ERKNet
The European Rare Kidney Disease Reference Network

european society for paediatric nephrology

IPNA International Pediatric Nephrology Association
GREAT CARE FOR LITTLE KIDNEYS. EVERYWHERE

WELCOME TO

ESPN/ERKNet
Educational Webinars on Pediatric Nephrology & Rare Kidney Diseases

Date: 02 June 2020

Topic: Nephronophtisis

Speaker: Marijn Stokman

Moderator: Elena Levtchenko

Disclosures

- Nothing to disclose

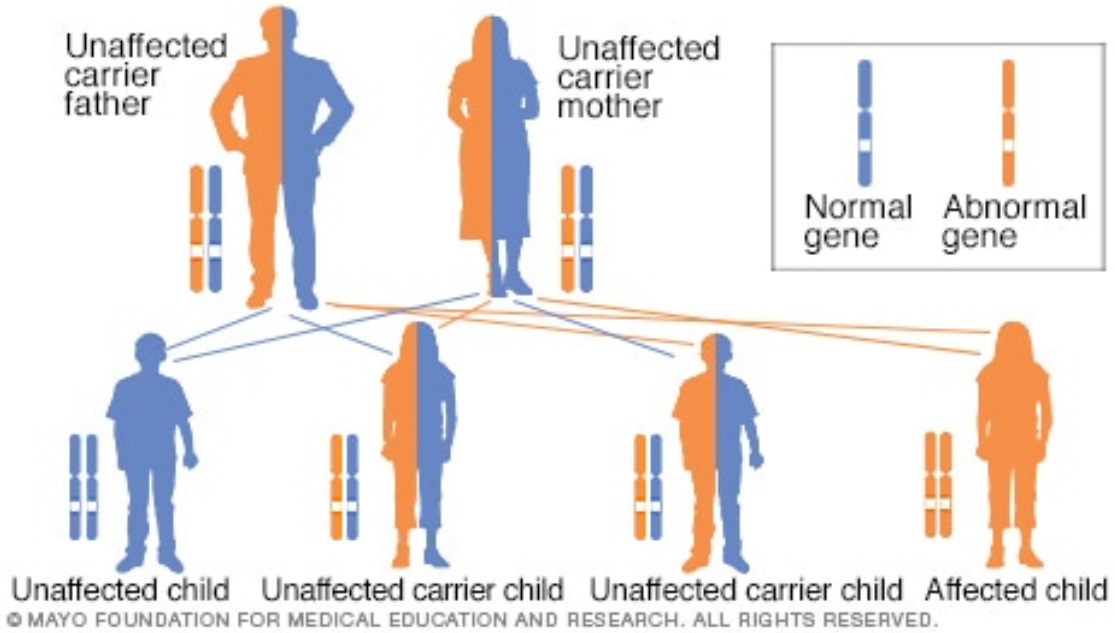
Outline presentation

- Clinical presentation
- Genetic testing
- Pathophysiology
- Road to therapy
- Research
- Take home messages

Nephronophthisis (NPH)

- Important hereditary cause of pediatric ESRD
  - Up to 15%
  - Incidence 1:50,000 to 1:1,000,000 live births

- Homozygous deletions in *NPHP1* are an important cause of ESRD in adults
- Autosomal recessive



Hildebrandt JASN 2009, Snoek JASN 2018

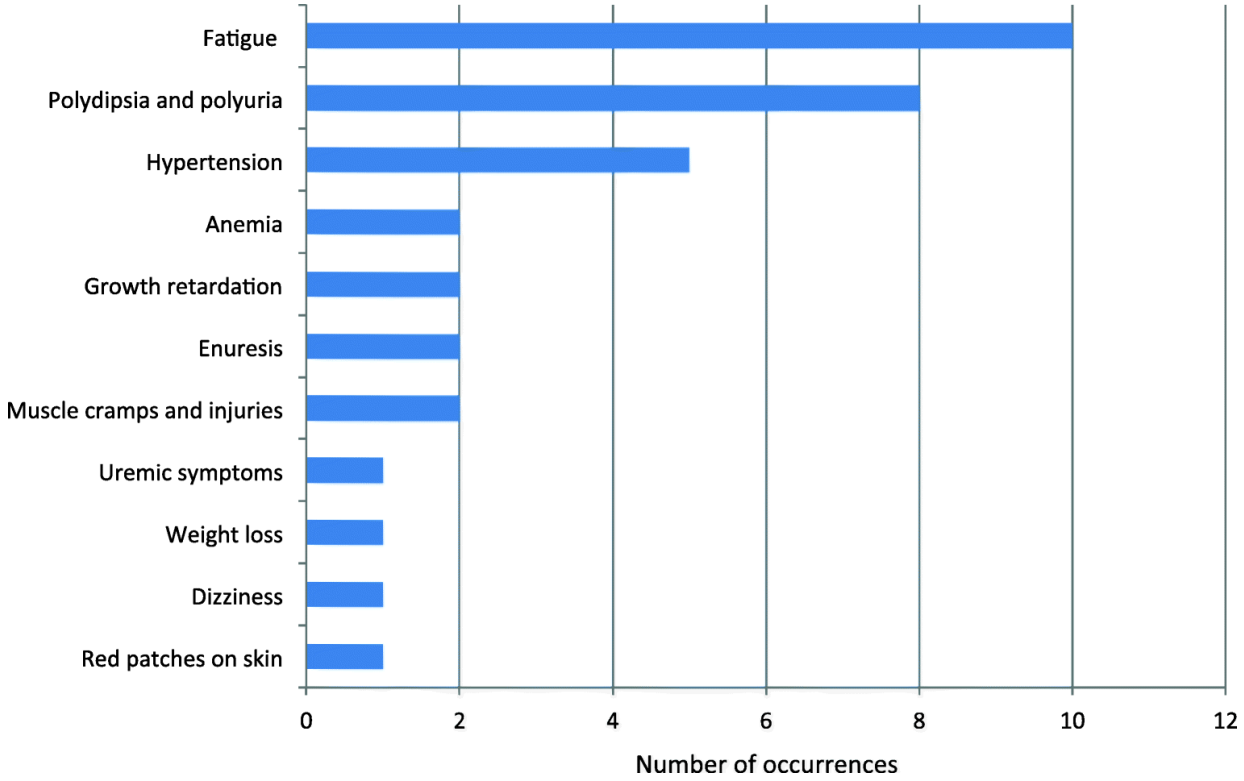
“Wasting of the nephrons”

- 3 subtypes
  - Infantile
  - Juvenile
  - Adult

- Juvenile and adult NPH
  - Classic triad:
    - Renal concentration defect
    - Tubulointerstitial nephritis
    - ESRD before age 30
  - Aspecific presenting symptoms lead to diagnostic delay

Presenting signs and symptoms

- Fatigue n=10
- Polydipsia and polyuria n=8
- Hypertension n=5
- Three patients had muscle complaints (uremic symptom?)



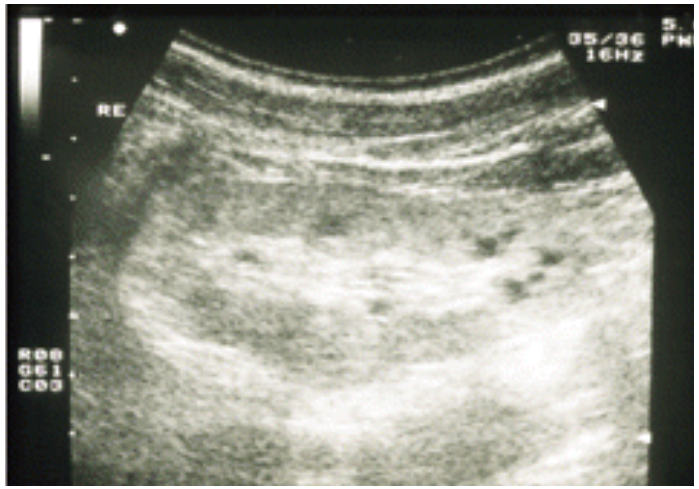
Stokman Pediatr Nephrol 2018

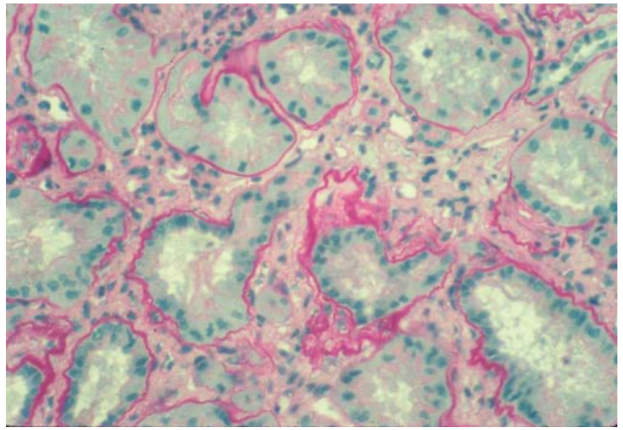
Juvenile/adult NPH

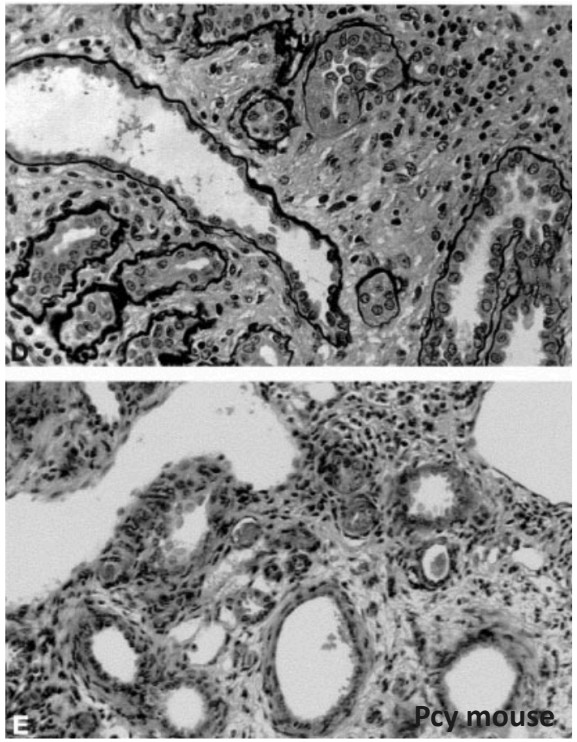
- Ultrasound:
  - Normal sized kidneys
  - Hyperechogenic kidneys
  - Reduced corticomedullary differentiation
  - Cysts on the corticomedullary junction (~50%)

