UniversitätsKlinikum Heidelberg

The Role of Genetics in Congenital Anomalies of the Kidneys and Urinary Tract

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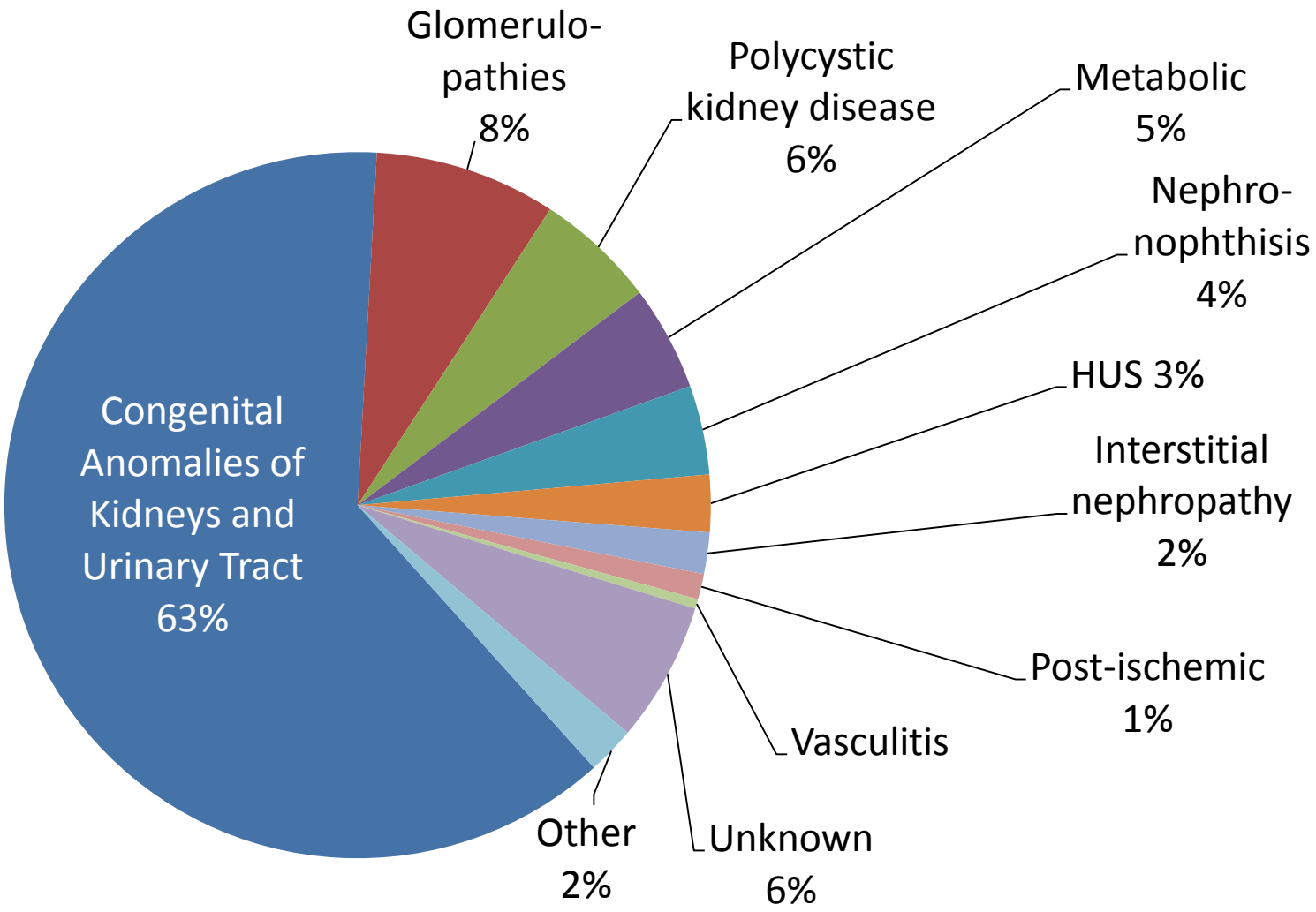
Congenital **A**nomalies of **K**idneys and **U**rinary **T**ract (CAKUT): Prevalence (per 1000 births)

Vesicoureteral reflux	20-30 (100 during first days of life)
Duplex kidney/ureter	8.0
Obstruction of kidney or ureter	1.0
Kidney agenesis/hypoplasia/dysplasia	0.8
Obstruction of bladder/urethra	0.2
Horseshoe kidney	0.15
Bladder ekstrophy	0.06
Cystic kidney diseases	0.04

Beetz et al. 1998

Causes of Chronic Kidney Disease in Children

4C Study, 700 European children with GFR 15-60 ml/min/1.73m^2

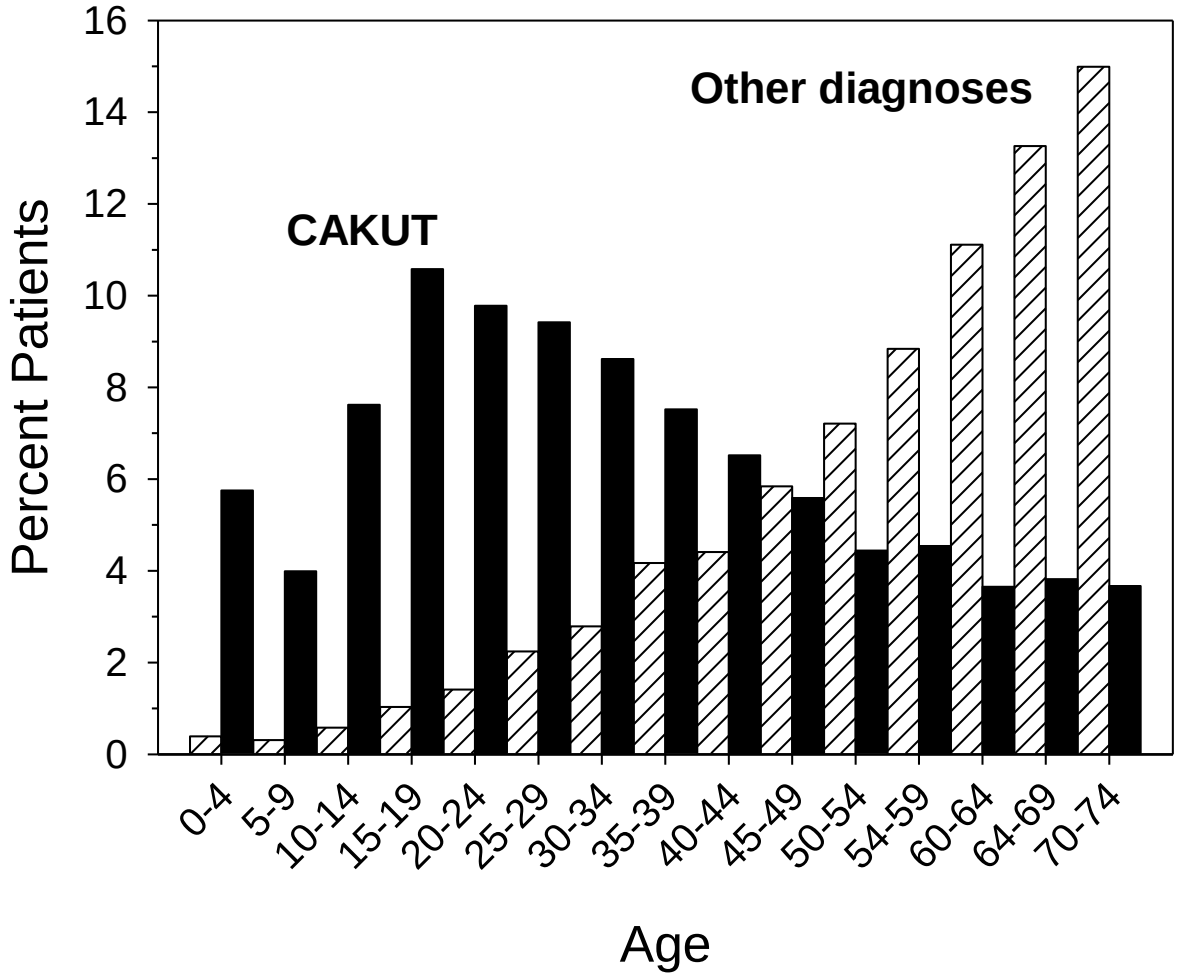


CAKUT Manifestations in CKD Cohort

Unilateral agenesis	12	
Unilateral hypoplasia	21	54%
Bilateral hypoplasia	21	
Unilateral dysplasia without cysts	14	
Bilateral dysplasia without cysts	12	
Unilateral cystic dysplasia	5	50%
Bilateral cystic dysplasia	8	
Multicystic-dysplastic kidney	11	
Associated VUR	48	
Associated distal ureteric obstruction	8	62%
Associated UPJ obstruction	6	

CAKUT: Age at Start of Renal Replacement Therapy

ERA-EDTA Registry: % patients starting RRT per age group



Wühl et al. cJASN 2012

CAKUT: Familial Clustering

Unilateral renal agenesis in 9% of relatives of fetuses with bilateral renal agenesis

