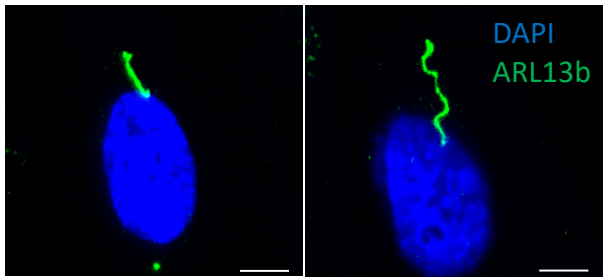


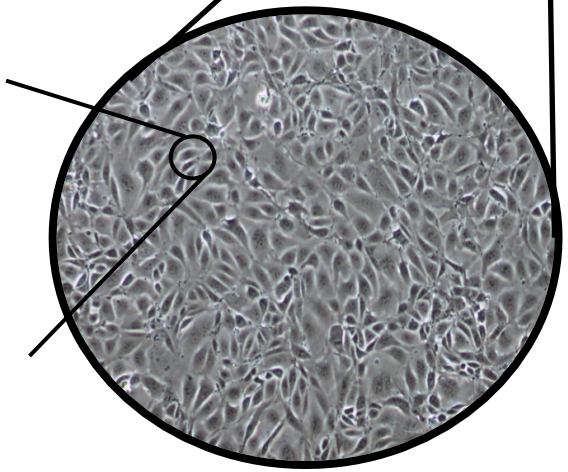


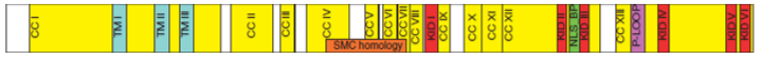




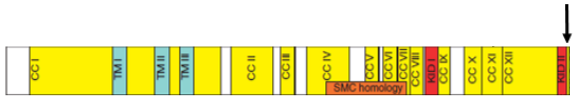
Wild type II:1



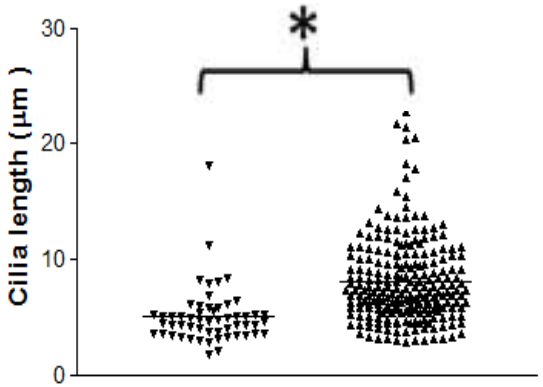


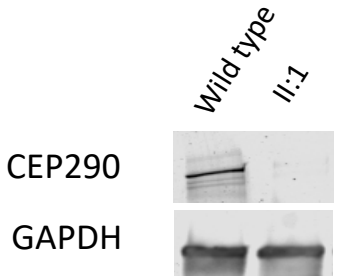


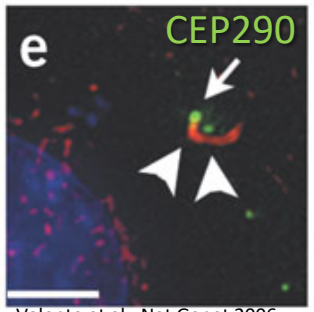
Wild type CEP290 protein



Mutated CEP290 protein



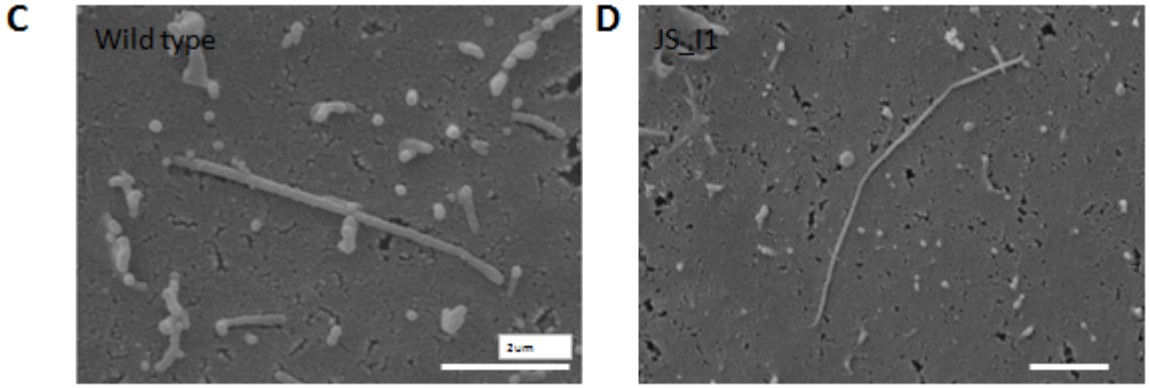




Valente et al., Nat Genet 2006

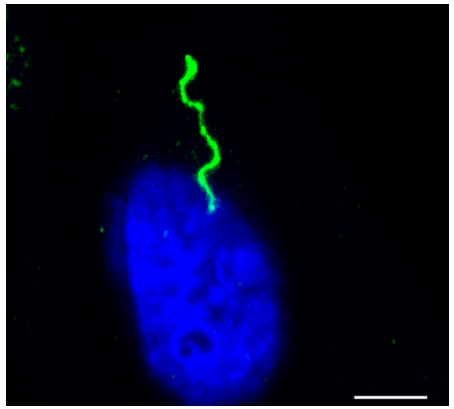
Family A and cells grown from urine

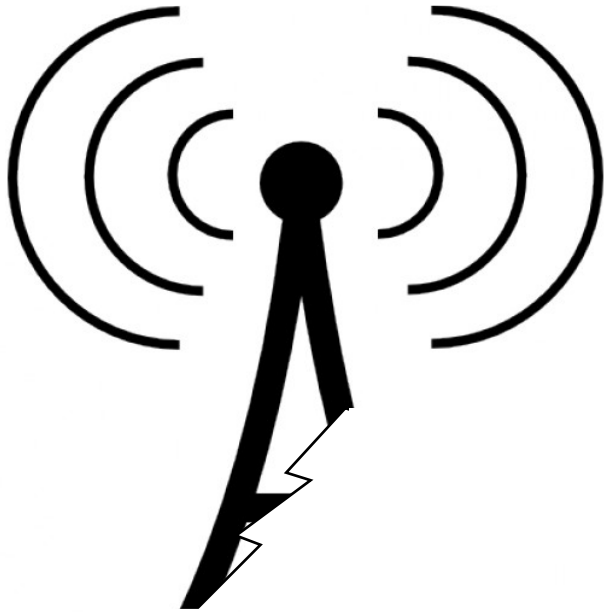
Cells grown from urine allow a “virtual renal biopsy” to be performed