- <u>Histology:</u>
  - Tubulointerstitial fibrosis
  - Cysts arise mainly from distal tubule
  - Thickened and disrupted tubular basement membrane

- **Inconclusive in advanced stages of CKD!**



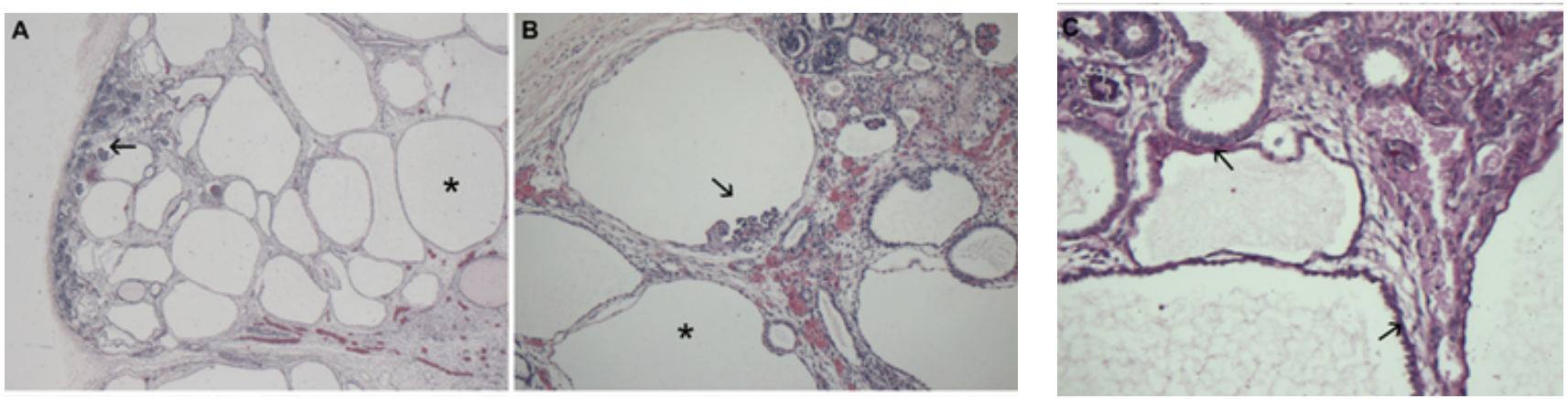




Hildebrandt JASN 2007, Salomon Pediatr Nephrol 2009, Omran JASN 2001

Infantile NPH

- Histology:
  - Cystic enlargement
  - Cortical cysts that arise from proximal tubule/collecting duct
  - Absence of tubular basement membrane thickening
  - Minimal fibrosis

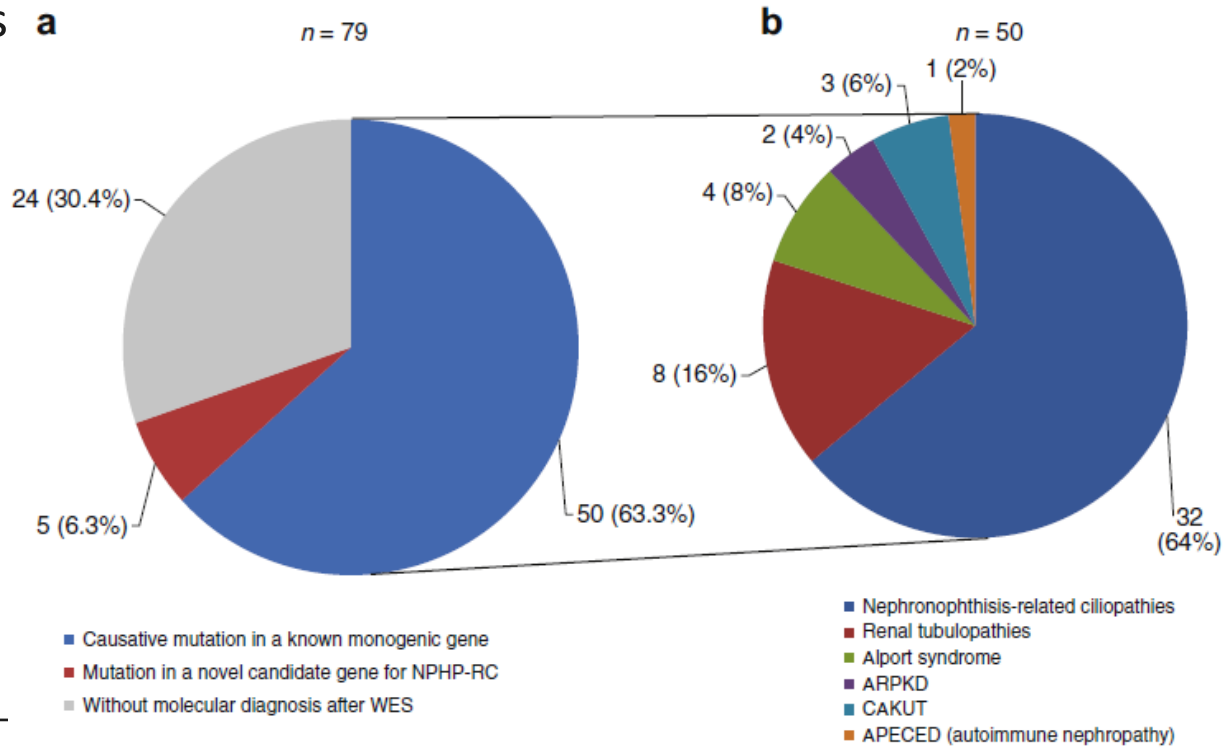


- Rapidly progressive course, resembles ARPKD
- Is infantile NPH part of the same phenotypic spectrum?

Oud AJMG 2014

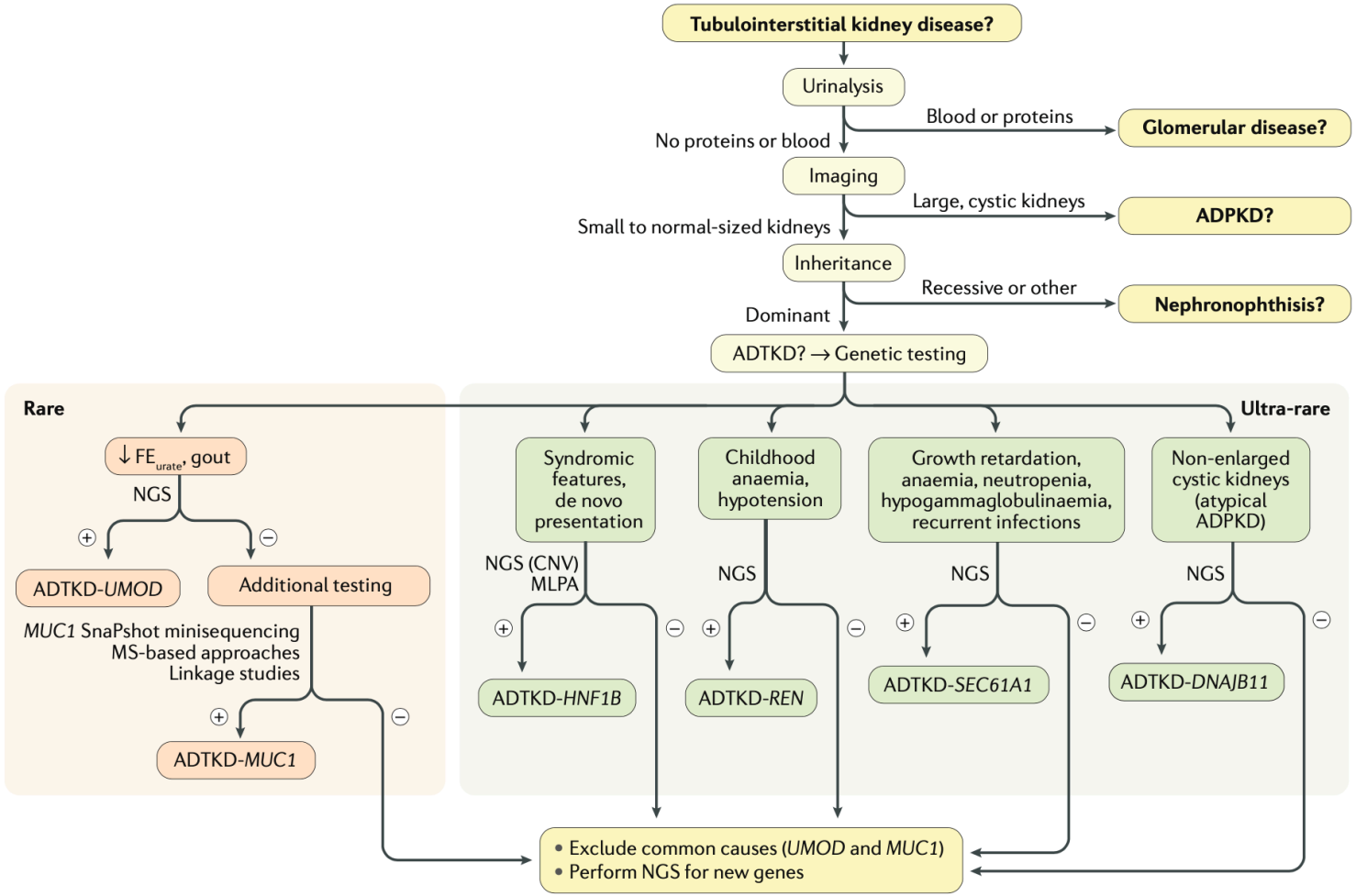
Diagnosis can be challenging

- Whole-exome sequencing (WES) in 79 families with pediatric onset CKD and suspected nephronophthisis based on renal ultrasound
- Consanguineous/familial cases
- 50 cases causal mutation:
  - 32 in NPH-RC gene
  - 18 in other genes:
    - Renal tubulopathies (n=8, 16%)
    - Alport syndrome (n=4, 8%)
    - CAKUT (n=3, 6%)
    - ARPKD (n=2, 4%)
    - APECED [autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy] syndrome (n=1, 2%).