Roddhoft et al. N Engl J Med 1984

Offspring of CAKUT patients: CAKUT risk 15-20%

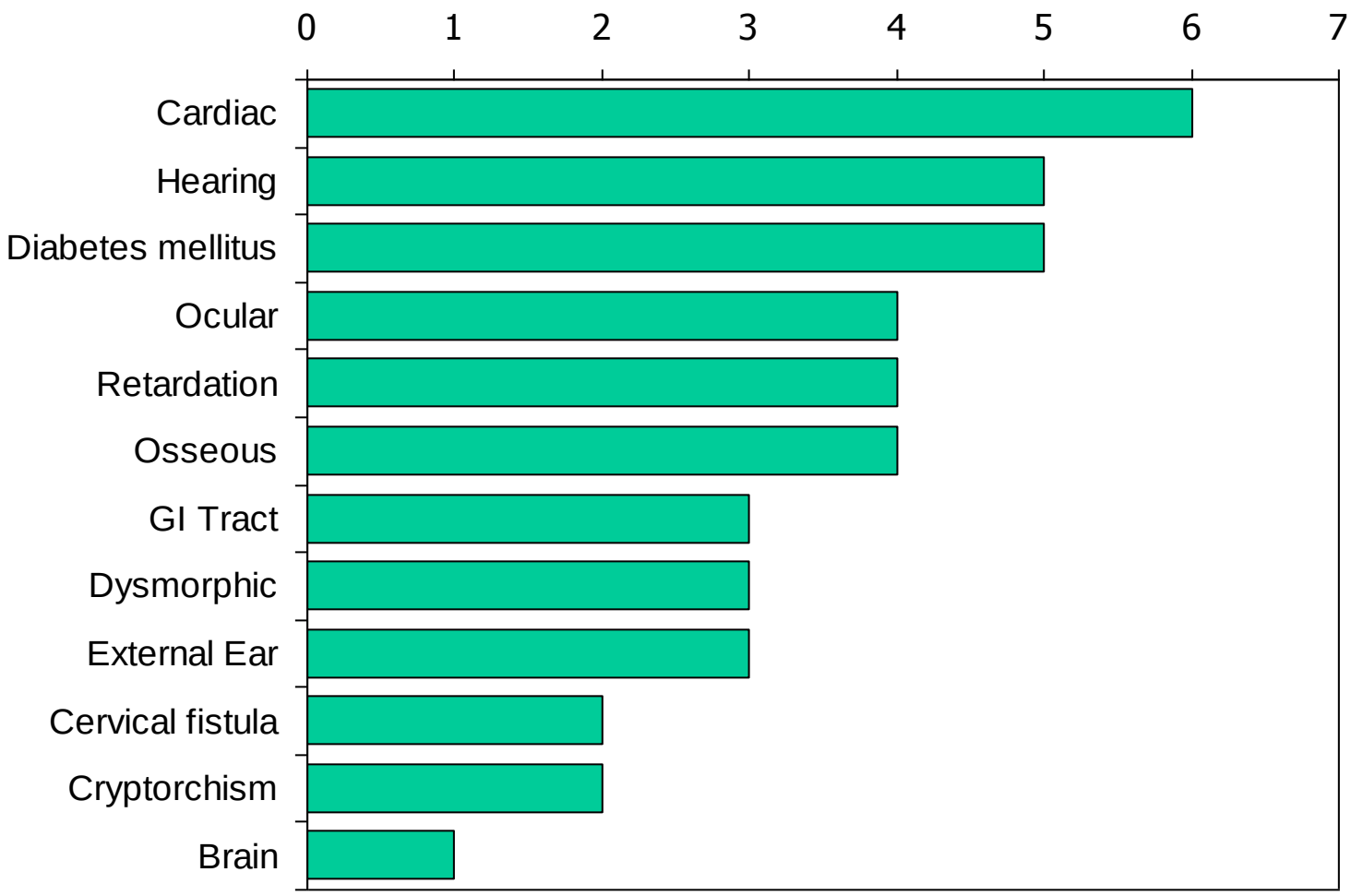
Carter et al. J Hum Genet 1984
McPherson et al. Am J Med Genet 1987

Solitary, duplex kidneys, UPJ obstruction in 15% of 1st/2nd degree relatives of children with renal agenesis/adysplasia

Schwaderer Pediatr Nephrol 2007

Positive family history: 12%

Extrarenal anomalies: 28%



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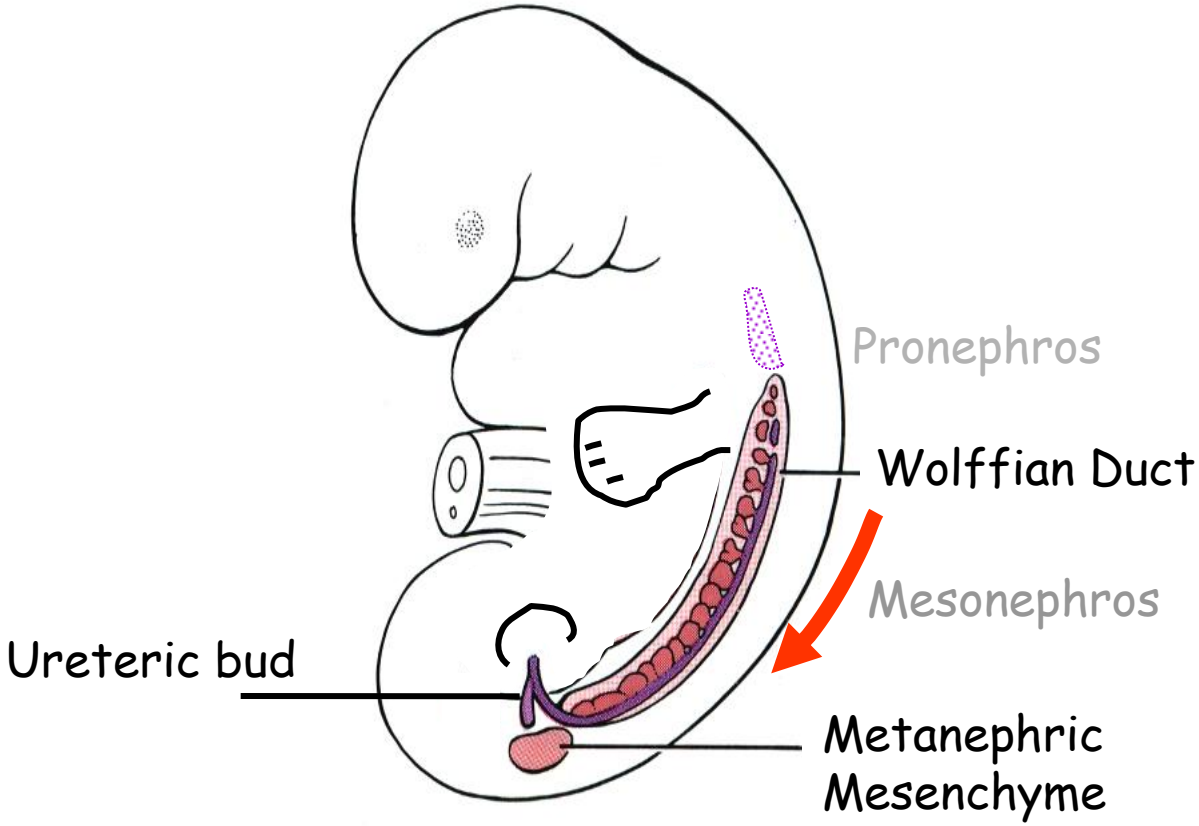
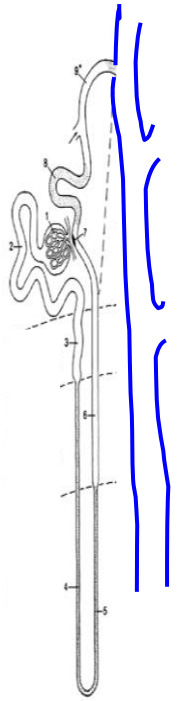
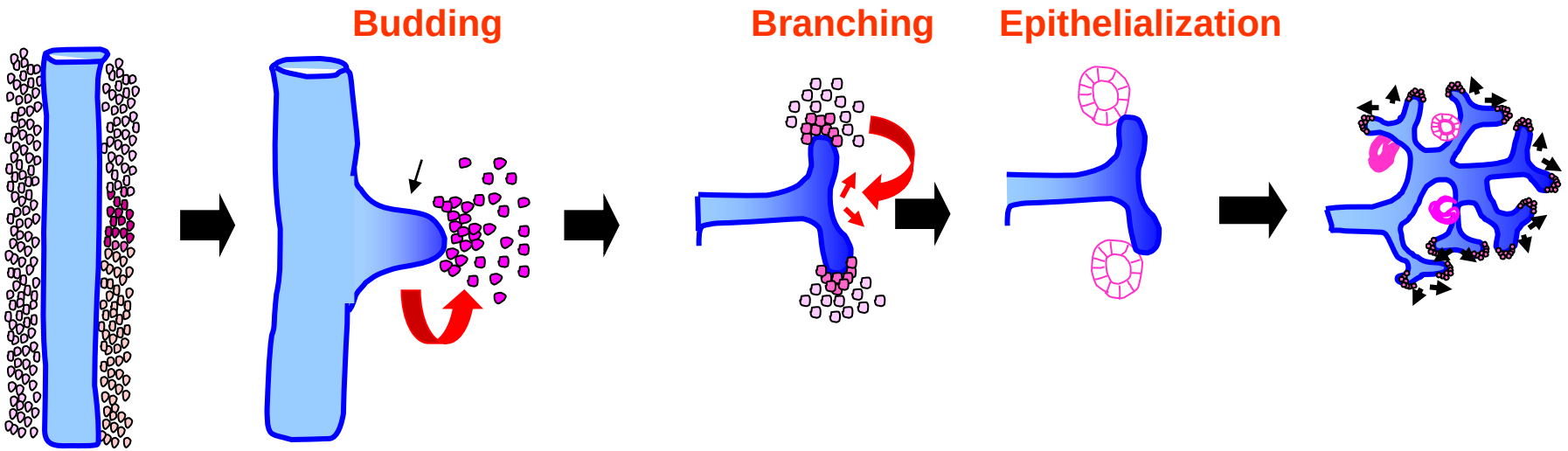
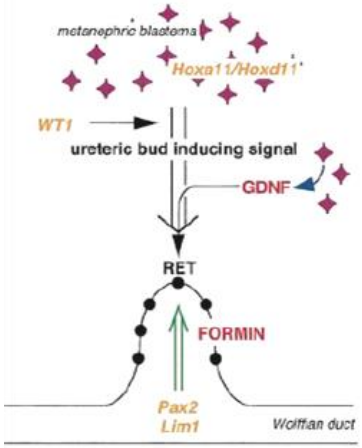
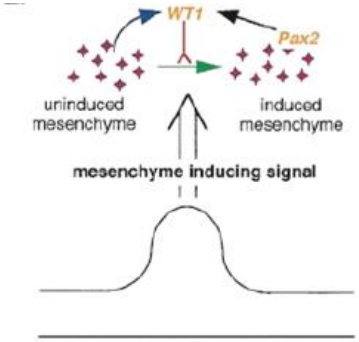
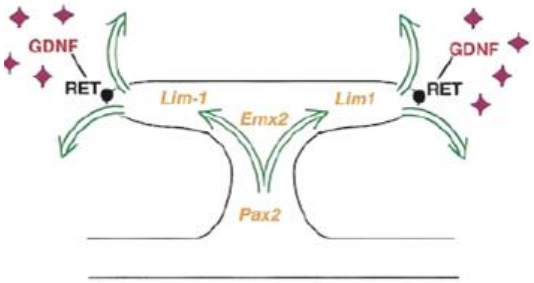