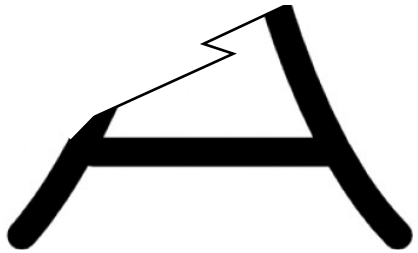


Cilia are abnormally long in JBTS patients

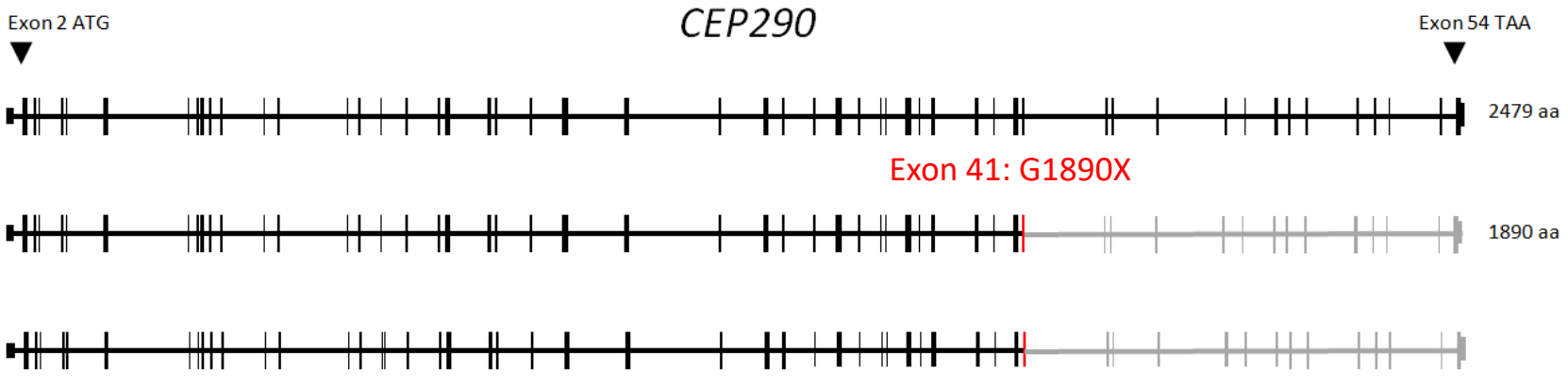
Can we fix the broken antenna?







Targeted exon skipping as a means to treat Joubert syndrome



Hom c.5668G>T

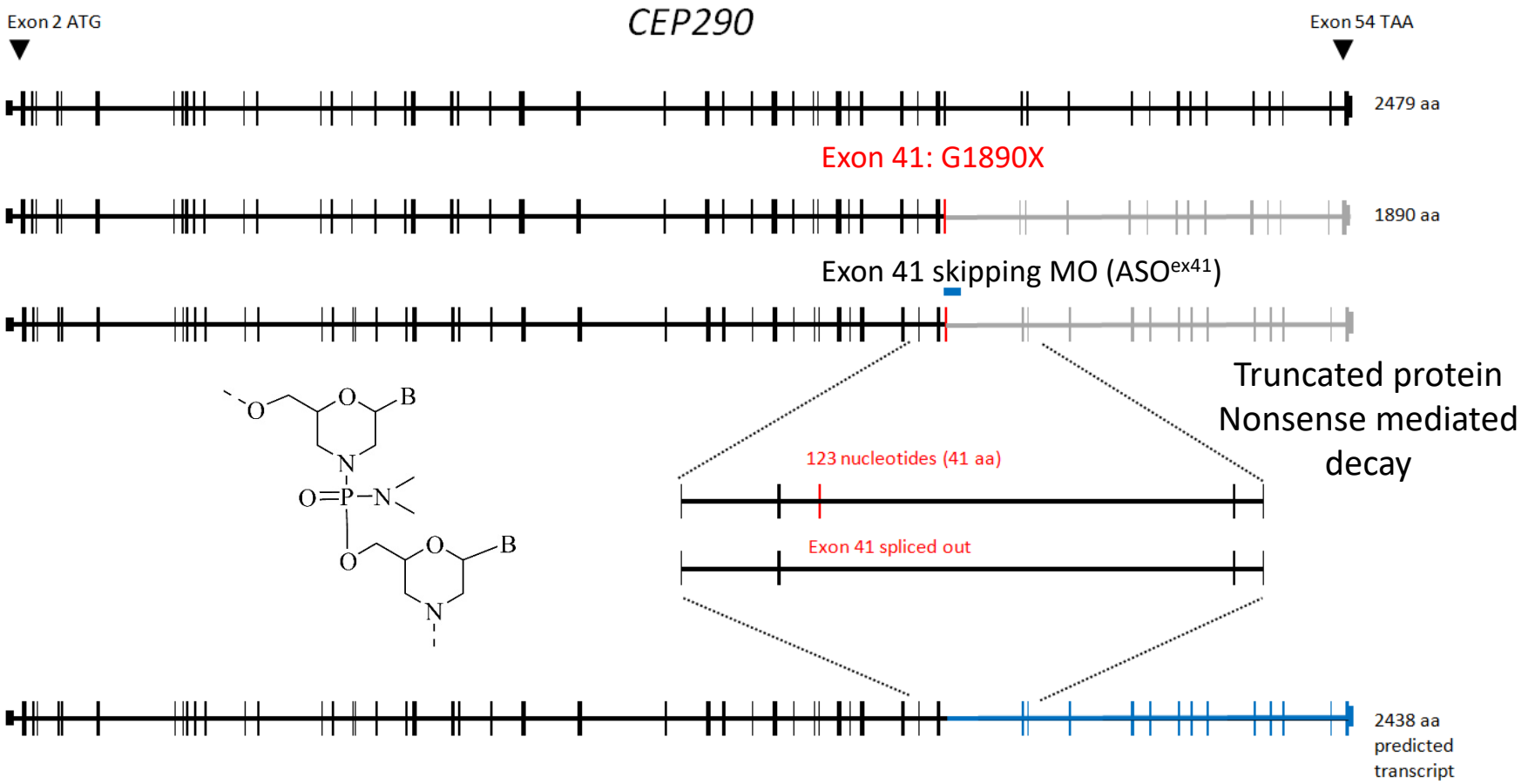
Hom p.G1890X

Exon 41 comprises 123 nucleotides (multiple of 3)
Can be skipped out without affecting reading frame