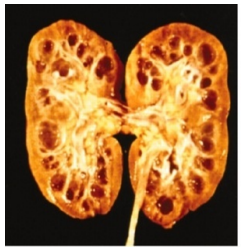
Braun, Kidney Int, 2016

Differential diagnosis

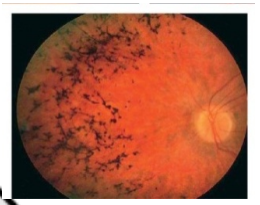


Devuyst Nat Rev Dis Primers 2019

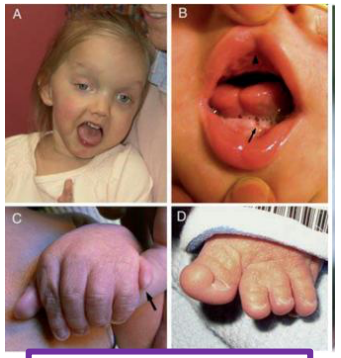
Syndromic forms



NPHP



Retinitis pigmentosa



Joubert syndrome

Ciliary dysfunction

Kidney cysts

Retinal degeneration

Mental retardation

Obesity

Deafness

Hepatic fibrosis/ cysts

cleft palate

nail/hair defects

Hypo-gonadism

Neural tube defects

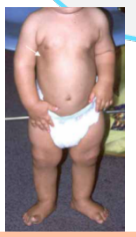
Hydro-cephalus

Bronchiolar dysfunction

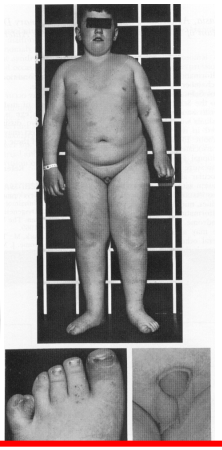
Laterality defects

Polydactyly

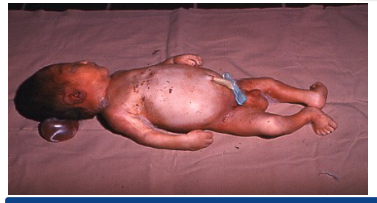
Anomalies of ribs/long bones



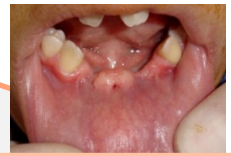
Jeune syndrome



Bardet-Biedl syndrome



Meckel-Gruber syndrome

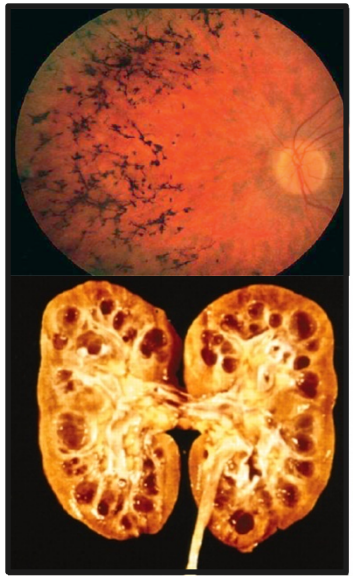


Ellis-van Creveld syndrome

Adapted from Gibson, 2006

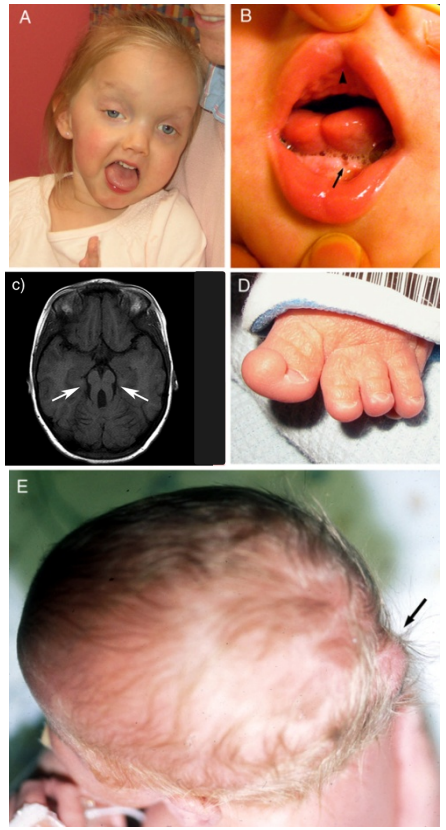
Phenotypic variability

Hildebrandt & Zhou, 2007
den Hollander et al., 2008



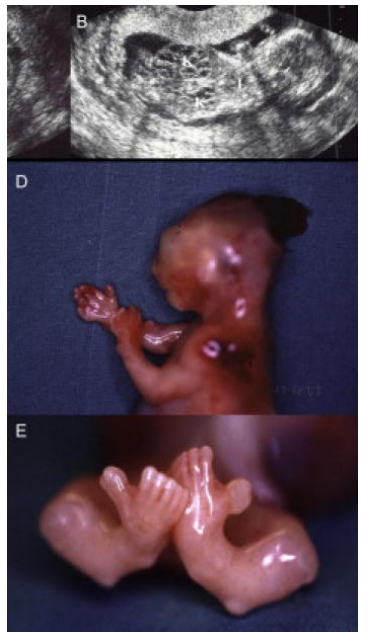
Senior-Løken

Parisi MA, 2009



Joubert

Tallila et al.,2008



Meckel-Gruber

Nature of mutations: hypomorphic ↔ null

mild

severe

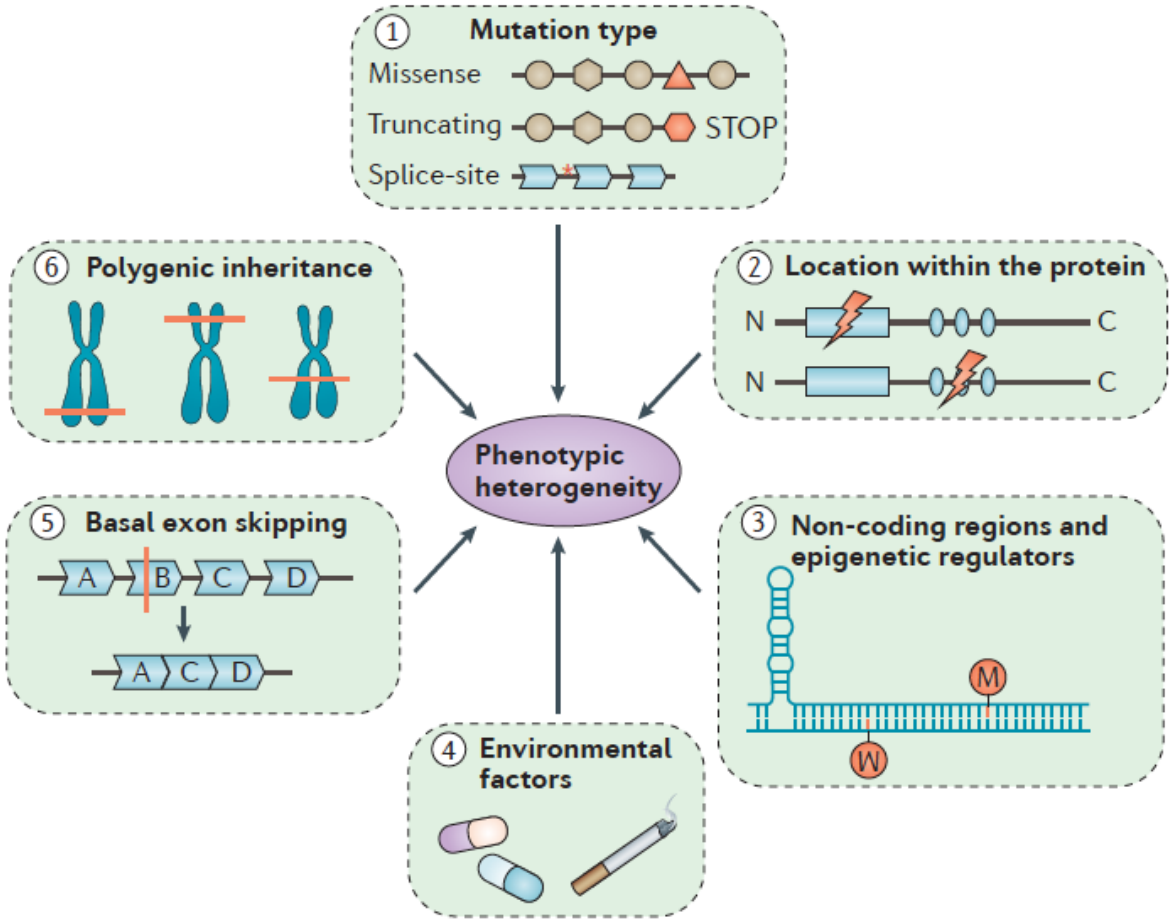
Phenotypic variability

TTC21B contributes both causal and modifying alleles across the ciliopathy spectrum

