Westfälische Wilhelms-Universität Münster

UKM Universitätsklinikum Münster

Bartter-like Syndromes
MAGE-D2 and the Regulation of Salt Transporters

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University Children ‘s Hospital
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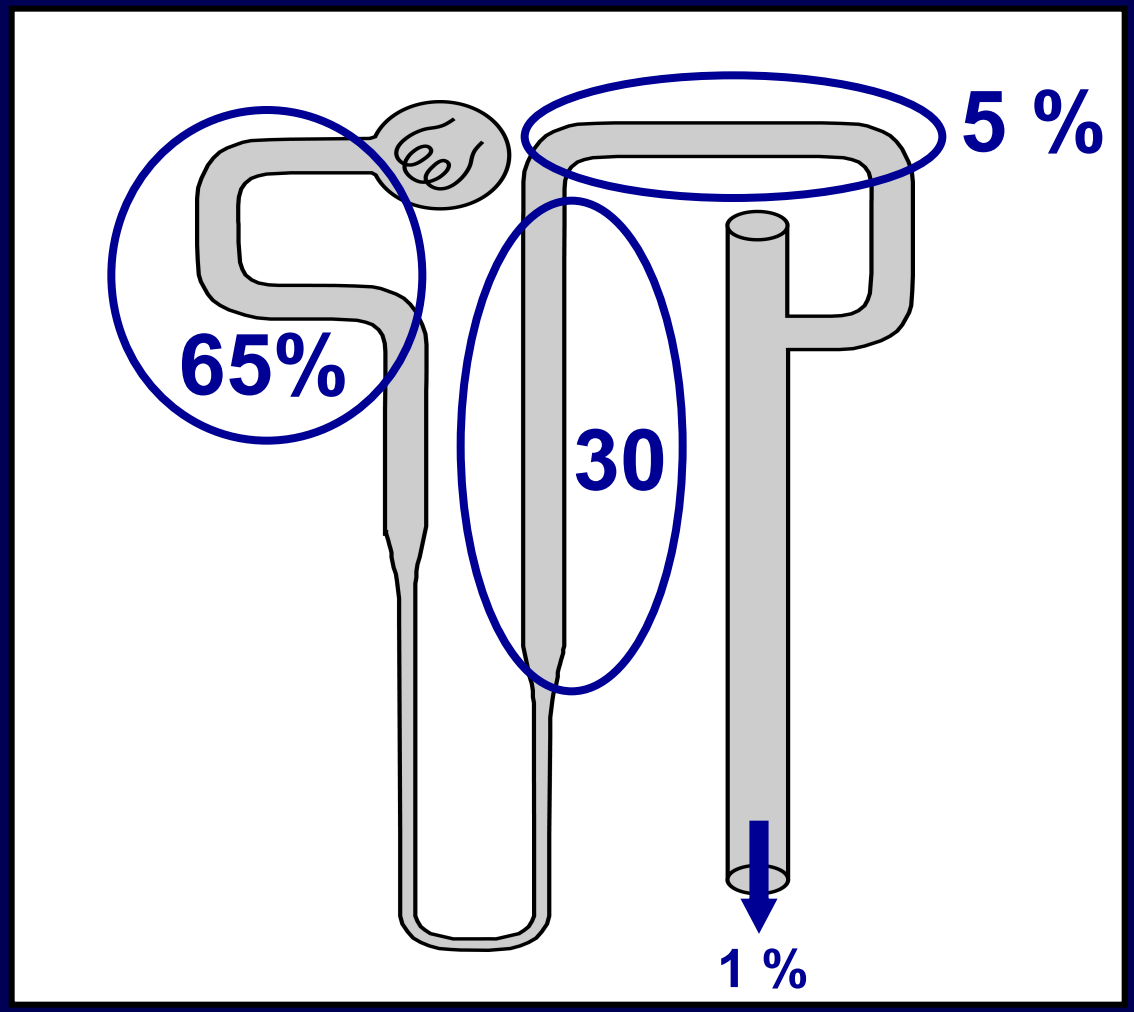
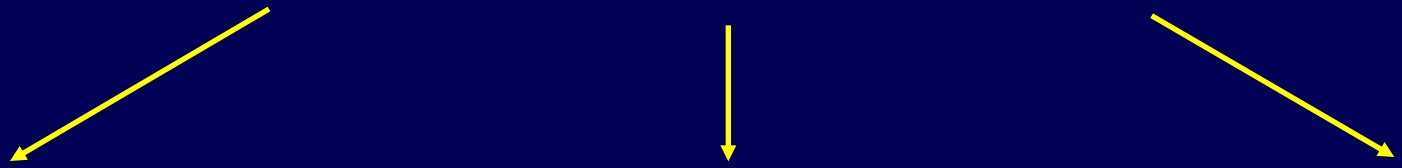
ERKNet / ERA / WKIKD CME Course, Heidelberg 2018