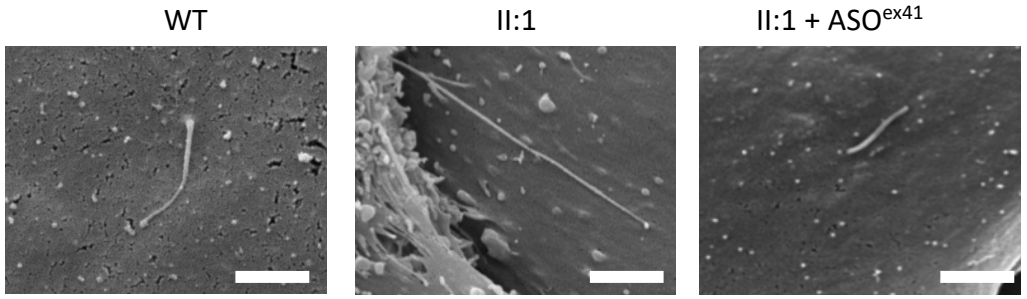
Targeted exon skipping as a means to treat Joubert syndrome

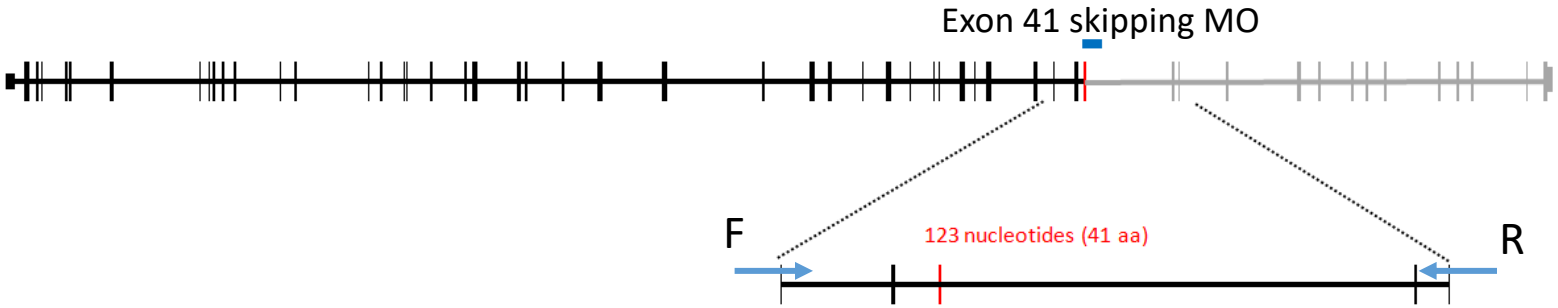
Hom c.5668G>T

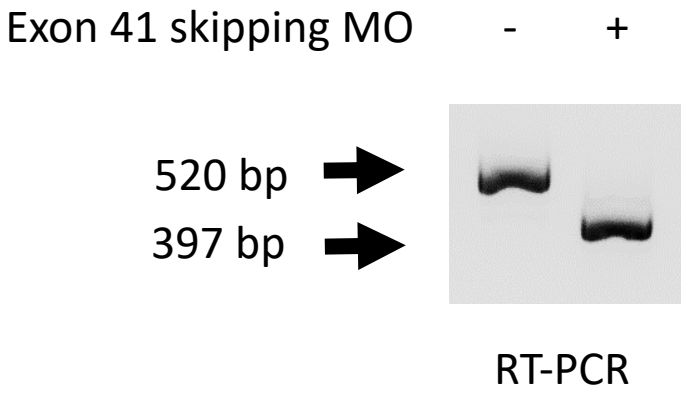
Hom p.G1890X

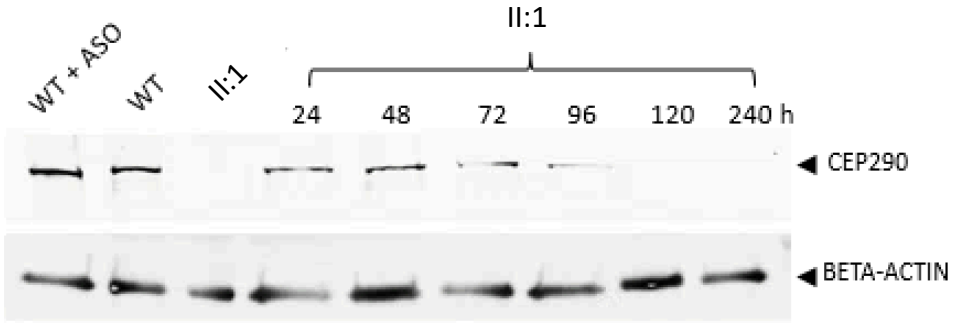


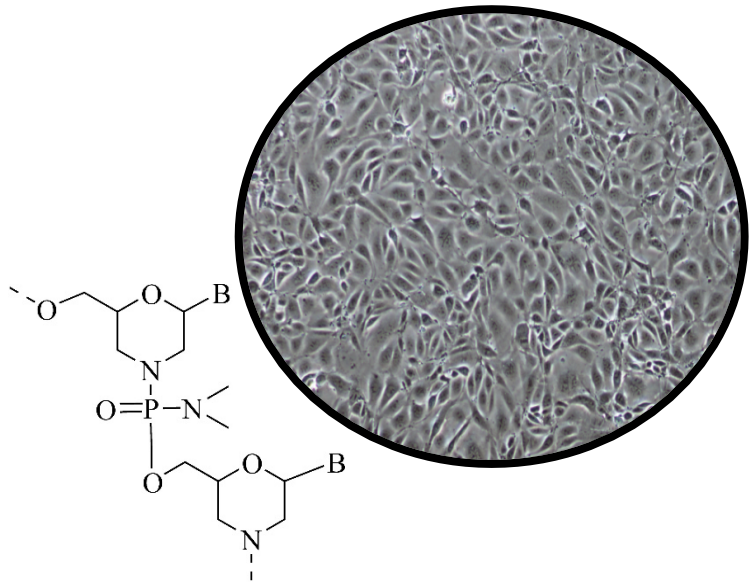
URECs

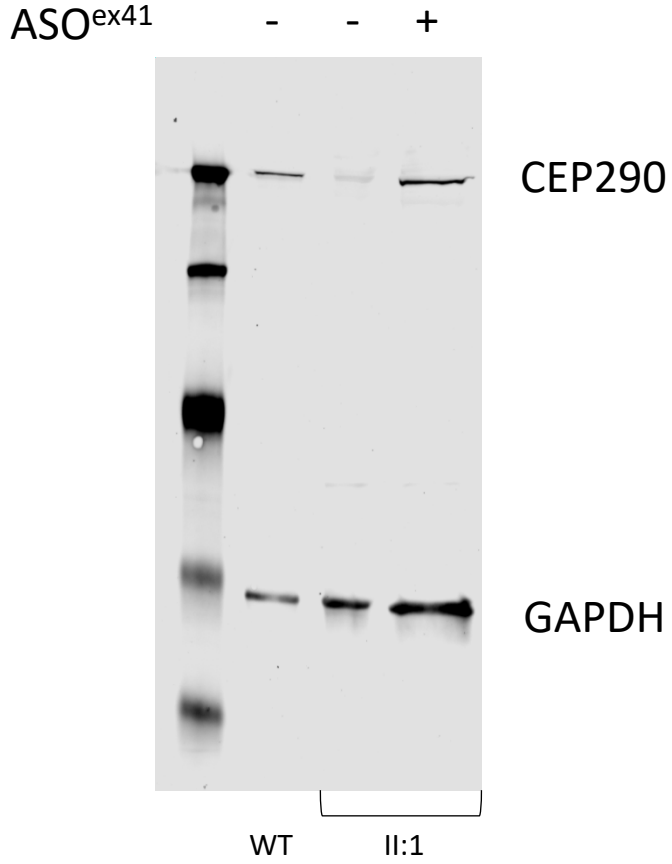

WT
II:1
II:1 + ASO^{ex41}

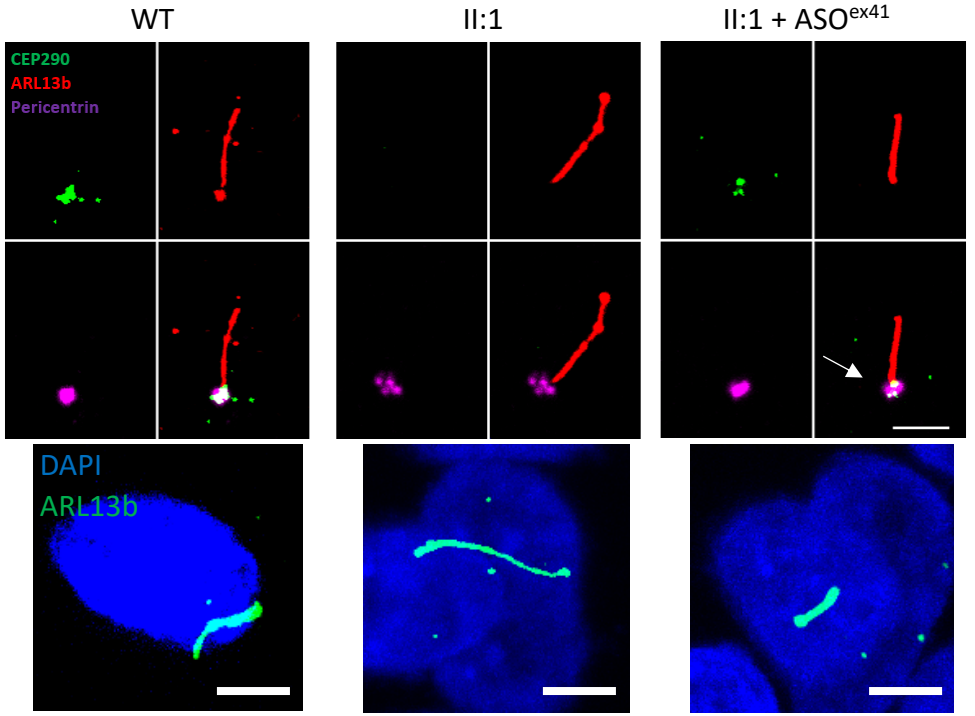

Exon 41 skipping MO