Pronephros
Wolffian Duct
Mesonephros
Ureteric bud
Metanephric
Mesenchyme

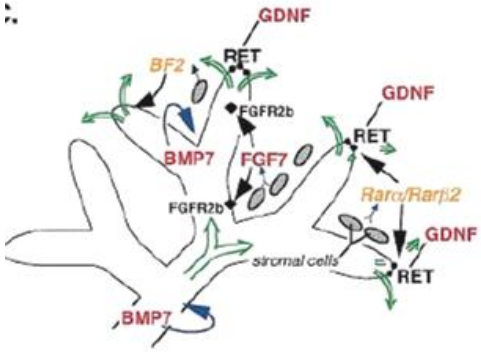


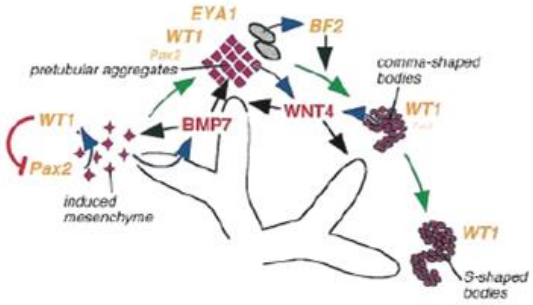

Budding
Branching
Epithelialization

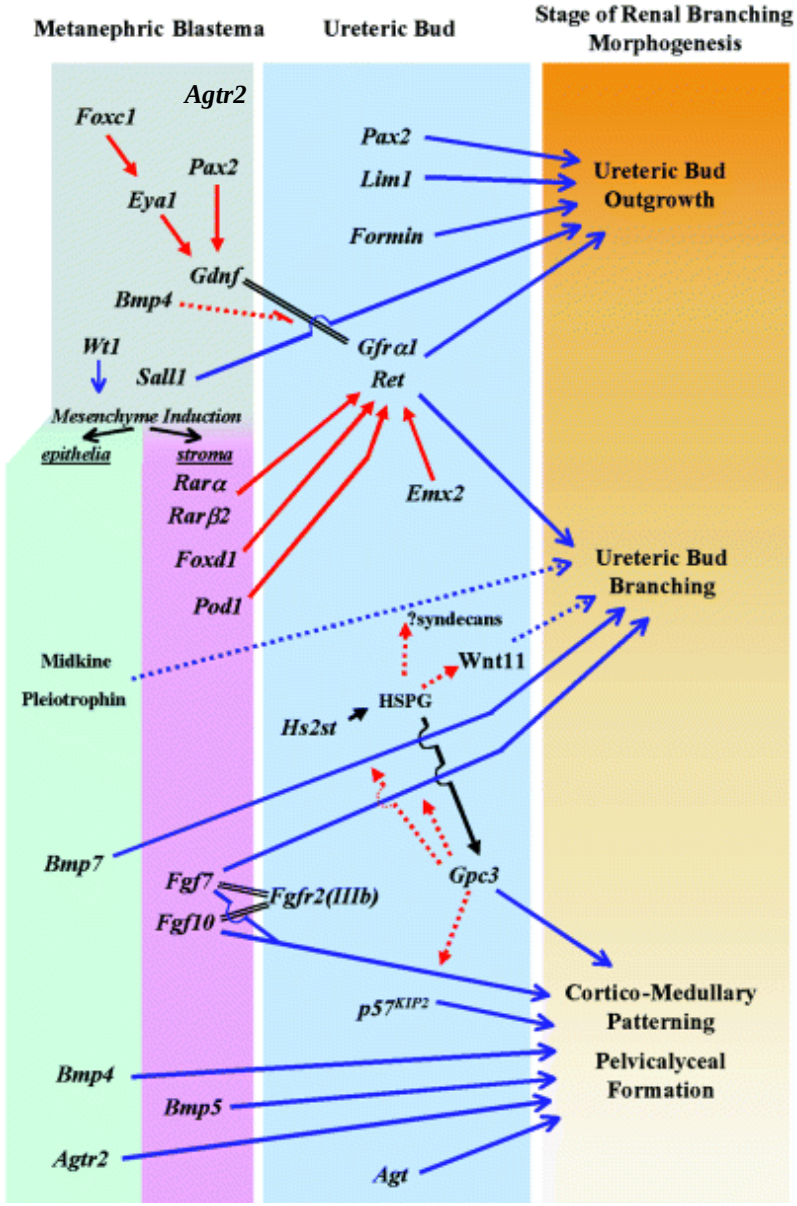

metanephric blastema
Hoxa11/Hoxd11
WT1
ureteric bud inducing signal
GDNF
RET
FORMIN
Pax2
Lim1
Wolffian duct


WT1
Pax2
uninduced mesenchyme
induced mesenchyme
mesenchyme inducing signal


GDNF
RET
GDNF
RET
Lim-1
Emx2
Lim1
Pax2


GDNF
RET
BF2
FGFR2b
BMP7
FGF7
FGFR2b
GDNF
RET
Rarα/Rarβ2
GDNF
stromal cells
RET
BMP7


EYA1
WT1
Pax2
BF2
pretubular aggregates
comma-shaped bodies
WT1
WT1
BMP7
WNT4
Pax2
induced mesenchyme
WT1
S-shaped bodies

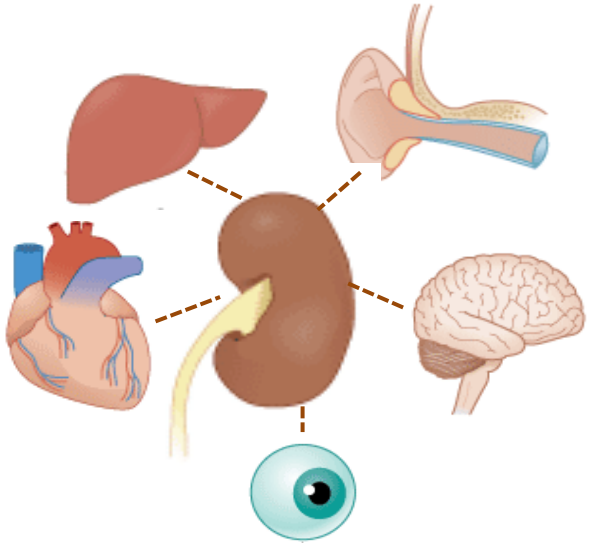

Metanephric Blastema
Ureteric Bud
Stage of Renal Branching Morphogenesis
Agtr2
Foxc1
Pax2
Eya1
Gdnf
Bmp4
Wt1
Sall1
Mesenchyme Induction
epithelia
stroma
Pax2
Lim1
Formin
Ureteric Bud Outgrowth
Gfrα1
Ret
Rarα
Rarβ2
Foxd1
Pod1
Emx2
Ureteric Bud Branching
?syndecans
Midkine
Pleiotrophin
Wnt11
HSPG
Hs2st
Bmp7
Fgf7
Fgf10
Fgfr2(IIIb)
Gpc3
Cortico-Medullary Patterning
p57KIP2
Pelvicalyceal Formation
Bmp4
Bmp5
Agtr2
Agt