Erica E Davis[1,2], Qi Zhang[3], Qin Liu[3], Bill H Diplas[1], Lisa M Davey[1], Jane Hartley[4], Corinne Stoetzel[5], Katarzyna Szymanska[6], Gokul Ramaswami[7], Clare V Logan[6], Donna M Muzny[8], Alice C Young[9], David A Wheeler[8], Pedro Cruz[9], Margaret Morgan[8], Lora R Lewis[8], Praveen Cherukuri[9], Baishali Maskeri[9], Nancy F Hansen[9], James C Mullikin[9], Robert W Blakesley[9], Gerard G Bouffard[9], NISC Comparative Sequencing Program[9], Gabor Gyapay[10], Susanne Rieger[11], Burkhard Tönshoff[11], Ilse Kern[12], Neveen A Soliman[13], Thomas J Neuhaus[14], Kathryn J Swoboda[15,16], Hulya Kayserili[17], Tomas E Gallagher[18], Richard A Lewis[19–22], Carsten Bergmann[23,24], Edgar A Otto[7], Sophie Saunier[25], Peter J Scambler[26], Philip L Beales[26], Joseph G Gleeson[27], Eamonn R Maher[4], Tania Attié-Bitach[28], Hélène Dollfus[5], Colin A Johnson[6], Eric D Green[9], Richard A Gibbs[8], Friedhelm Hildebrandt[7,29], Eric A Pierce[3] & Nicholas Katsanis[1,2,30]

Basal exon skipping and genetic pleiotropy: A predictive model of disease pathogenesis

Theodore G. Drivas[1,*], **Adam P. Wojno**[1,*], **Budd A. Tucker**[2], **Edwin M. Stone**[2,3], and **Jean Bennett**[1,†]



Davis Nat Genet 2011, Drivas Sci Transl Med 2015, Stokman Nat Rev Nephrol 2016

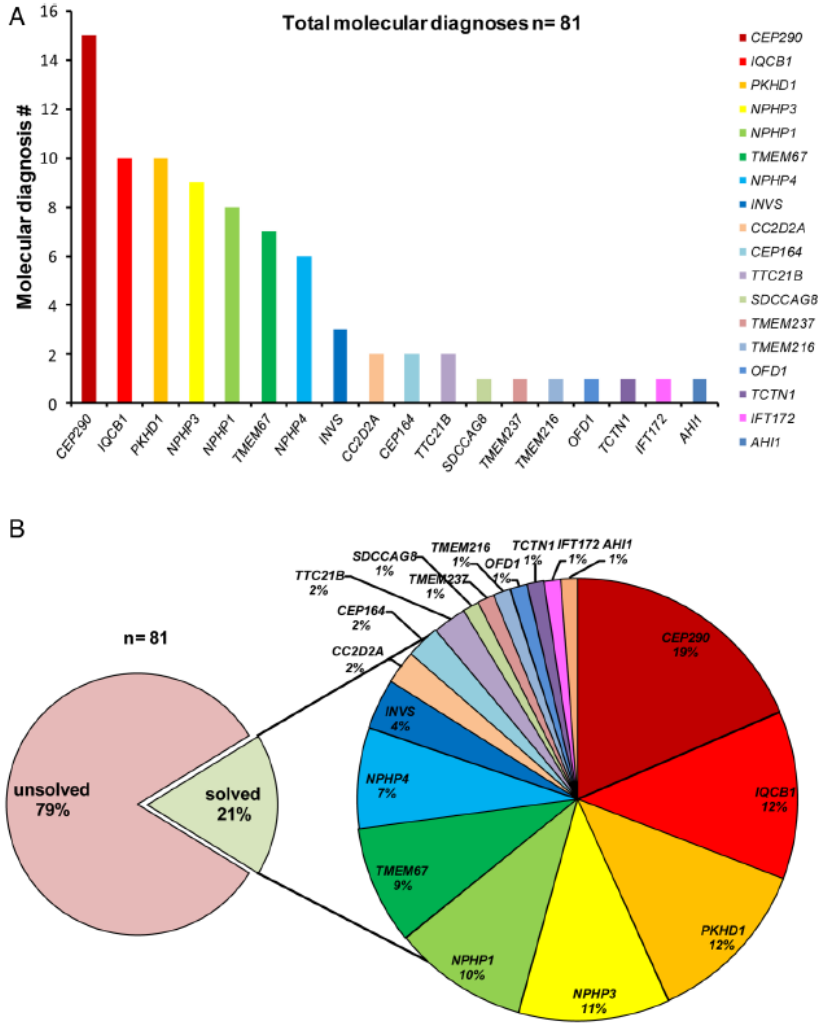
Genetic testing

Genetically heterogeneous disease

- Most common: Homozygous deletion in *NPHP1*

Gene	Locus
NPHP1	NPHP1
INVS	NPHP2
NPHP3	NPHP3
NPHP4	NPHP4
IQCB1	NPHP5
CEP290	NPHP6
GLIS2	NPHP7
RPGRIP1L	NPHP8
NEK8	NPHP9
SDCCAG8	NPHP10
TMEM67	NPHP11
TTC21B	NPHP12
WDR19	NPHP13
ZNF423	NPHP14
CEP164	NPHP15
ANKS6	NPHP16
IFT172	NPHP17
CEP83	NPHP18
DCDC2	NPHP19
MAPKBP1	NPHP20
ADAMTS9	NPHP21

- 384 individuals with NPH-RC
- Homozygous deletion in *NPHP1* excluded
- Targeted MIP-based sequencing of 34 NPH-RC genes
- Molecular diagnosis in 81/384 patients (21,1%)



Schueler, J Med Genet, 2016

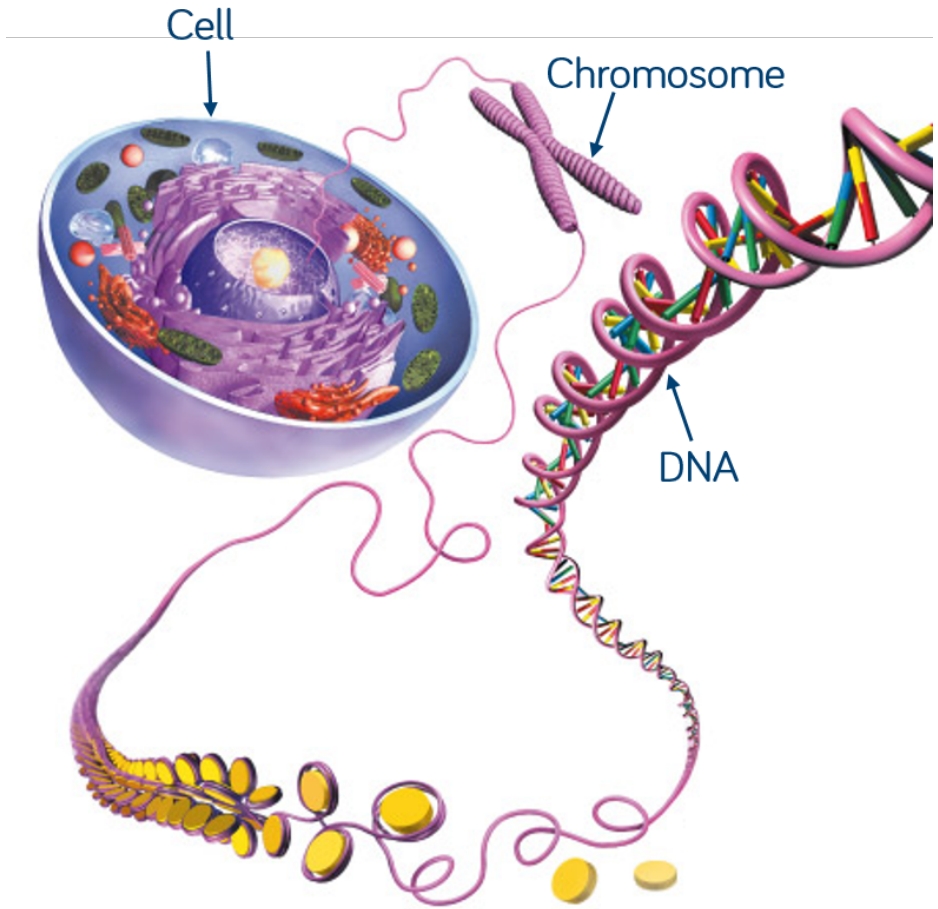
Genetic testing

- Who to test:
  - Children and adults with clinical signs and symptoms of NPH or ciliopathy
  - Siblings of children with NPH
  - Anyone with unexplained CKD (including adults!)

- Why is genetic testing relevant?
  - Establish diagnosis
  - Prognosis and surveillance
  - Genetic and reproductive counseling
  - Testing of at risk relatives (potential donors!)