F
123 nucleotides (41 aa)
R

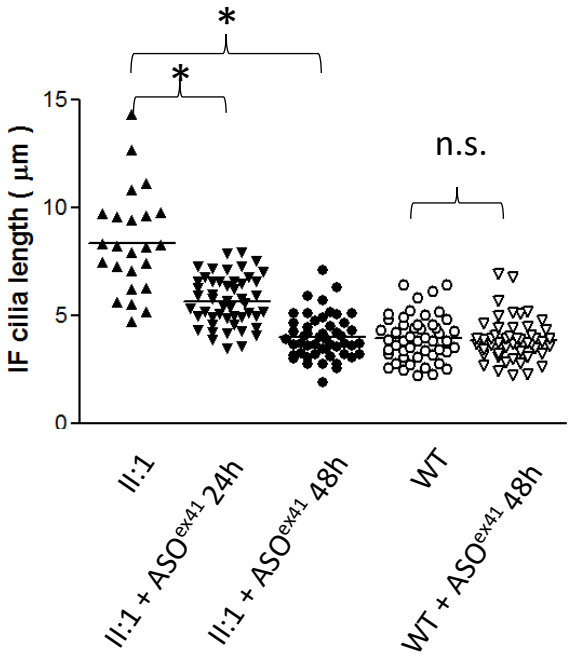

Exon 41 skipping MO
-
+
520 bp
397 bp
RT-PCR


II:1
WT + ASO
WT
II:1
24
48
72
96
120
240 h
CEP290
BETA-ACTIN

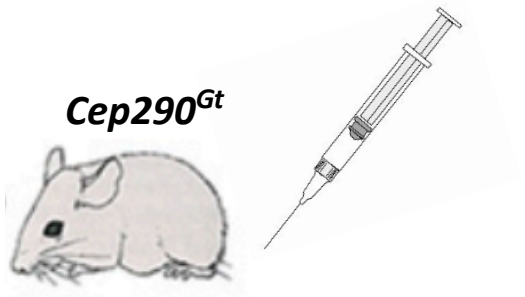


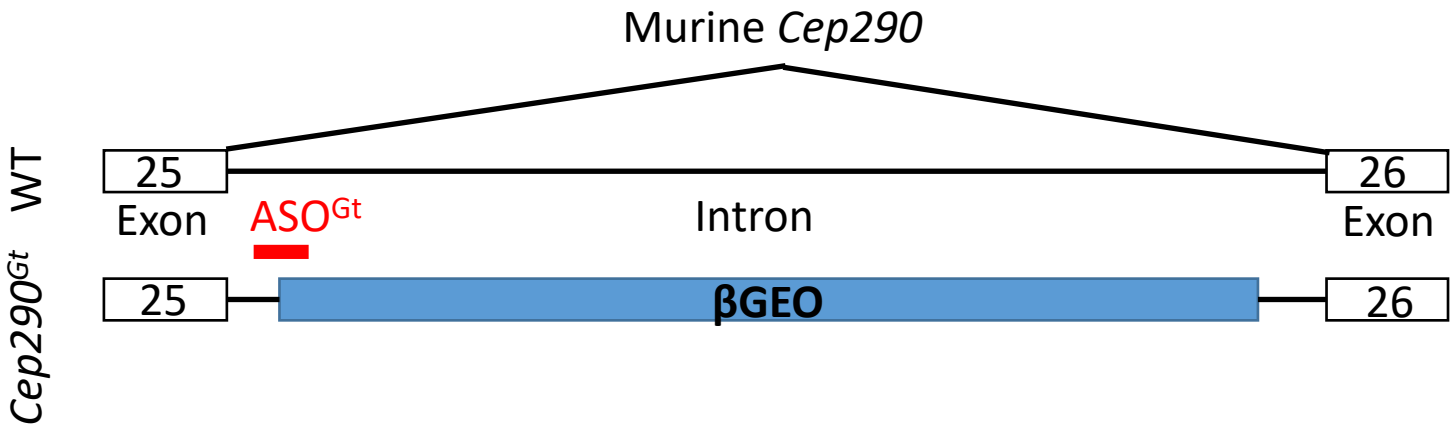


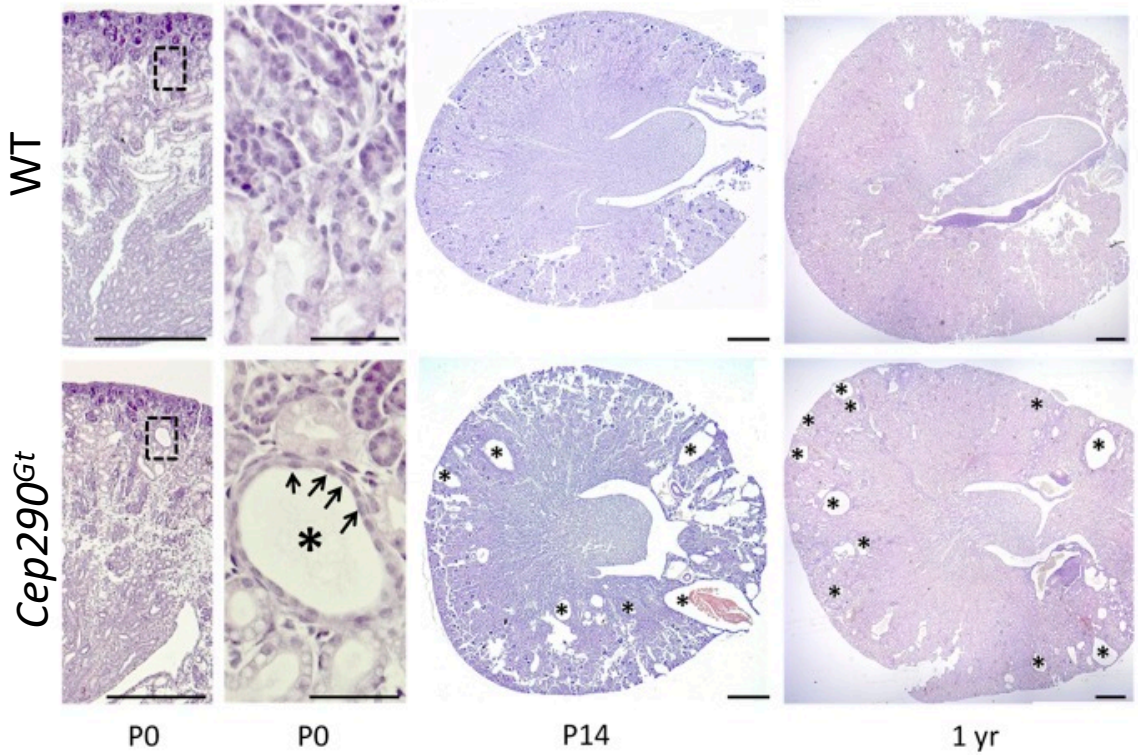




- Does rescue of ciliary morphology have consequences on renal phenotype?
- Can ASO be delivered to the kidney?