Knock-Out Mouse Models with Renal Phenotype

Gene	Function	Renal phenotype
WT1	transcription factor	bilateral agenesis
Pax2	transcription factor	-/-: bilateral agenesis; +/-: hypoplasia
EYA-1	transcription factor	-/-: bilateral aplasia; +/-: hypoplasia
LIM1	transcription factor	bilateral agenesis
Foxc1/2	transcription factor	double kidneys, hydroureter, hypoplasia
BMP-4	secreted signaling molecule	+/-: hypo/dysplasia, hydronephrosis
BMP-5	secreted signaling molecule	hydronephrosis
BMP-7	secreted signaling molecule	dysplasia
FGF-7	secreted signaling molecule	-/-: hypoplasia
WNT4	secreted signaling molecule	dysplasia
GDNF	secreted signaling molecule	+/-: unilat.agenesis, bilat.dysplasia -/-: bilat.agenesis
RET	receptor tyrosine kinase	uni/bilat.aplasia/dysplasia
AGTR2	angiotensin receptor	hypo/dysplasia, hydronephrosis
ADAMTS-1	metalloproteinase	-/-: stenosis of uretero-pelvic junction

Bardet-Biedl → ***BBS1-12***
Beckwith-Wiedemann → ***p57KIP2***
Cat-Eye
Renal cysts and diabetes → **HNF1β**
EEC
Fanconi
Fraser → ***FRAS1***
Fryns
Jeune (thoracic dystrophy)
Johanson-Blizzard
Klippel-Feil (sequence)
Lateral (sequence)
Meckel-Grubel
Melnick-Fraser (BOR) → ***EYA1, SIX1, SIX5***
MURCS (association)
Oral-Facial-Digital → ***OFD1***
Pallister-Hall
Peter's-Plus
Renal-coloboma → ***PAX2***
Rubinstein-Taybi
Schinzel-Gideon
Tuberous sclerosis
Saldino-Noonan
Majewski
Townes-Brocks → ***SALL1***
VATER (association)
Zellweger → ***PAF1***

Aarskog
Acrodysostose
Apert → ***FGFR2***
Baller-Gerold
Branchio-Oculo-Facial
Campomelic dysplasia → ***SOX9***
CHARGE (association)
Chondroectodermal dysplasia
Elhers-Danlos
Gorlin
HDR → ***GATA3***
Kabuki
Kallmann → ***KAL1, FGFR1, PROK2***
Langer-Gideon
Nail-patella → ***LMX1B***
Opitz
Robinow
Russell-Silver
Saethre-Chotzen
Simpson-Golabi-Behmel →***GPC3***
Smith-Lemli-Opitz → ***D(7)HCR***
Von Hippel Lindau
Williams → ***elastine***
Deletions 3p, 4p, 5p, 9p,13q, 18q
Duplications 4p, 9p
Trisomies 9 mosaïque, 13, 18



Identifying human CAKUT genes:

The **Syndrome Candidate Gene** Approach

100 CAKUT Patients from ESCAPE Study:

Search for Mutations in Genes Causing Syndromes with Mild Clinical Phenotype

PAX2: Renal-Coloboma Syndrome (RCS)

RHD, MCDK, oligomeganephronia, VUR,
optic nerve dysplasia / coloboma, rarely hypoacusis

HNF1b (TCF2): Renal Cysts and Diabetes Syndrome

RHD, glomerulocystic nephropathy,
MODY, hyperuricemia, hepatopathy

EYA1 and *SIX1*: Branchio-Oto-Renal Syndrome (BOR)

RHD, lateral cervical cysts/fistulae, ear anomalies, hypoacusis

SALL1: Townes-Brocks Syndrome (TBS)

RHD, VUR, PUV, skeletal, cardiac, hepatic and cerebral anomalies

PAX2

Heterozygous *PAX2* mutations detected
in 7 patients from 6 families

Multicystic-dysplastic kidney in 2 pts, UPJ obstruction in 1 patient

Ocular anomalies in 5 of 7 patients and 1 of 2 affected parents

Hypoacusis in 3 of 7 patients

Extrarenal anomalies minimal in 5 of 7 affected patients,
diagnosed only after mutation findings

Weber et al. JASN 2007

HNF1B

- *HNF1b* anomalies in **8/100** patients with renal hypo/dysplasia
- 4 heterozygous *HNF1b* deletions, 1x *de novo*
  3 heterozygous mutations in 4 patients
- **Cystic** RHD in 6 of 8 affected patients
- Extrarenal manifestations: 1 DM (13 y), 2 hyperuricemia
- Diabetic relatives in 3 of 8 families

Weber et al. JASN 2007

HNF1B

Ulinski et al. *JASN* 2006:

- *HNF1b* mutations and gene deletions in **25/80** patients with mainly **cystic RHD**
- ***de novo*** in 9 / 17 families

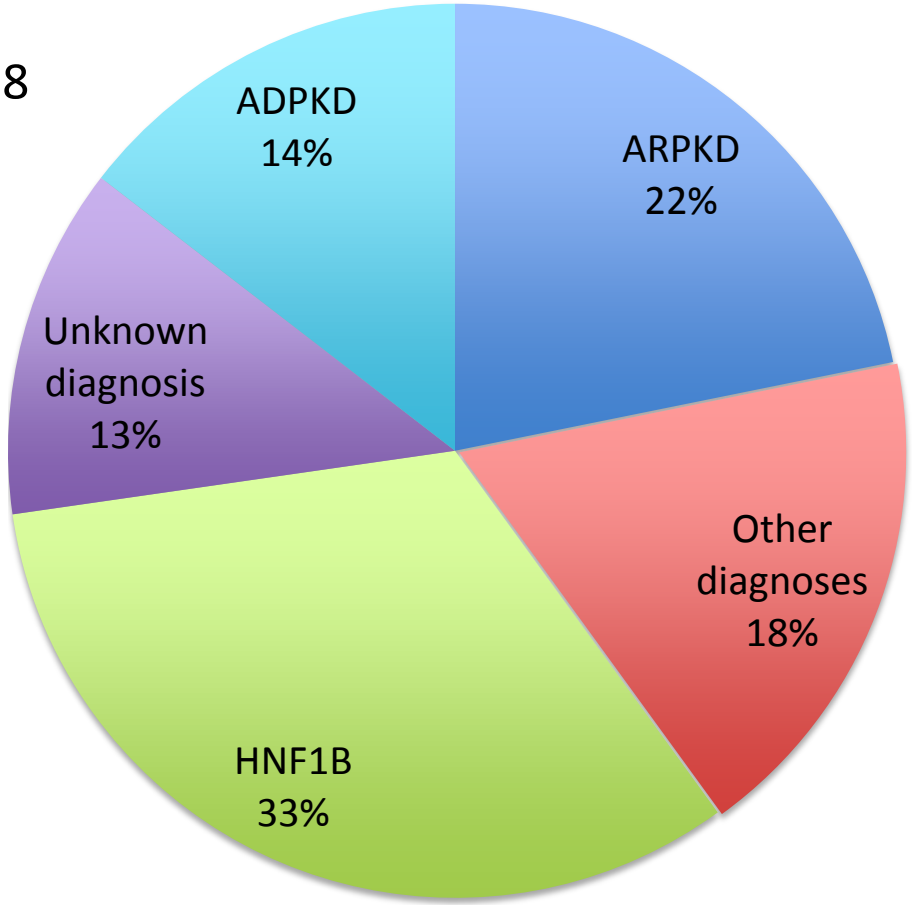
-> Systematic *HNF1b* screening relevant

- in children with **cystic RHD**
- because of **metabolic risks** (diabetes, hyperuricemia, liver dysfunction) for patient and affected family members

Anomalies of the *TCF2* Gene Are the Main Cause of Fetal Bilateral Hyperechogenic Kidneys

Stéphane Decramer,*¶ Olivier Parant,† Sandrine Beaufils,‡ Séverine Clauin,‡ Cécile Guillou,¶ Sylvie Kessler,† Jacqueline Aziza,§ Flavio Bandin,*¶ Joost P. Schanstra,* and Christine Bellanné-Chantelot‖

- Antenatal cysts: 11 of 18 pts (unilateral in 8/11)
- Cysts appeared during 1st YoL in 17/18
- GFR decreased with time in all pts