Genetic testing strategy

- MLPA *NPHP1* -> homozygous deletion?
- WES-based gene panel ‘Renal cysts and ciliopathies’ (118 genes)
- Option to remove the filter and analyze the whole exome



https://www.umcutrecht.nl/en/ziekenhuis/genoomdiagnostiek

Question 1

- Juvenile nephronophthisis is characterized by
  A. Large kidneys, cortical cysts, normal basement membranes
  B. Small kidneys, glomerular cysts, fibrosis
  C. Fibrosis, cysts, thickened tubular basement membranes
  D. Diffuse cysts, large kidneys, fibrosis

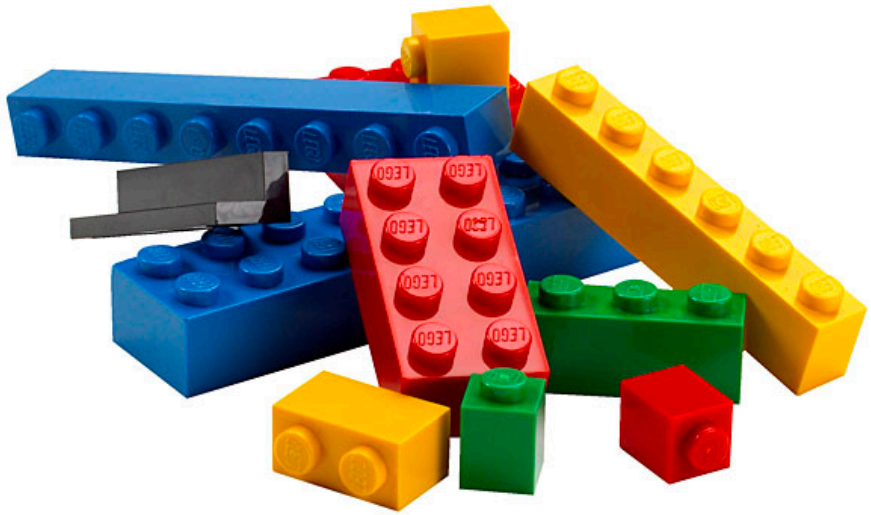
Question 2

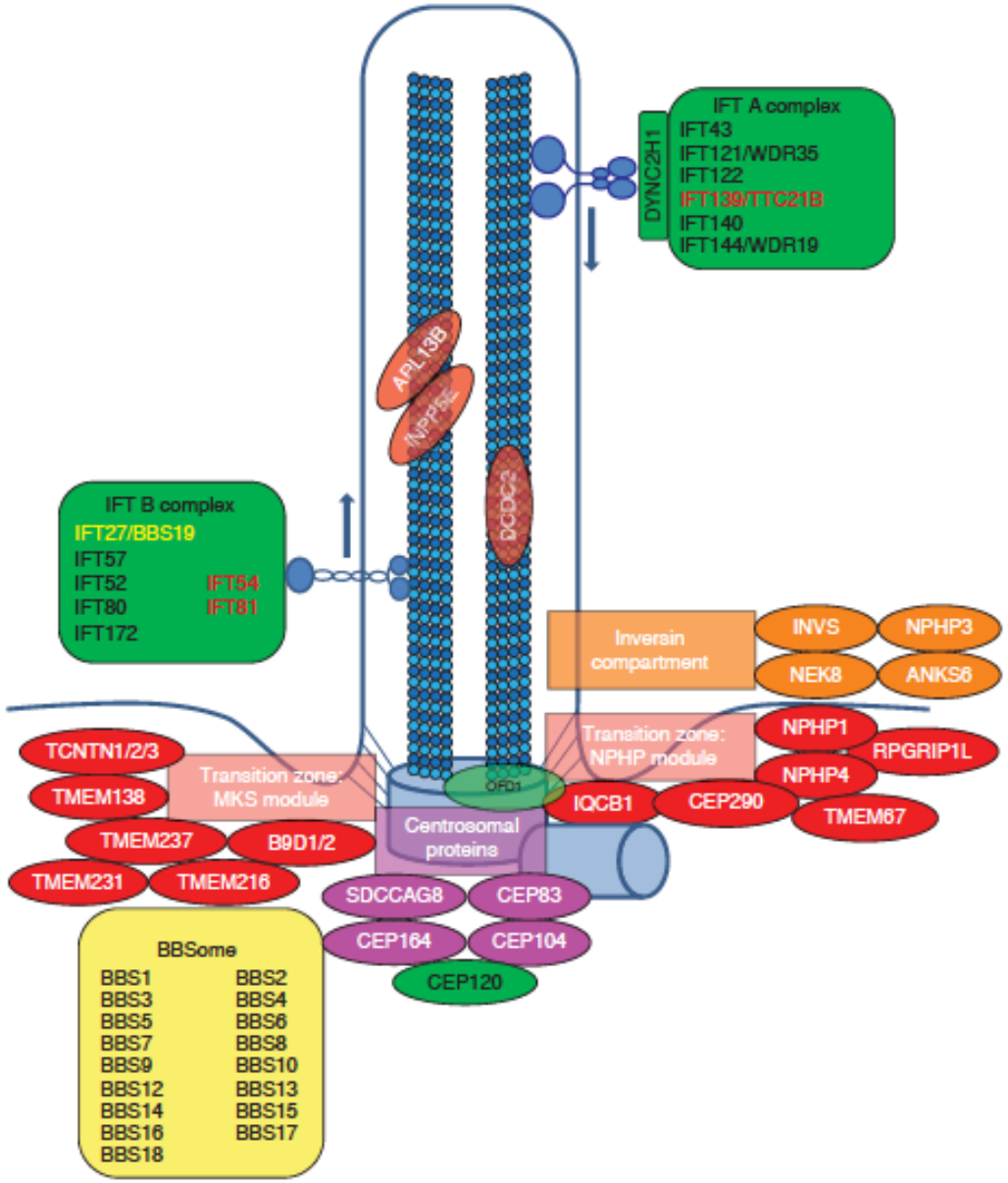
- Nephronophthisis is the main monogenic cause of pediatric end-stage renal disease
  A. True
  B. False

Pathophysiology

NPH is a ciliopathy

- NPHP genes localize to distinct ciliary compartments
- Compartments are associated with specific extrarenal phenotypes