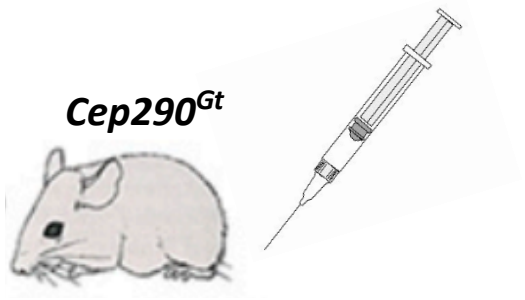


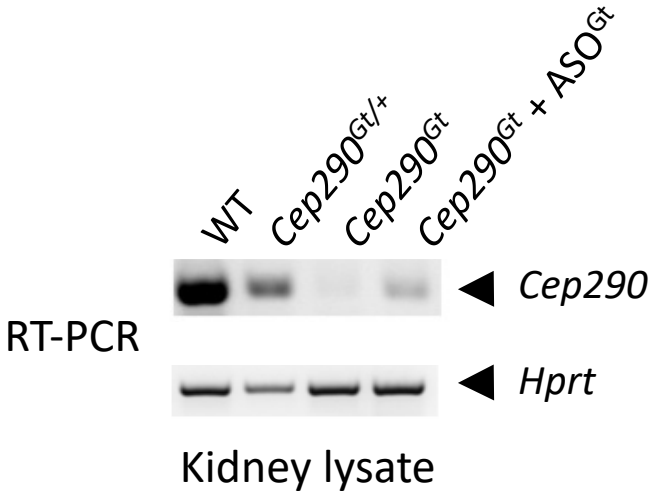


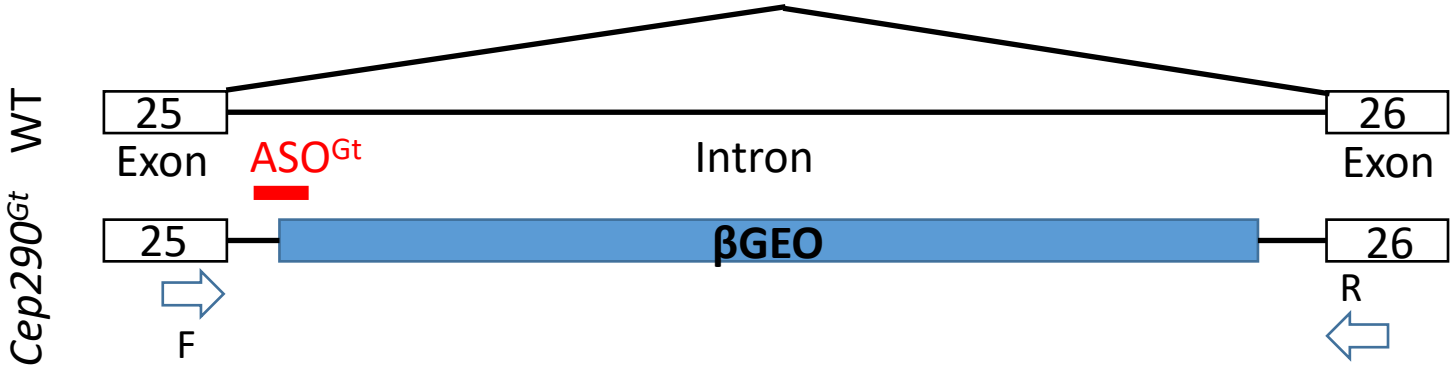


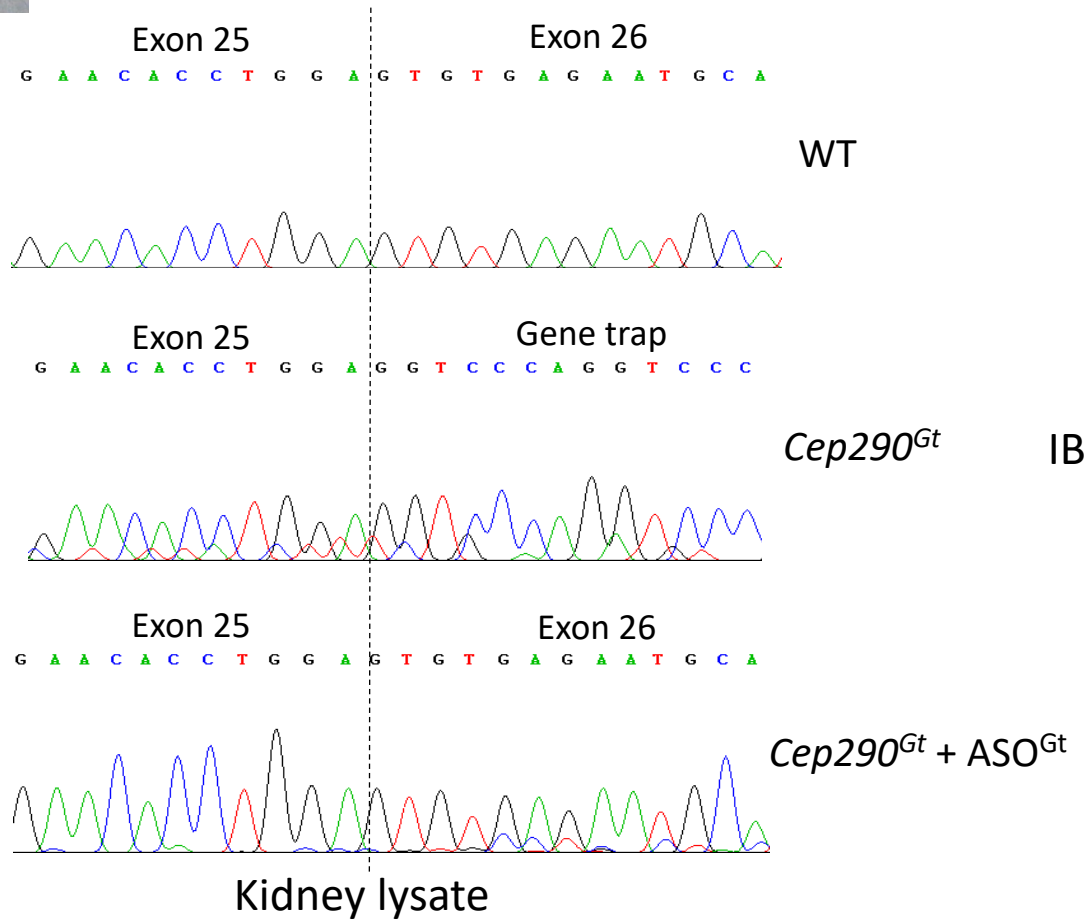
Hynes et al., 2014

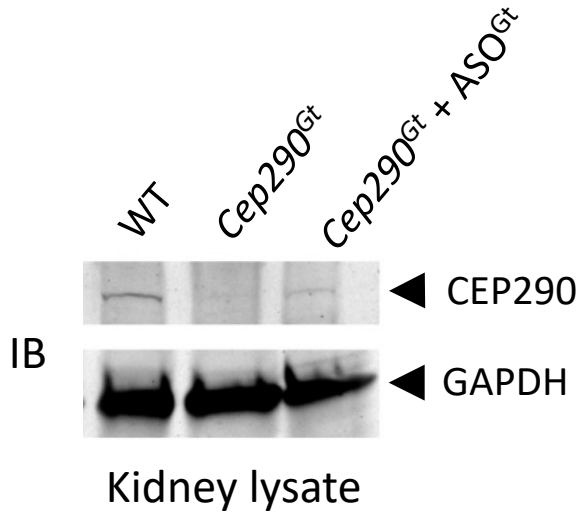

WT
Cep290Gt

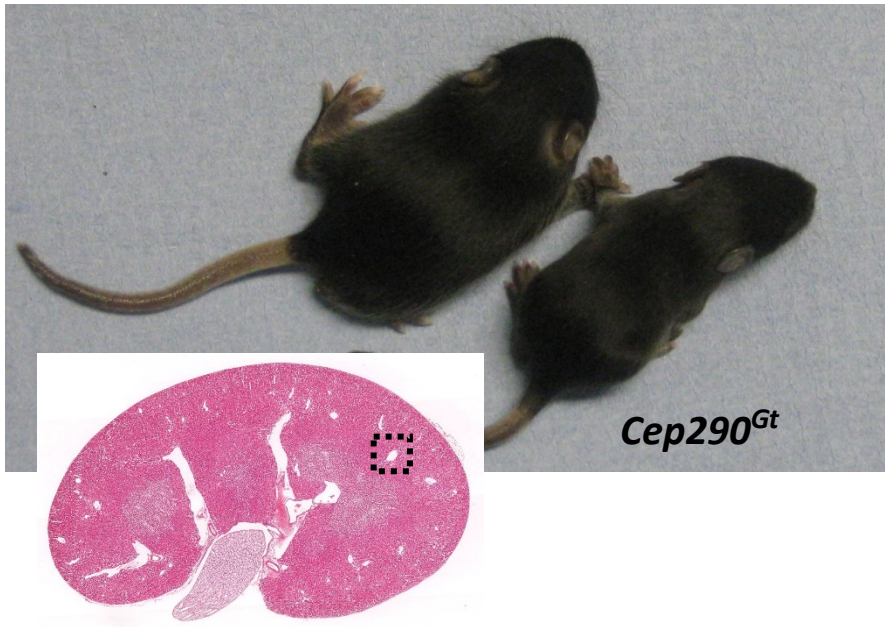

Cep290Gt

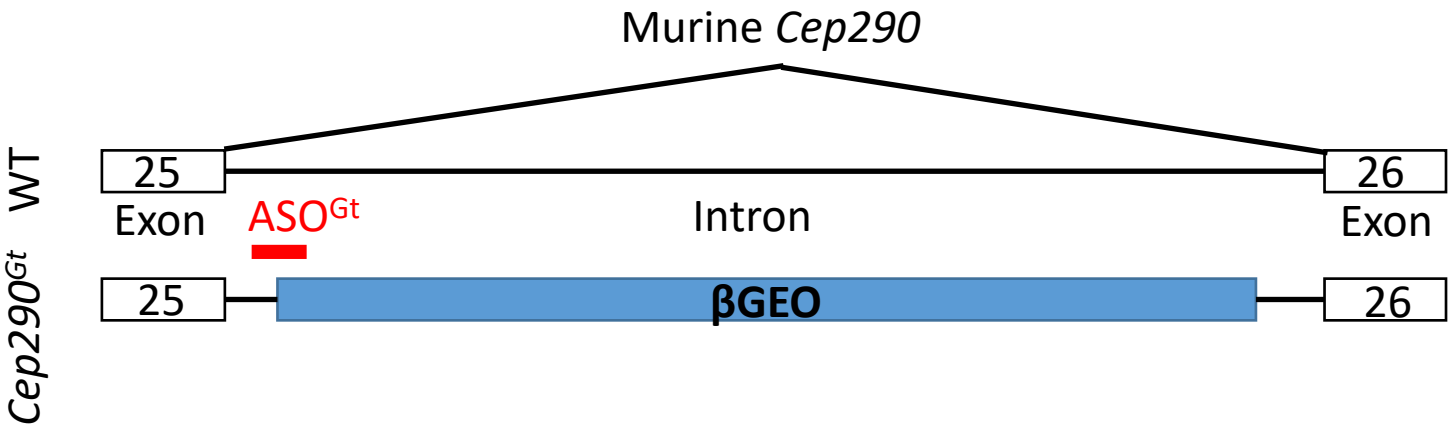

WT
Cep290Gt/+
Cep290Gt
Cep290Gt + ASOGt
RT-PCR
Cep290
Hprt