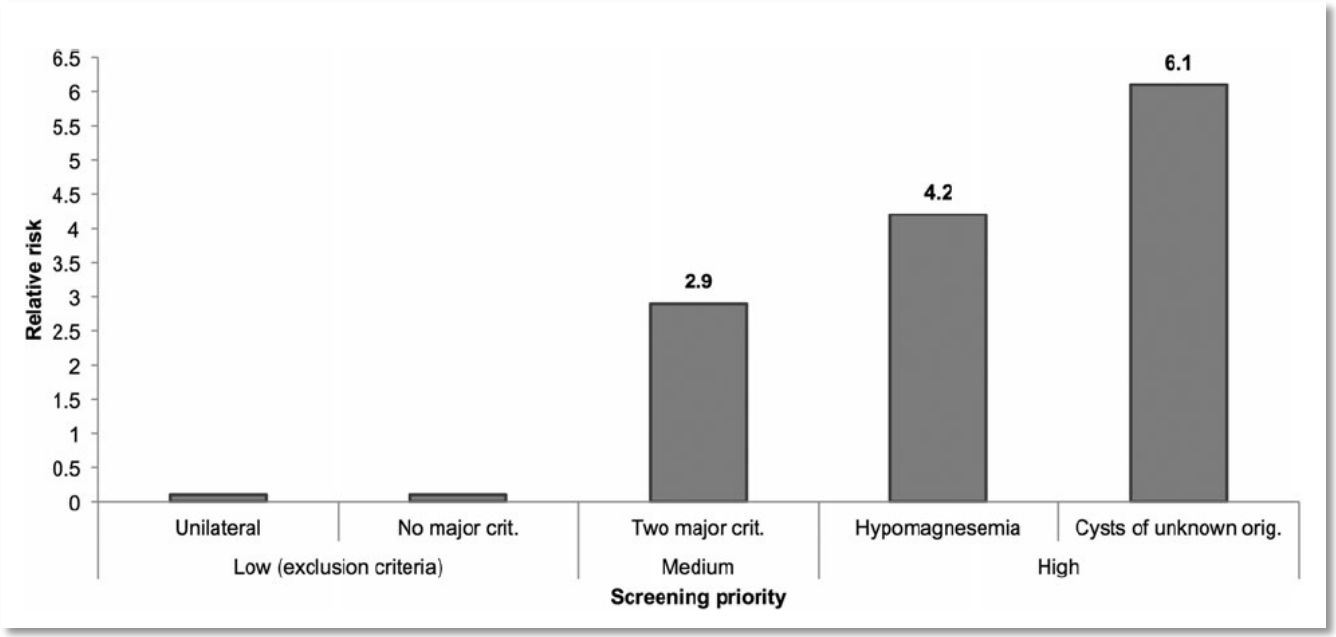


Decramer et al. JASN 2007

Indications for HNF1B Screening

- 200 CAKUT patients screened in HNF1B
- Screening criteria:
  1 major renal criterion,
  or 1 minor + extrarenal / family history
- 20 positive (10%)
  (10 mutations, 10 deletions)

Major criteria	Minor criteria
Fetal bilateral hyperechogenic kidneys	Ectopic kidney
Multicystic dysplastic kidney	Vesico-ureteral reflux
Renal agenesis	Hydronephrosis
Hypoplastic or dysplastic kidneys	
Cysts from unknown origin	
Extra-renal criteria	**Positive familial history**
Diabetes mellitus	Abnormalities in kidney
Hypomagnesaemia	Abnormalities in urogenital tract
Hypokalaemia	Gout
Hyperuricaemia	Liver function abnormalities
Liver function abnormalities	Diabetes or exocrine pancreas dysfunction



Raajmakers et al. NDT 2015

ESCAPE CAKUT Cohort:
Gene Variations in 99 Patients with Renal Hypodysplasia

PAX2:	7 patients with mutations
***HNF1b*:**	8 patients with deletions/mutations/variants
***EYA1*:**	1 patient with mutation
SALL1:	1 patient with mutation

-> **17% prevalence** of mild syndromic forms in CAKUT with CKD

HNF1B and Pax2 Mutations in Fetuses with Severe CAKUT

103 fetuses from 91 families with termination of pregnancy due to severe CAKUT on ultrasound

HNF1B mutations in 12 fetuses from 11 families
- all bilateral hyperechogenic kidneys, cystic dysplasia
- 2 unilateral duplex ureter

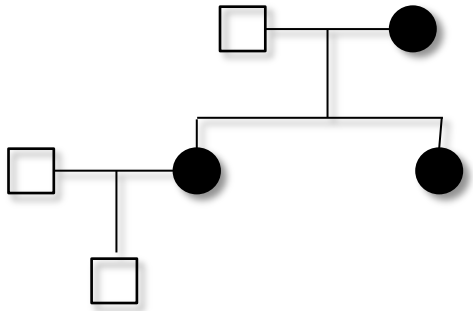
PAX2 mutations in 5 unrelated fetuses
3 bilateral hypoplasia, 1 hypodysplasia, 1 single dysplastic kidney

50% isolated, 50% syndromic features

No *HNF1B/PAX2* mutations in 27 cases of bilateral renal agenesis

-> **23% prevalence** of *HNF1B/PAX2* anomalies in **non-BRA CAKUT**

Madriaga et et al. CJASN 2013



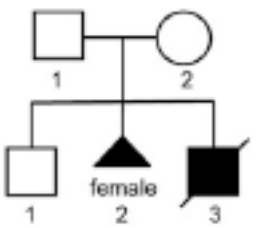
Identifying human CAKUT genes:

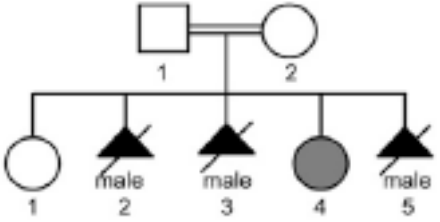
The **Family Gene Study** Approach

(genome-wide linkage, whole exome sequencing)

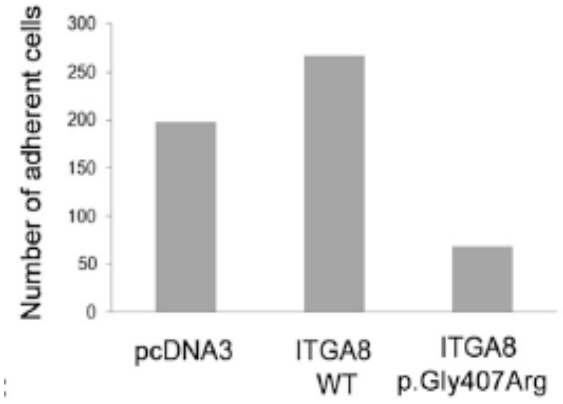
Bilateral Renal Agenesis

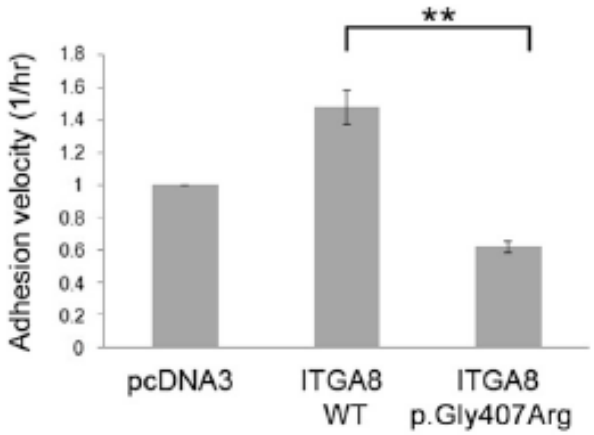
- **Recessive ITGA8** mutations identified by WES in 2/10 families





- Integrin-α8 expressed in metanephric mesenchyme
- Absent kidney development in KO mice
- Pathogenicity of mutations demonstrated

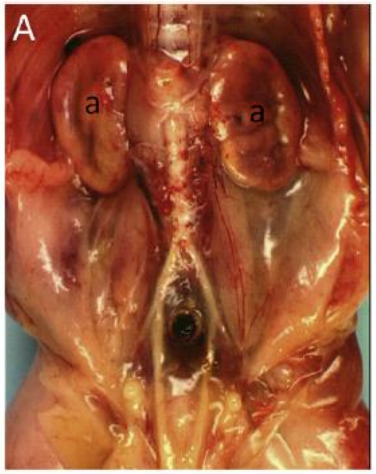


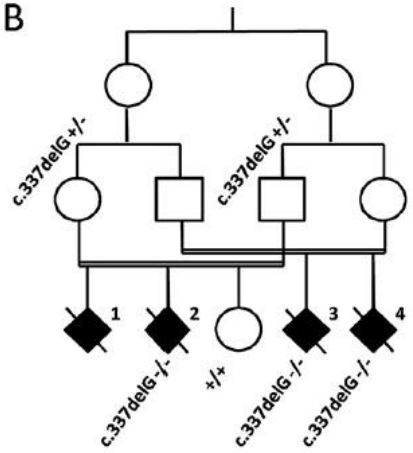


Humbert et al. Am J Hum Genet 2014

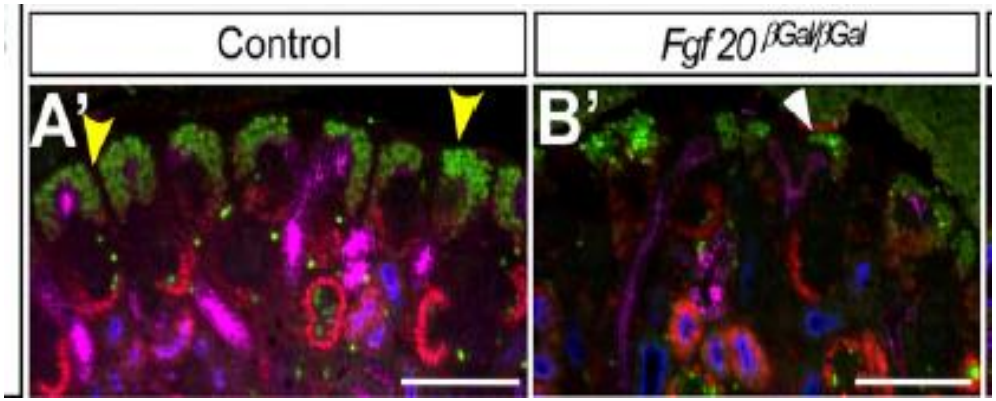
Bilateral Renal Agenesis

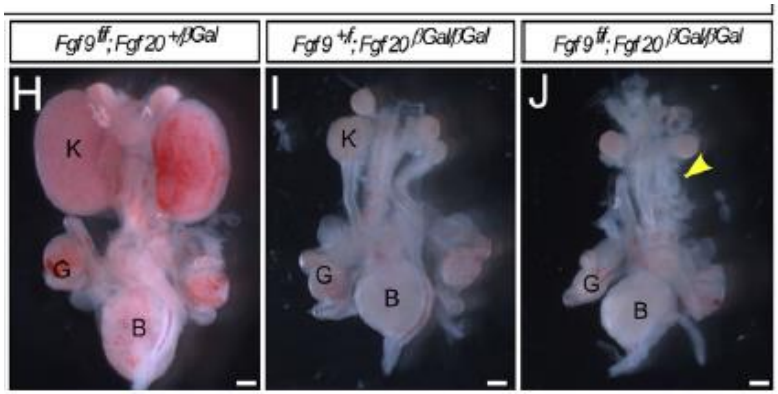
- Recessive **FGF20** mutation identified in family with BRA