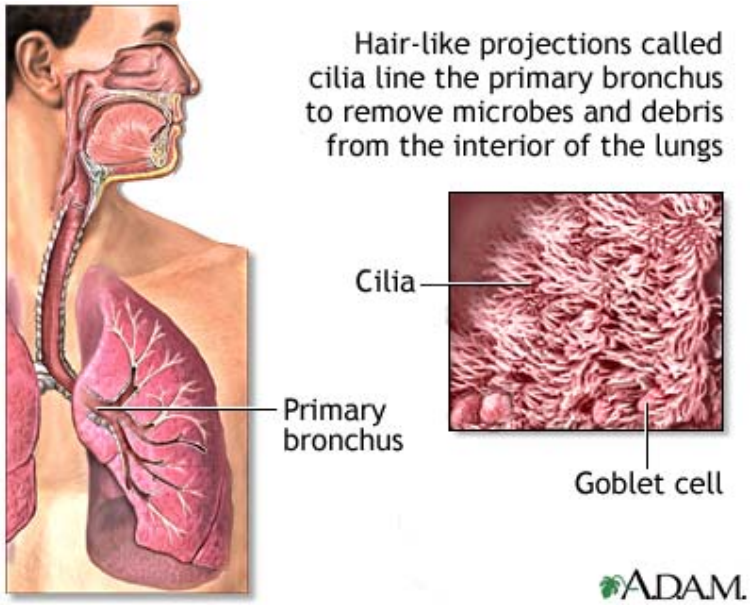


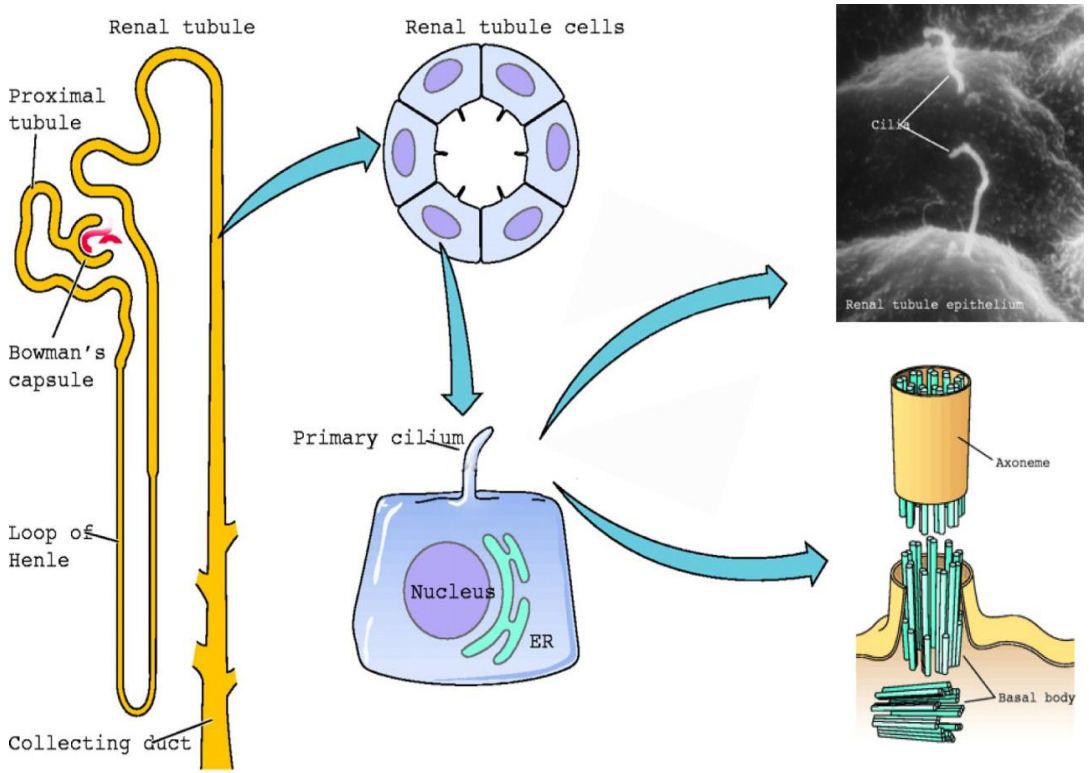


Braun Cold Spring Harb Perspect Biol 2017

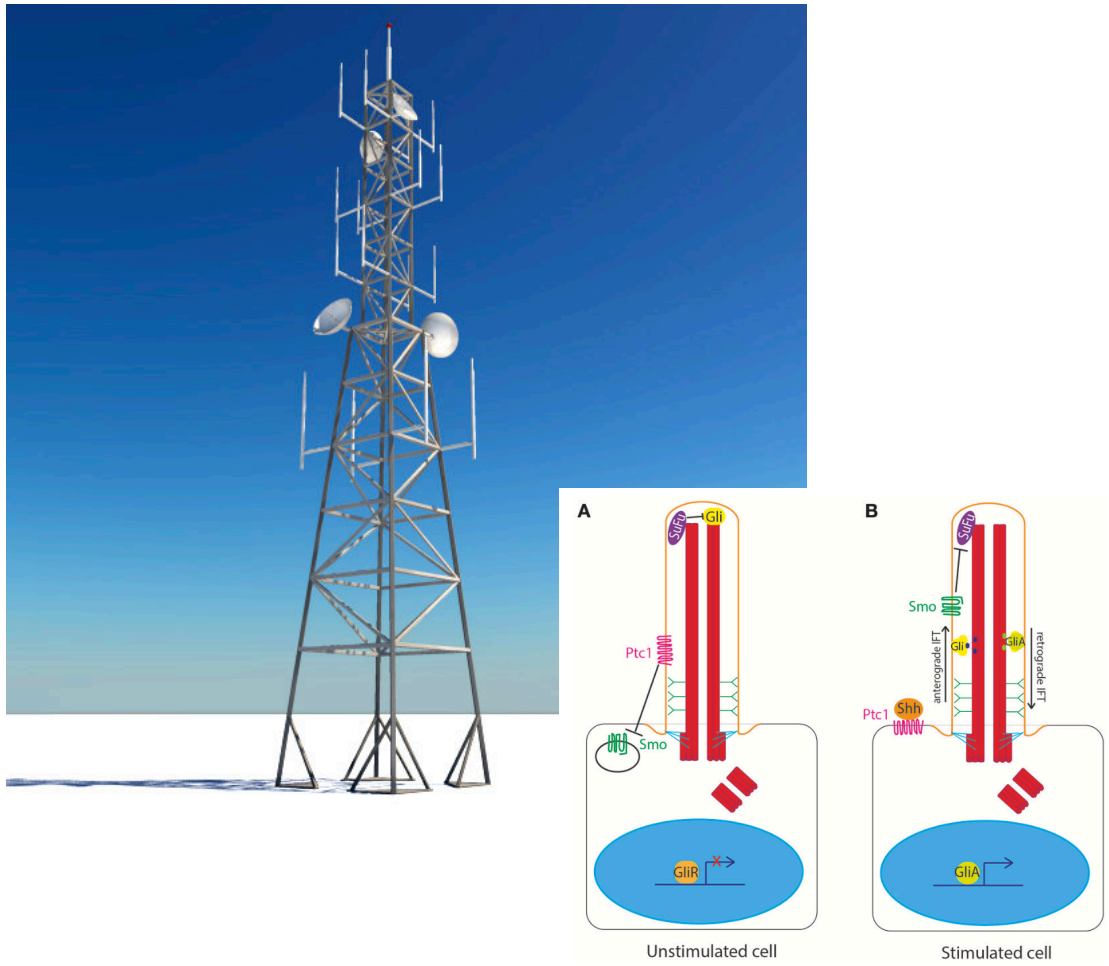
NPH is a ciliopathy

- Motile vs. primary cilia





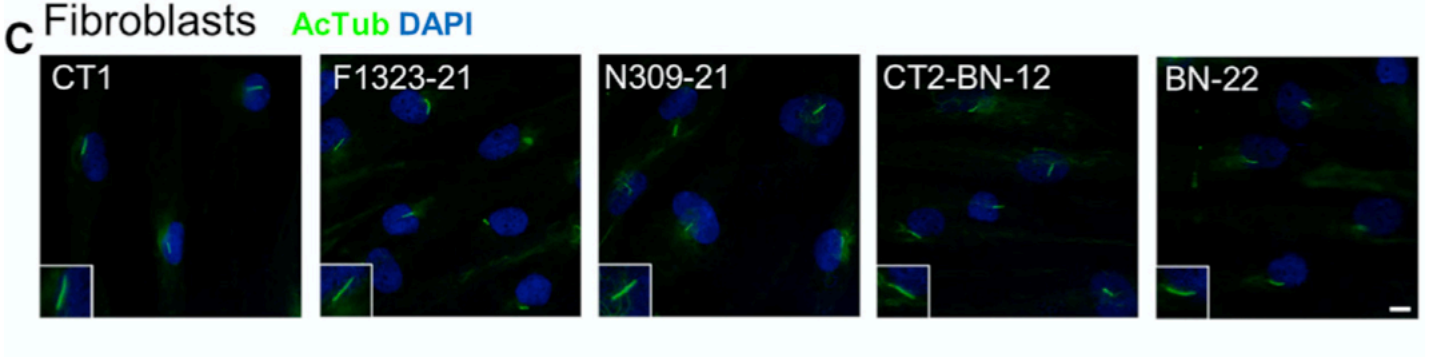
Ciliary signaling pathways

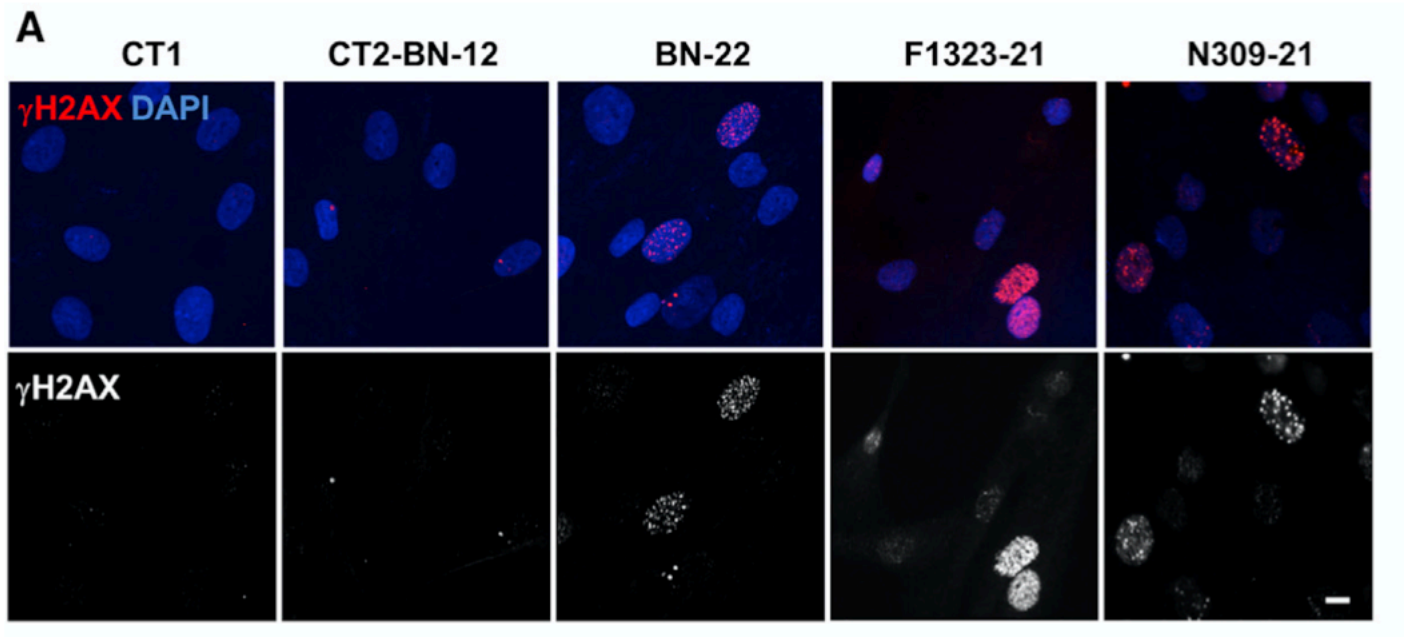


- Signaling pathways
  - Shh: differentiated state tubular epithelial cells (*GLIS2, RPGRIP1L, ZNF423*, IFT)
  - GPCR (AVPR2): CFTR, proliferation, AQP2 water reabsorption
  - Wnt: maintenance planar cell polarity in response to urine flow (*INVS*)
  - Hippo: suppresses growth and transcription (*NPHP3, NPHP4, NEK8*)
  - MTOR
  - Notch
  - TGFB
  - …
- Development and maintenance of kidney architecture

Wheway Front Cell Dev Biol 2016

Extra ciliary functions of NPHP proteins





- Like MAPKBP1/NPHP20, ZNF423/NPHP14, CEP164/NPHP15, CEP290/NPHP6, NEK8/NPHP9 and SDCCAG8/NPHP10 have functions in DNA-damage response (DDR) signaling and cell-cycle checkpoint control
- Disruption can lead to degenerative phenotypes

Macia AJHG 2017

Road to therapy

Current therapy is supportive

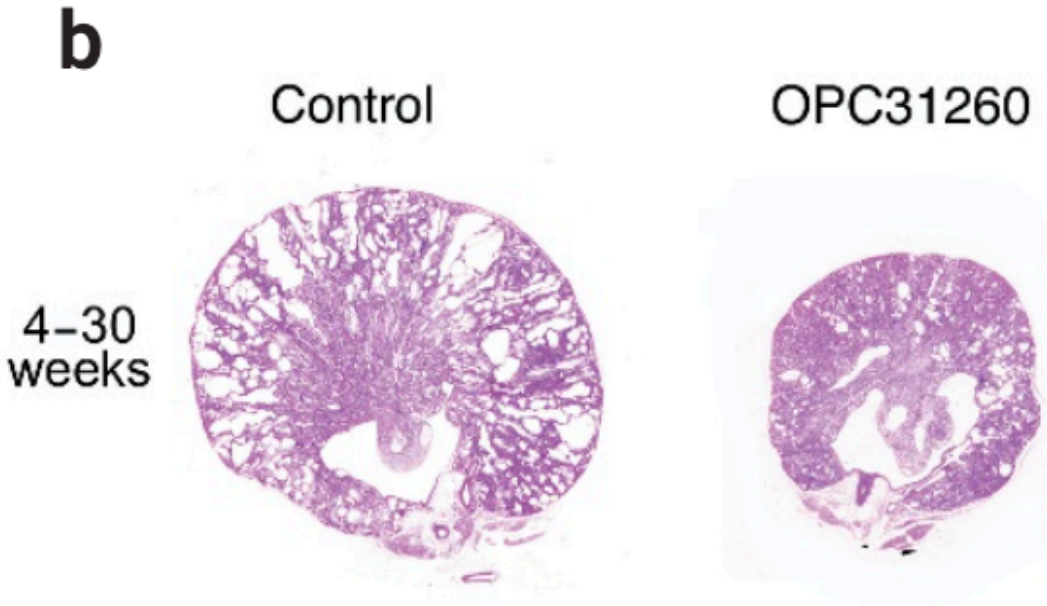
- Treatment of CKD: anemia, hypertension, proteinuria
- Renal replacement therapy
- NPH does not recur in transplanted kidney