Kidney lysate

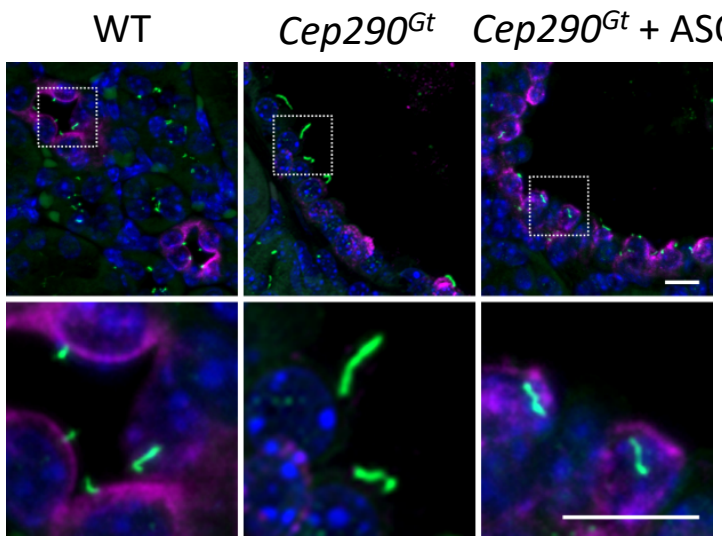

Murine Cep290
WT
25
Exon
ASOGt
Intron
26
Exon
Cep290Gt
25
βGEO
26
F
R

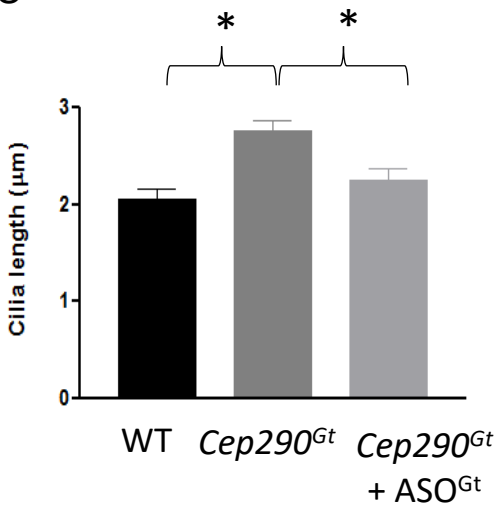

Exon 25
Exon 26
WT
Exon 25
Gene trap
Cep290Gt
Exon 25
Exon 26
Cep290Gt + ASOGt
Kidney lysate

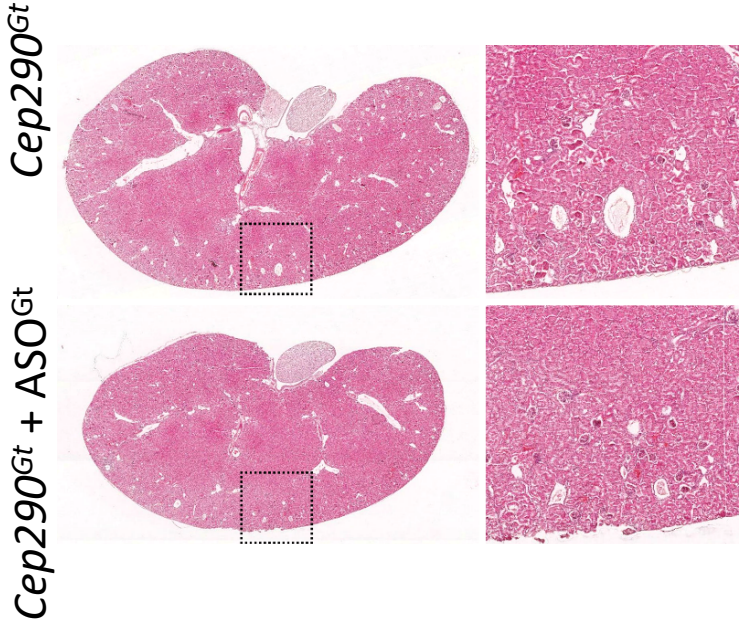

WT
Cep290Gt
Cep290Gt + ASOGt
IB
CEP290
GAPDH
Kidney lysate

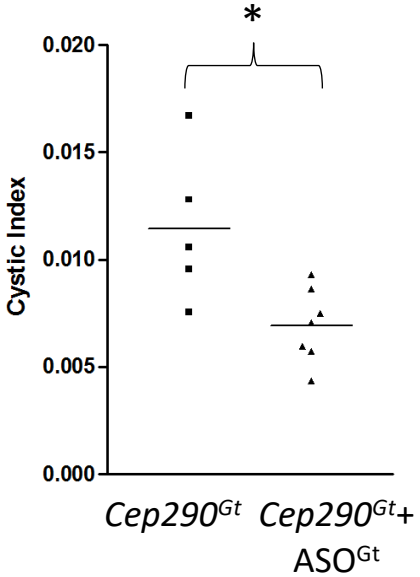

Cep290Gt


Murine Cep290
WT
25
Exon
ASOGt
Intron
26
Exon
Cep290Gt
25
βGEO
26


WT
Cep290Gt
Cep290Gt + ASOGt


*
*
Cilia length (µm)
3
2
1
0
WT
Cep290Gt
Cep290Gt + ASOGt


Cep290Gt
Cep290Gt + ASOGt


*
Cystic Index
0.020
0.015
0.010
0.005
0.000
Cep290Gt
Cep290Gt+ ASOGt

Targeted exon skipping of a *CEP290* mutation rescues Joubert syndrome phenotypes in vitro and in a murine model