- FGF20 deficiency in mice induces renal hypoplasia due to premature differentiation of nephron progenitors (‚loss of stemness‘)





Barak et et al. Dev Cell 2012

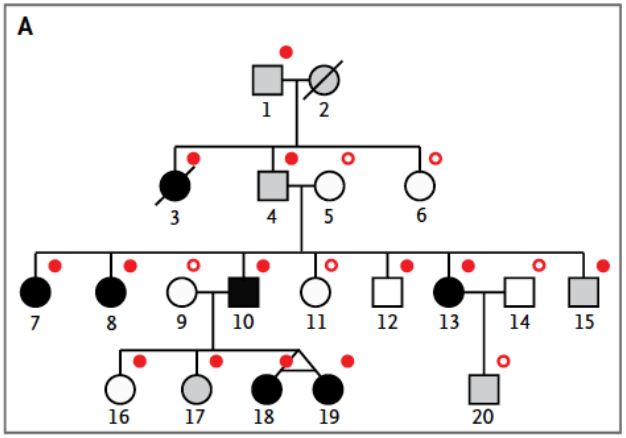
DSTYK: Novel Genetic Cause of CAKUT

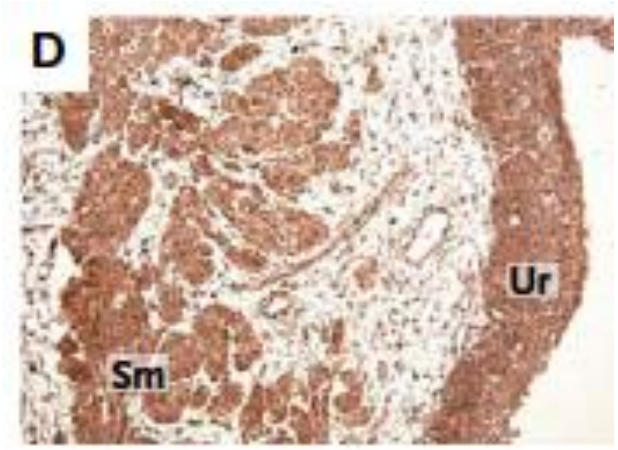
Dual **S**erine-**T**hreonine and t**y**rosine protein **K**inase (DSTYK) gene identified by GWLA/WES in **dominant CAKUT** family

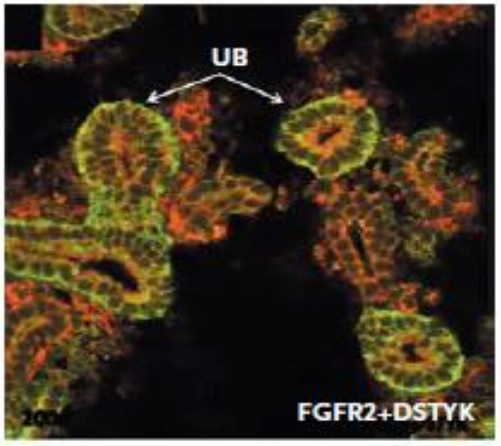
Additional DSTYK mutations found in **7 of 311** CAKUT patients (2.3%)

Phenotype: **UPJO, UVJO, renal hypodysplasia**

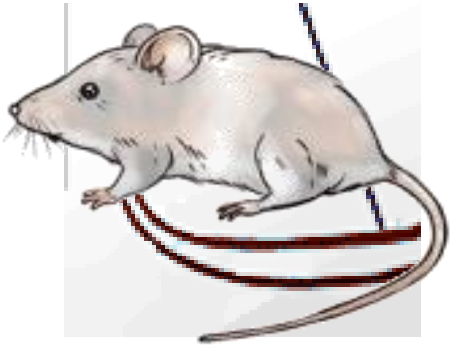
DSTYK defects cause **loss of FGF signaling** in UB/MM







Sanna-Cherchi et al. NEJM 2013

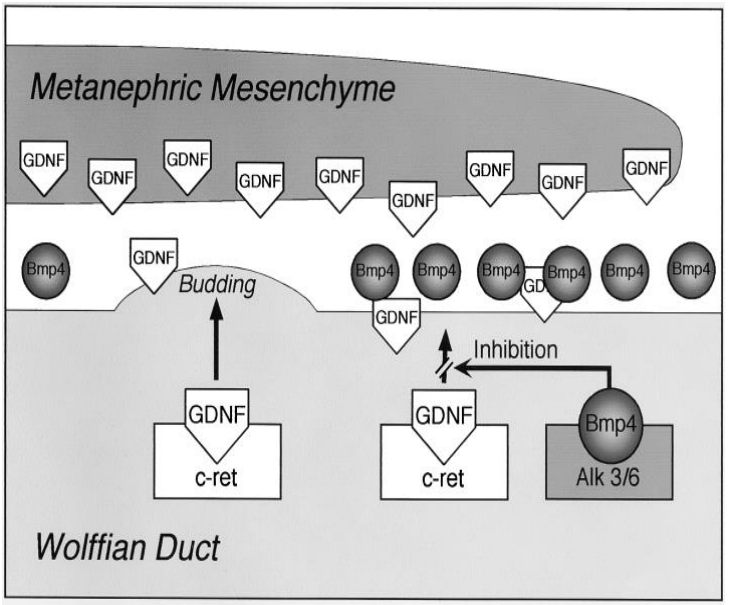


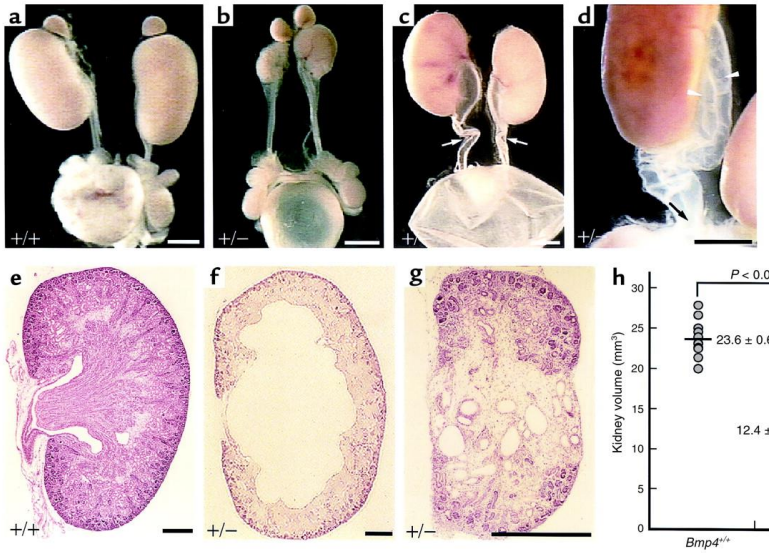
Identifying human CAKUT genes:

The **Murine Candidate Gene** Approach

CAKUT Candidate Gene: BMP4

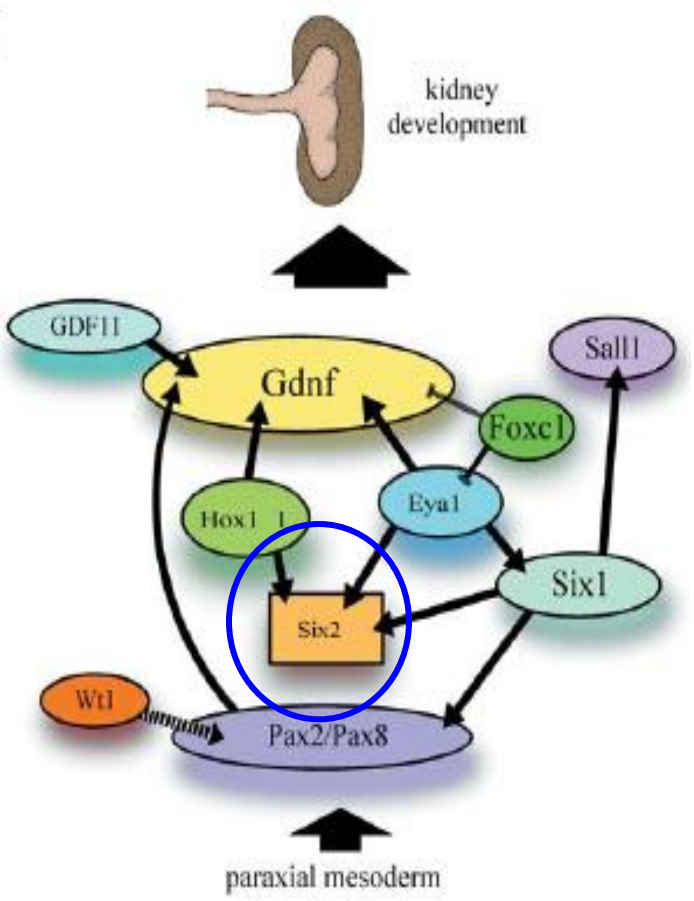
- Bone morphogenetic proteins: secreted signaling proteins involved in **body patterning and morphogenesis**
- Bmp4 highly expressed in cells surrounding the Wolffian Duct, **inhibits abnormal ureteric budding**
- Bmp4 **promotes elongation of the branching ureter**
- Murine *Bmp4* knock-out: Homozygous lethal, heterozygous **renal hypo/dysplasia, anomalies of urinary tract**