Therapies investigated in animal models

- Pharmacological therapies
- Gene-based therapies

Targeting signaling pathways

- AVPR2 antagonist reduced cyst formation and fibrosis in *pcy* mouse model of NPHP3
- mTOR inhibitor rapamycin restored renal size and morphology in morphant zebrafish embryos
- Shh agonists purmorphamine rescued impaired spheroid formation from collecting duct cells from *Cep290*-mutant mice
- YAP inhibitor verteporfin rescued pronephric cysts in zebrafish embryos overexpressing human *NEK8*

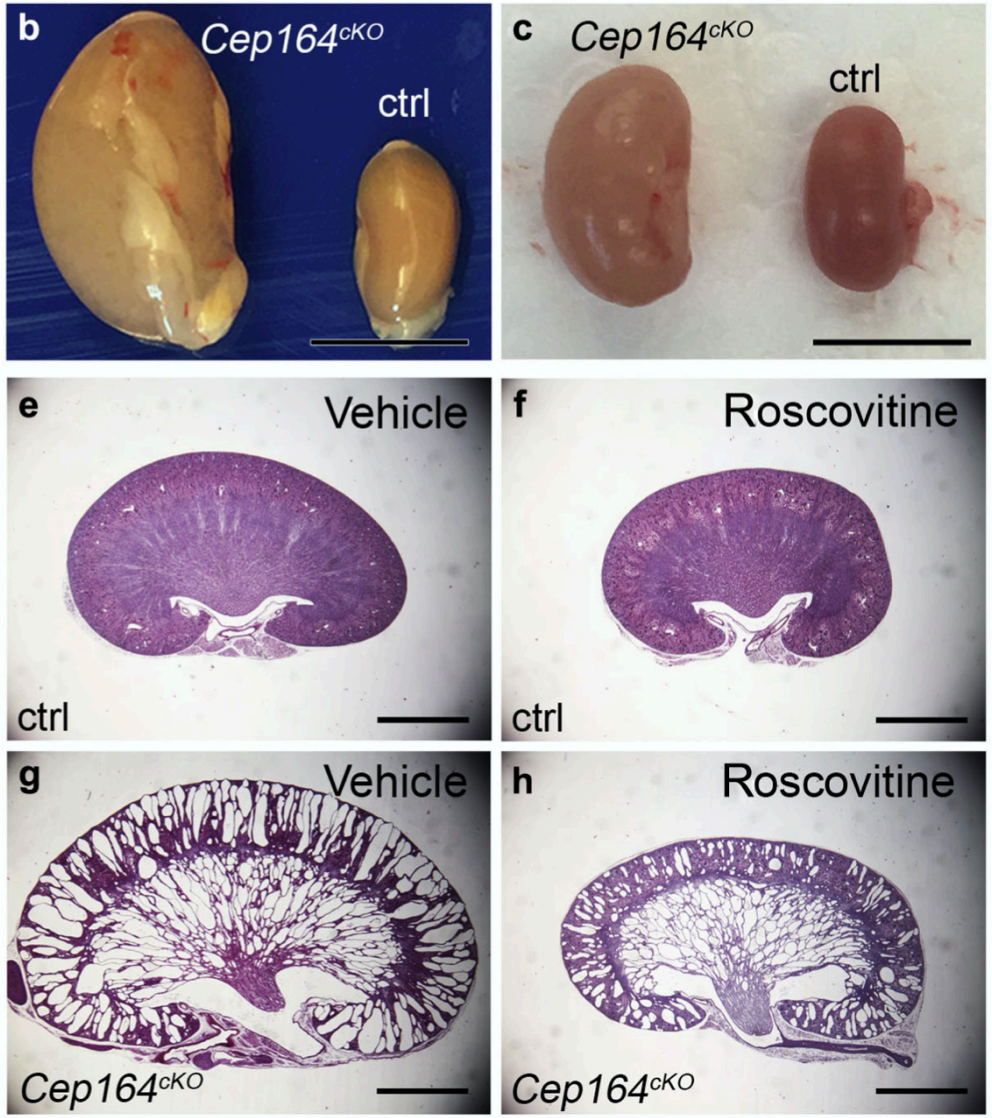


Gattone Nat Med 2003, Tobin and Beales Pediatr Nephrol 2008, Hynes PNAS 2014, Grampa PLOS Genet 2016

Targeting cell cycle

- CDK inhibitors
  - roscovitine attenuated renal cyst progression in a *Cep164*-knockout mouse model