Simon A. Ramsbottom, Elisa Molinari, Shalabh Srivastava, Flora Silberman, Charline Henry, Sumaya Alkanderi, Laura A. Devlin, Kathryn White, David H. Steel, Sophie Saunier, Colin G. Miles, and John A. Sayer

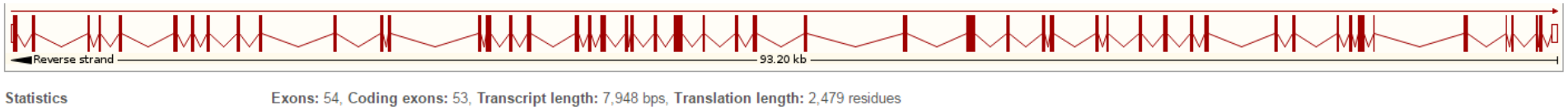
PNAS December 4, 2018 115 (49) 12489-12494; published ahead of print November 16, 2018
https://doi.org/10.1073/pnas.1809432115

- Targeted exon skipping of mutated in frame exon 41 restores near full-length CEP290 protein expression and rescues ciliary phenotype in patient-derived cells
- Exon skipping of the gene trap in a Joubert syndrome mouse model halts renal disease progression and provides a proof of concept for the delivery of ASO to the kidney via intra-venous injection

- Inject ASO^{ex40} in a humanized mouse carrying patient G1890X mutation

Precision medicine in ciliopathies

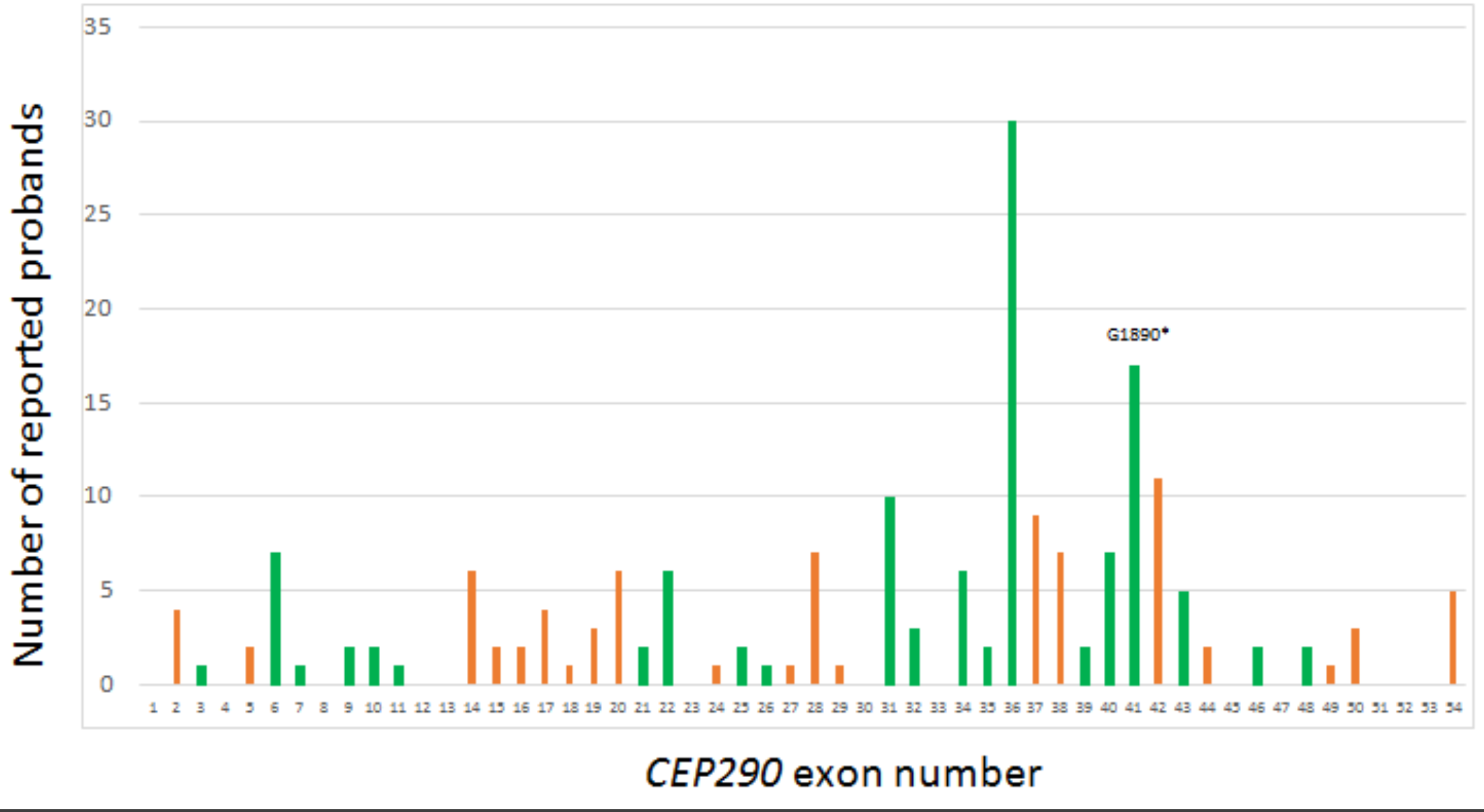
- CEP290 (and other ciliopathy genes?)
- CRISPR Murine cep290 model Gly1890X



54 coding exons for CEP290

26 skippable – in terms of no change in reading frame…functional sig. needs to be assessed

CEP290 mutation database – over 50% of mutations in “skippable” exons


Number of reported probands
35
30
25
20
15
10
5
0
G1890*
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54
CEP290 exon number

Conclusions

- Nephrology – every patient a research opportunity
- Phenotyping is important
- Urine – liquid biopsy of the kidney
- NPHP – gene discovery and therapeutics becoming translational
- Team science and collaborations are key
- RaDaR NPHP/ARPKD group

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