CAKUT Candidate Gene *SIX2*

- Six protein family has important functions as transcription factors for **early organogenesis**
- Human mutations in *SIX1* and *SIX5* are associated with BOR syndrome
- In the kidney, *SIX1* and *SIX2* have **similar expression patterns**
- Murine *Six2* knock-out is associated with **bilateral kidney dysplasia** (Self et al., ASN 2004)



Brodbeck & Englert, Ped Nephrol 2004

(Dominant?) Heterozygous Mutations in *SIX2* and *BMP4* in 250 Children with Renal Hypodysplasia

BMP4

Patient	Origin	*BMP4* mutation analysis Nucleotide exchange	*BMP4* mutation analysis Amino acid exchange	Kidney ultrasound
P6	Poland	272 C->G	Ser91Cys (het)	AGEN(r)
P7	Germany	272 C->G	Ser91Cys (het)	DYS(l)/VUR(r)
P8	Turkey	347 C->G	*de novo* Thr116Ser (het)	HYPO(r)/VUR(l)
P9	Turkey	450 C->G	Asn150Lys (het)	HYPO(r)
P10	Turkey	450 C->G	Asn150Lys (homo)	CYS-DYS(r,l)

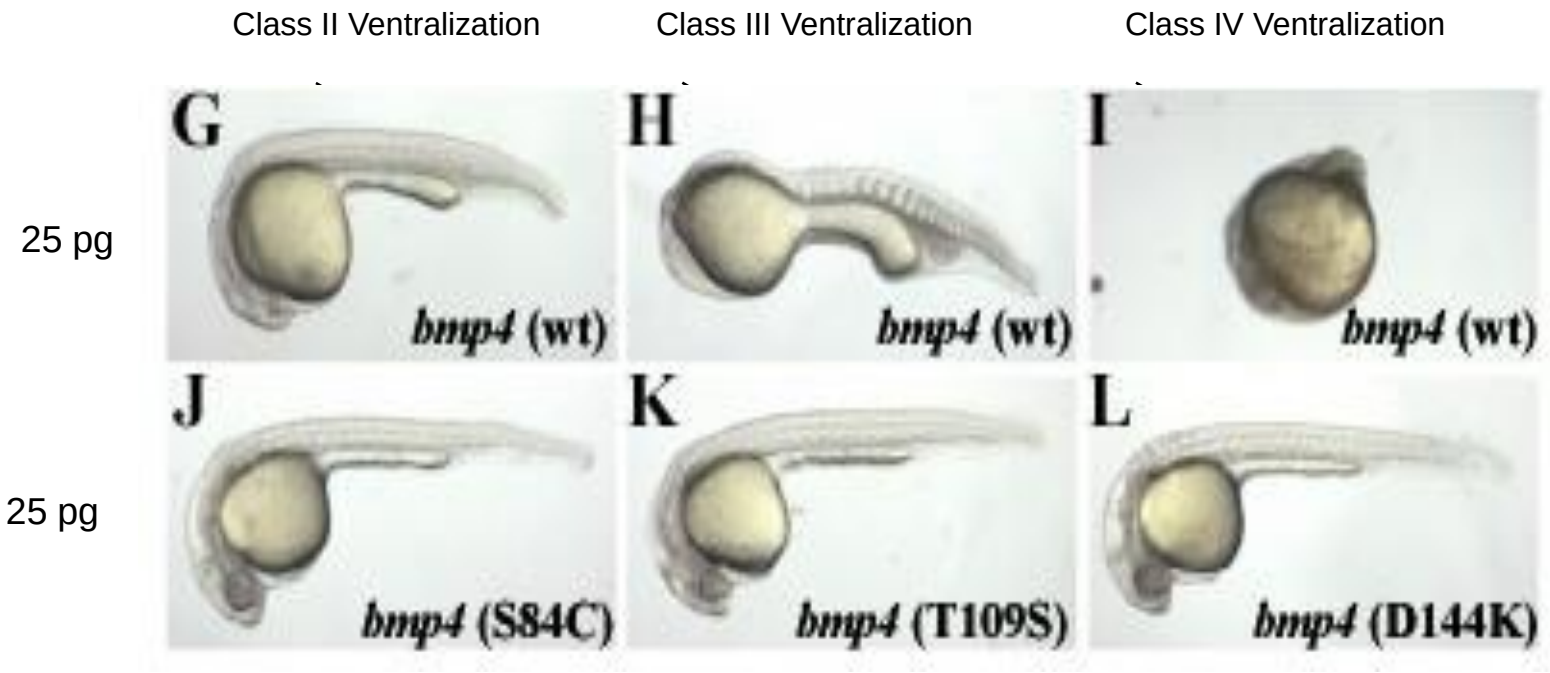
SIX2

Patient	Origin	*SIX2* mutation analysis Nucleotide exchange	*SIX2* mutation analysis Amino acid exchange	Kidney ultrasound
P1	Poland	402 C->T	Leu43Phe (het)	DYS(l)/VUR(r)
P2	Poland	997 C->T	Pro241Leu (het)	CYS-DYS(r,l)/VUR(r,l)
P3	Germany	997 C->T	Pro241Leu (het)	CYS-DYS(r,l)
P4	Italy	997 C->T	Pro241Leu (het)	HYPO(r)/VUR(r)
P5	Poland	1100-1101 GG->AA	Asp276Asn (het)	CYS-DYS(r,l)/ HYPO(r)

ESCAPE

Weber et al. JASN 2008

Human mutations abolish *bmp4* function in zebrafish overexpression assay



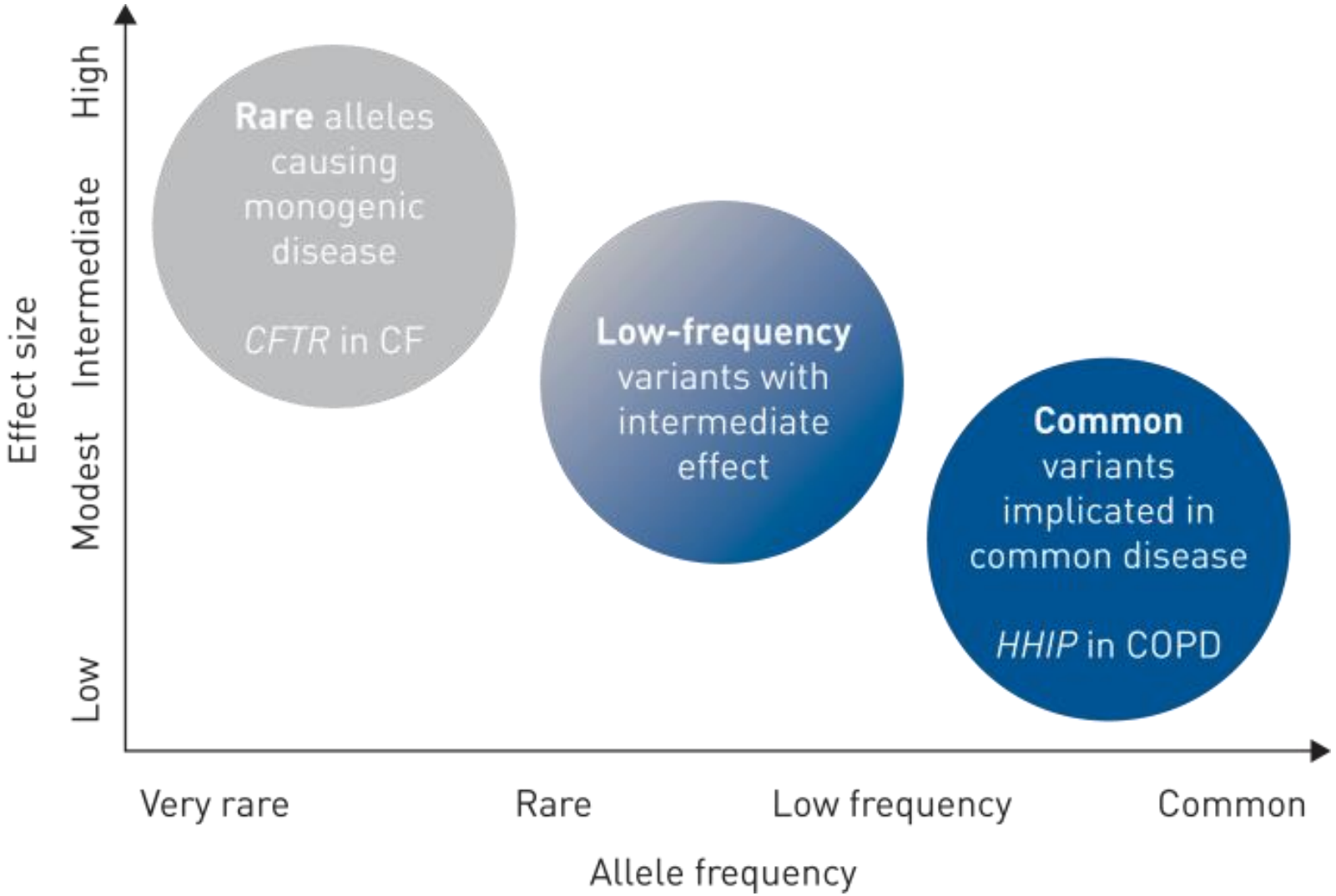
Weber et al. JASN 2008

ESCAPE CAKUT Cohort:
Gene Variations in 99 Patients with Renal Hypodysplasia

***BMP4*:**	3 patients with variants
***SIX2*:**	3 patients with variants
***SIX1*:**	2 siblings with variant
***RET*:**	6 patients with mutations, 5 patients with variants
***GDNF*:**	1 patient with variant

But: Some variants also found in healthy family members and patients with non-renal disease!