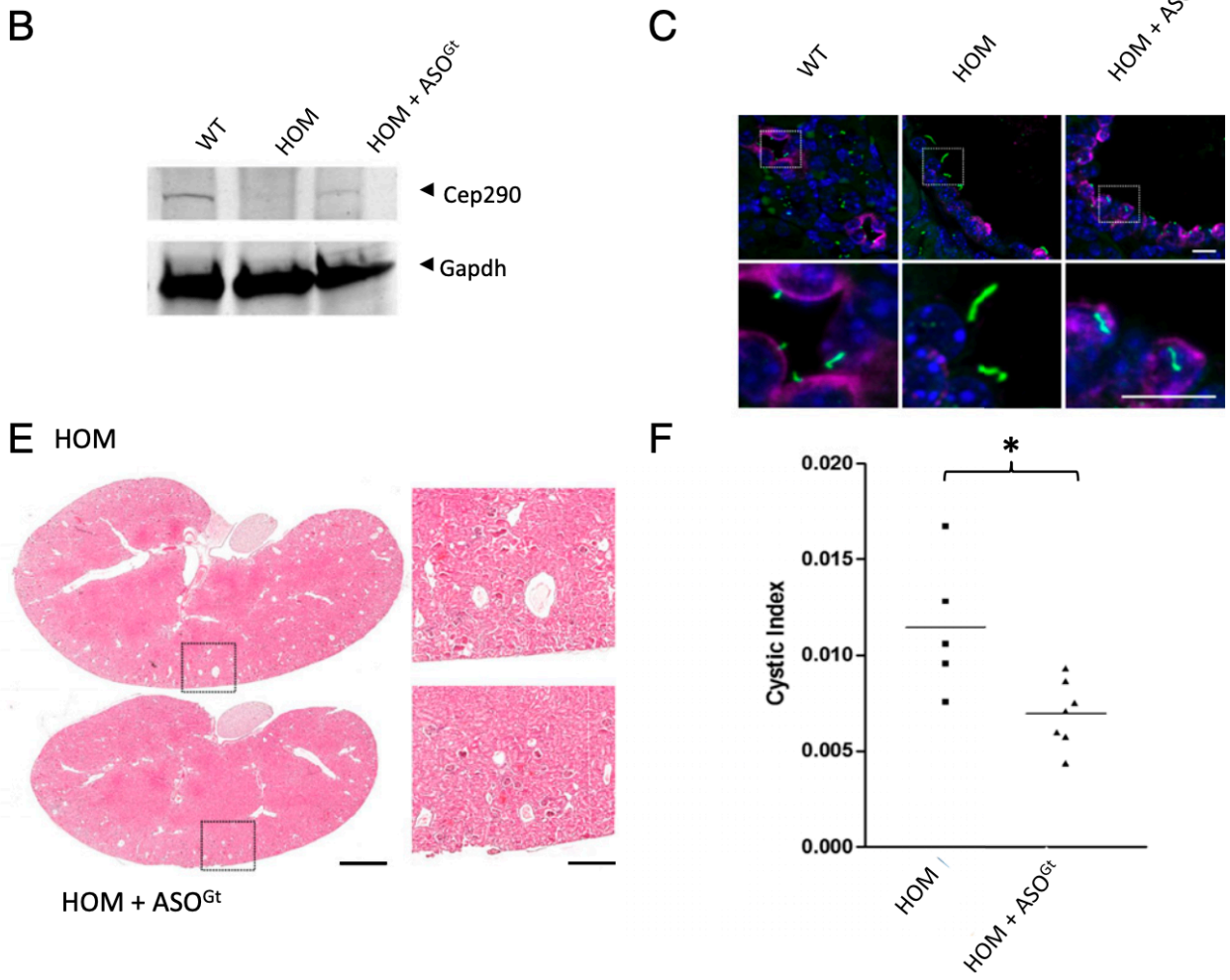
Targeting the immune response



Airik Kidney Int 2019

Gene-based therapy

- Antisense-oligonucleotide treatment
- CRISPR/Cas
- Gene replacement therapy



Forbes AJHG 2018, Ramsbottom PNAS 2018

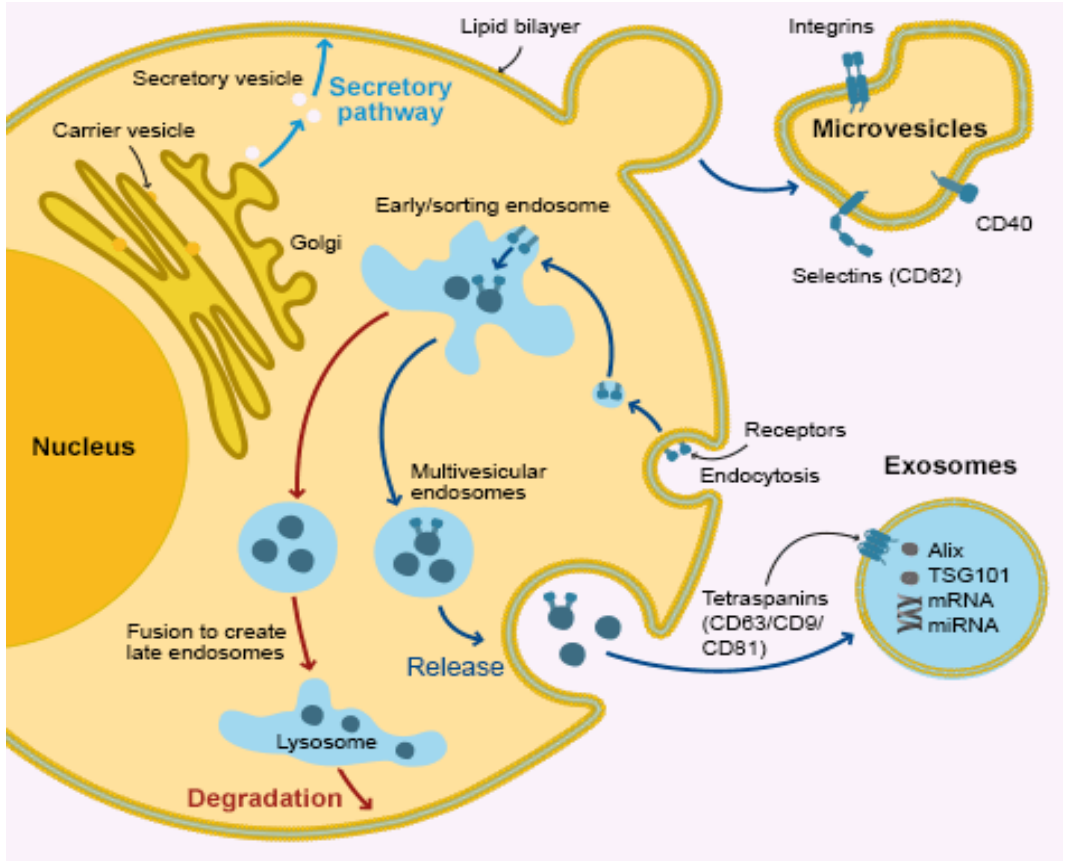
Areas of research

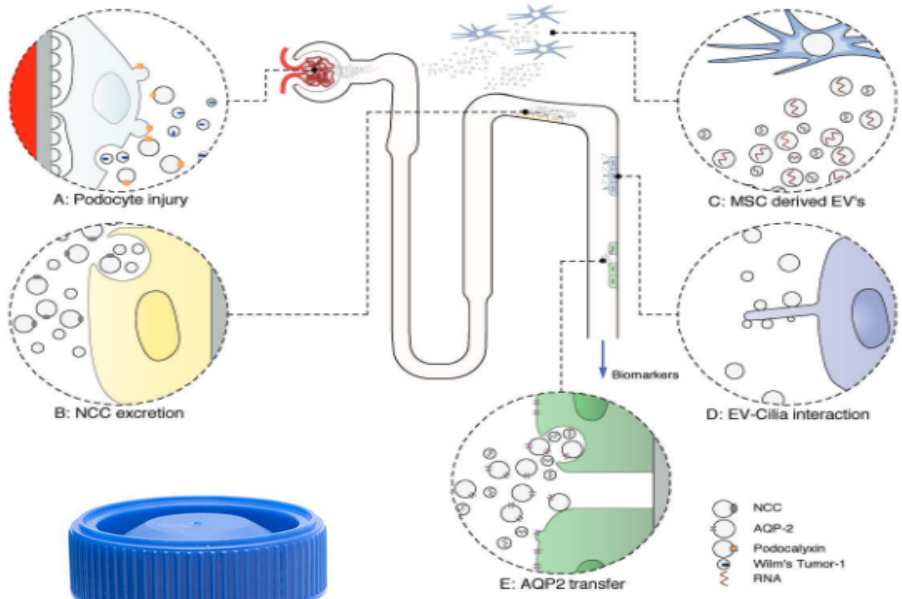
- <u>Key issues:</u>
  - NPH is often diagnosed in advanced stages of CKD
  - Genetic and phenotypic heterogeneity
  - There is currently no therapy that can delay or prevent renal replacement therapy

- Therapeutic window

Urinary extracellular vesicles for biomarker discovery

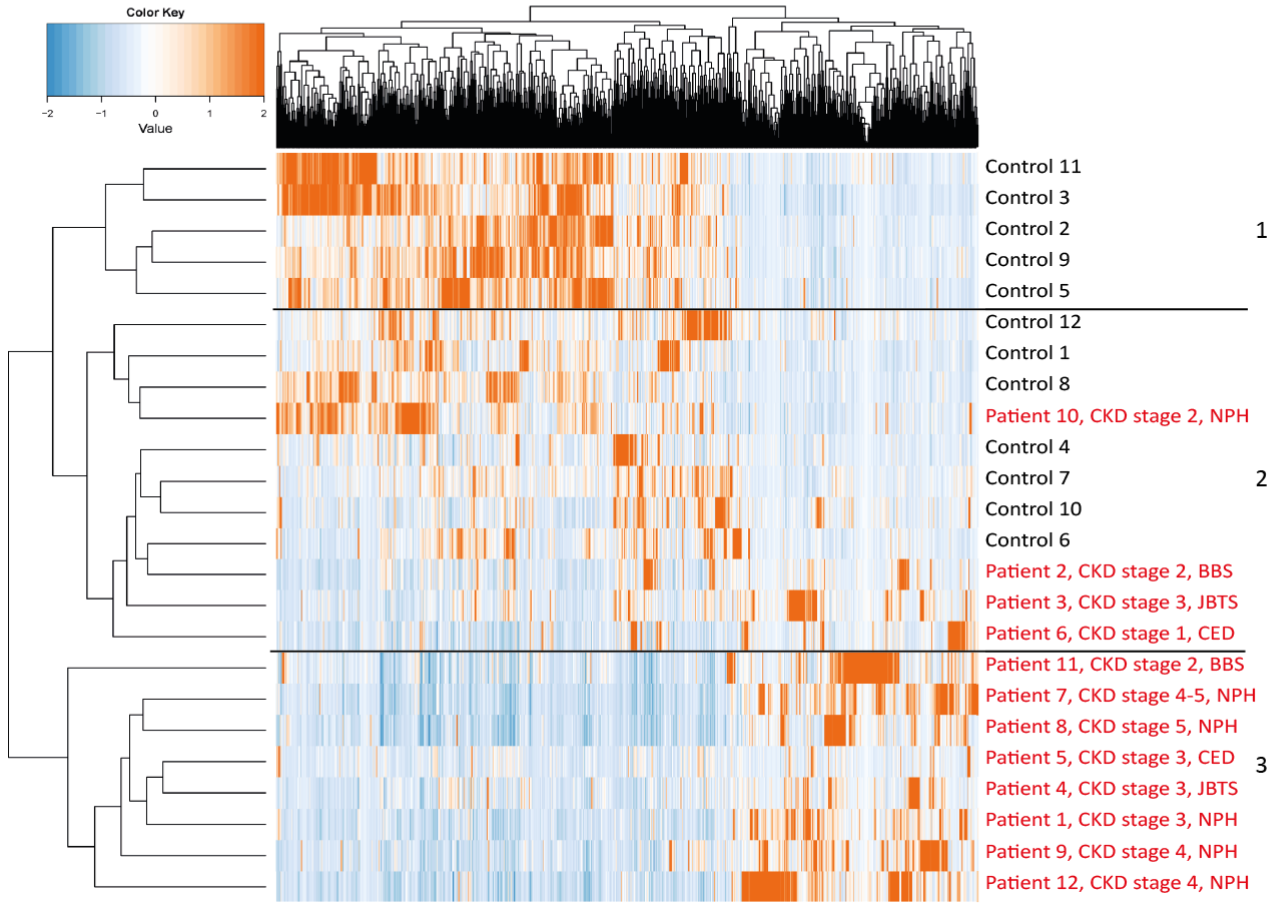
- Aim: Identification of non-invasive protein biomarkers for NPH





Liquid biopsy

Protein profiles separate patients from controls



- Patients with advanced stages of CKD cluster together
- 156 differentially expressed proteins meet strict selection criteria (114 down, 42 up)

- Candidate biomarkers
- Next: validation with disease controls

Stokman J Proteom 2018

Question 3

- Disruption of which functions are considered most important in the pathophysiology of nephronophthisis?
  A. Ciliary signaling and mitochondrial function
  B. Ciliary signaling and DNA damage response signaling
  C. Autophagy and apoptosis
  D. DNA damage response signaling and immune regulation

Question 4

- Which gene-based therapy for nephronophthisis has successfully reduced cyst burden in a mouse model?
  A. CRISPR/Cas therapy
  B. Gene replacement therapy
  C. Antisense-oligonucleotide therapy
  D. siRNA therapy

Take home messages

- NPH is a major cause of ESRD in children
- Symptoms and findings are not always specific (especially without extrarenal manifestations of a ciliopathy)
- Low threshold for genetic testing (also in adults!)

Acknowledgments

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Next Webinars

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era edta WGIKD working group
Working Group on Inherited Kidney Disorders

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european society for paediatric nephrology

ERKNet/ERA-EDTA Advanced Webinars on Rare Kidney Disorders

Date: **23 June 2020**

Speaker: **Albertien van Eerde**

Topic: **Genetic causes and genetic testing in ESRD in adults**

IPNA Clinical Practice Webinars

Date: **25 June 2020**

Speaker: **Katharina Hohenfellner**

Topic**: Management of bone disease in cystinosis: Statement from an international conference.**

ESPN/ERKNet Educational Webinars on Pediatric Nephrology & Rare Kidney Diseases

Date: **08 Sept 2020**

Speaker: **Bert Bammens**

Topic: **ADPKD**

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