CAKUT: A Disease with Complex Genetics?



Human Mutations in Genes Causing Recessive Unilateral Renal Agenesis in Mice

NGS panel sequencing of 12 recessive murine candidate genes

574 individuals with isolated CAKUT from 590 families

- **15 of 590 families**: recessive mutations in FRAS1 (n=7), FREM2, GRIP1, FREM1, ITGA8, and GREM1
- All genes involved in UB – MM interaction
- Missense FRAS mutations cause isolated CAKUT, truncating mutations multiorgan Fraser syndrome
- Mutations in 6 identified genes accounted for **2.5% of patients** in CAKUT cohort

Kohl et et al. JASN 2014

Identifying human CAKUT genes:

The **Next Generation Panel Gene Sequencing** Approach

NGS Panel Sequencing of CAKUT Cohort

749 individuals from 650 families with CAKUT screened in

17 known dominant CAKUT-causing genes

39% VUR, 15% renal hypodysplasia, 12% unilateral renal agenesis

37 heterozygous mutations in 47 patients from 41 families (6.3%):

HNF1B (6), *PAX2* (5), *SALL1* (9), *CHD1L* (5), *ROBO2* (4), *EYA1* (3), *RET* (3), *GATA3* (2), *BMP7* (1), *CDC5L* (1), *SIX2* (1), *SIX5* (1)

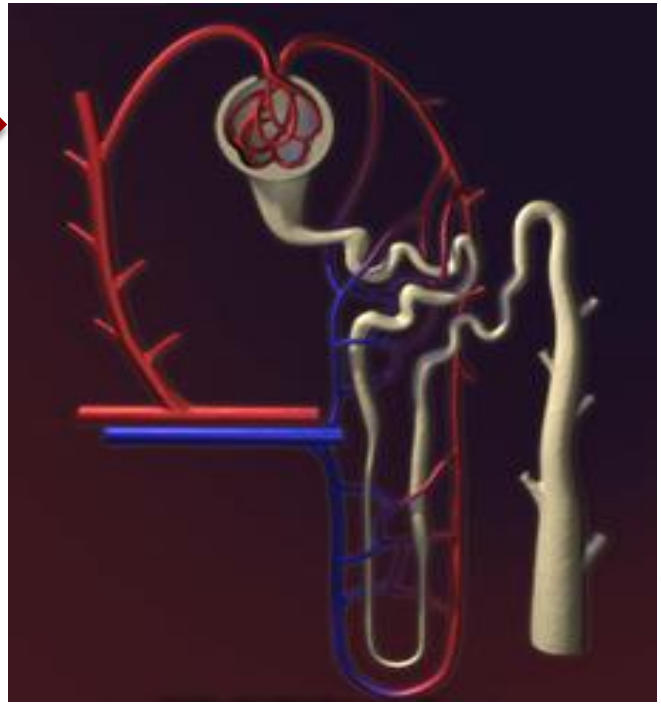
-> Cohort differences in CAKUT phenotype spectrum

-> Challenge to verify pathogenicity of mutations

Hwang et et al. KI 2014

WP2/3:
Nephrotic glomerulopathies

WP5:
Complement-mediated nephropathies



WP4:
Tubulopathies

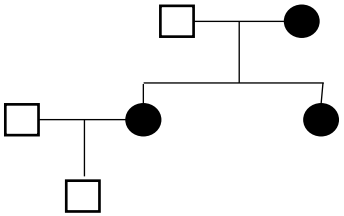
WP6: Kidney malformations

Targeted NGS Approach

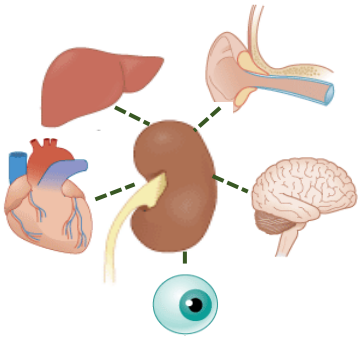
Nayia Nicolaou

UMC Utrecht

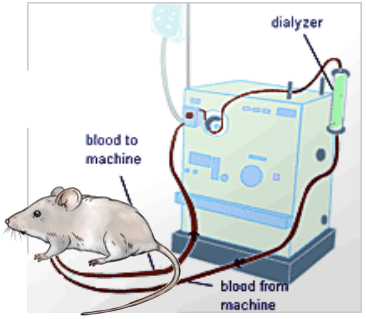
Gene mutations identified in family studies



Human malformation syndromes



Animal models of CAKUT



CAKUTOME NGS
Panel: 208 genes

453 CAKUT patients

predominantly mild disease (VUR, PUJO…)

Massive parallel sequencing of pooled samples (196 samples per run)

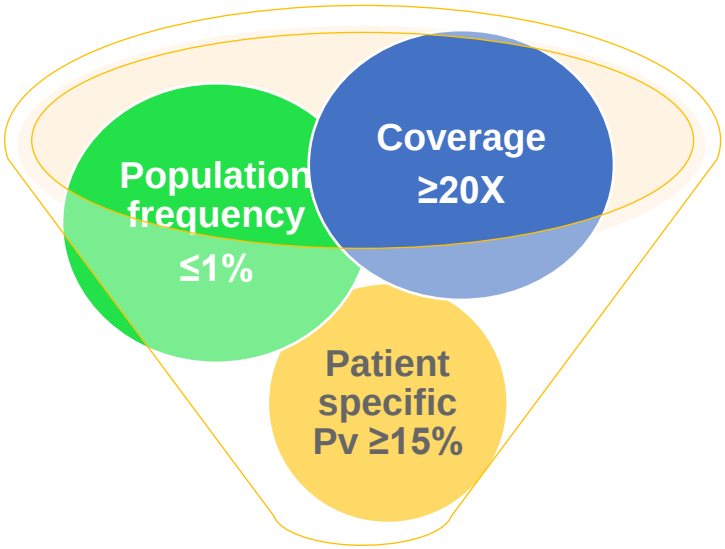
Mean target coverage per sample of 135X

Variant Filtering

208 genes; 453 patients
11,588 variants

remove synonymous

3,142 variants



1,928 variants

Prioritization

Prioritization

Missense variants	Truncating	HGMD variants
n=1,642 Mean Pv ≤ 2.5 Mean Po ≤ 2.5 Novel, Deleterious n=145	n=78	n=75 Kidney disease-related genes n=44

Validated by Sanger sequencing n=180

Classification

Classification of the Validated Variants

Group 1: Causal Mutations (n=5)		
Criteria	**1**	Known or truncating mutations in known CAKUT-causing genes with a high penetrance (no frequency in healthy individuals)
Group 2: candidate variants of pathogenicity (n=15)		
Criteria	**1**	Rare truncating variants in known kidney disease-related genes
	2	Variants previously reported in CAKUT patients with experimental evidence supporting the pathogenic effect on the protein function
Group 3: Probably deleterious (n=128)		
Criteria	**1**	Novel missense variants predicted to be deleterious (PolyPhen-2, SIFT) and conserved (GERP>4)
	2	Rare truncating variants in any of the 208 genes
Group 4: Variants of uncertain significance (VUS) (n=32)		
Criteria	**1**	HGMD variants with no functional evidence supporting their causality
	2	HGMD variants found in population databases and have no statistical evidence for an association with disease.

Conclusions: CAKUT NGS Gene Panel Sequencing

- Successful high-throughput sequencing with high coverage
- Small patient subset carries known or novel pathogenic mutations in a known causal CAKUT gene
- 148 candidate variants in **82 genes in 151/453 (33%) CAKUT patients** of which **143 variants** need further investigation
- ~98% of these variants were predicted to be among 1% most deleterious of all possible substitutions in the human genome
- Testing of **second CAKUT cohort** with more severe kidney disease (4C/ESCAPE) underway

Can CAKUT Genetics Become Clinically Useful?

- Currently limited impact of genetic diagnosis on clinical management: Genetic counseling, syndromic cases
- NGS technologies making diagnostics quick and cheap
- Lack of detailed genotype-phenotype correlation studies
- Crucial issue:
  Does genetic defect predict risk and rate of renal failure progression?

➢ Comprehensive NGS gene panel studies in large, well characterized CAKUT cohorts required

Role of Epigenetic Modifications in CAKUT?

Jin et et al. AJKD 2014

- Monozygotic twins **discordant for unilateral renal agenesis**
- No discordant SNPs or DNA copy number variations
- 514 Differentially Methylated Regions,
  localized to 10 signaling pathways and 25 genes,
  including MAP kinase pathway and 6 genes involved in organ development (FGF18, FGF12, PDGFRA, MAPK11, AMH, CTBP1)

➢ Epigenetic mechanisms involved in renal agenesis?

Potential Causes for Impaired Nephrogenesis in Humans