Renal Tubular Salt Reabsorption



„Bartter-like syndromes“

Set of inherited tubular disorders with
hypokalemic metabolic alkalosis,
activated RAAS,
increased prostaglandin synthesis

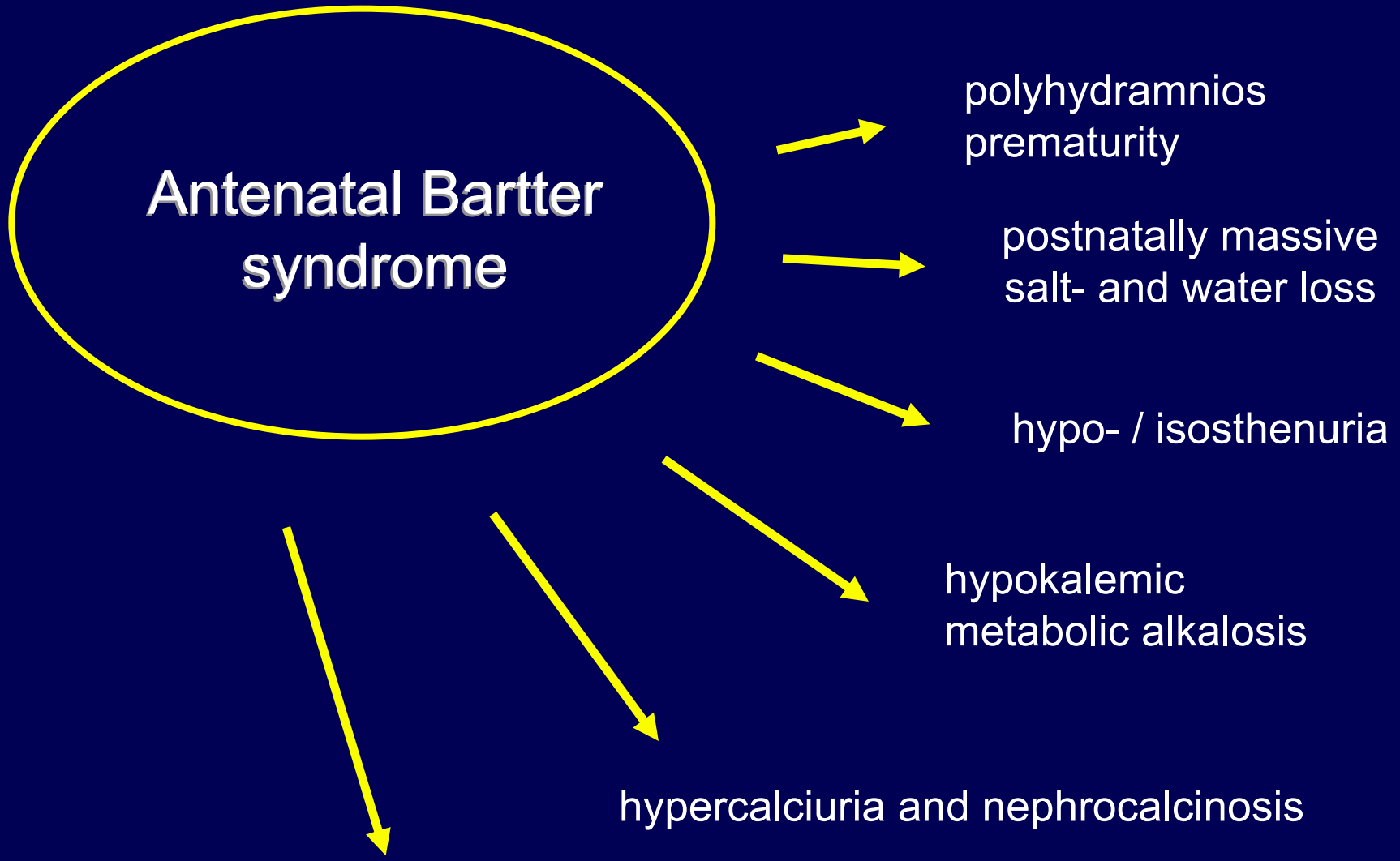


antenatal
Bartter-syndrome

+/- deafness

classic
Bartter-syndrome

Gitelman syndrome

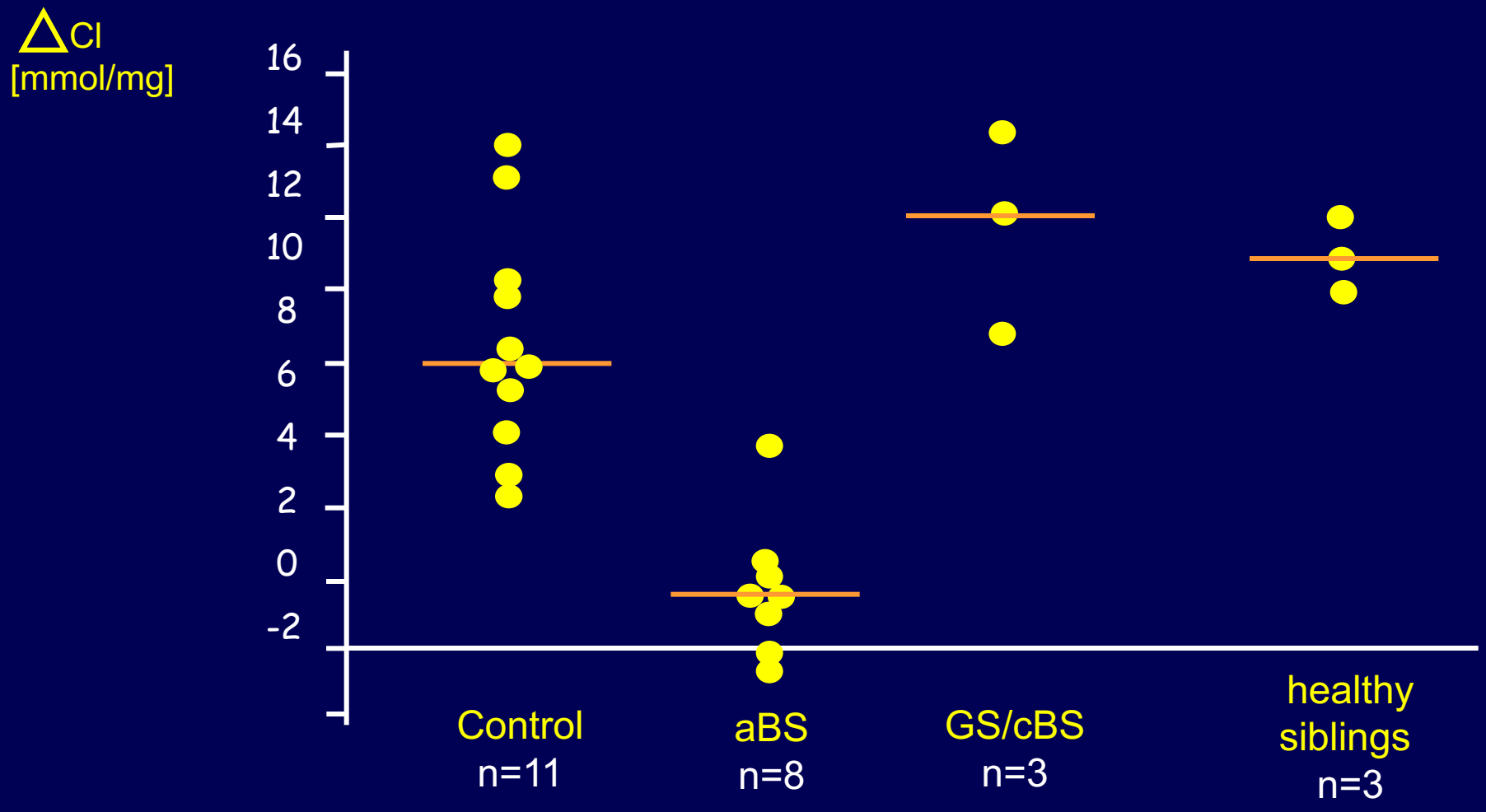

Antenatal Bartter syndrome
polyhydramnios
prematurity
postnatally massive
salt- and water loss
hypo- / isosthenuria
hypokalemic
metabolic alkalosis
hypercalciuria and nephrocalcinosis
Salt / fluid supply, indomethacin therapy

Pathophysiology of antenatal Bartter syndrome

Furosemid	Antenatal BS
mTAL	mTAL
Diuresis ↑	Diuresis ↑
Saliuresis ↑	Saliuresis ↑
Urinary calcium ↑	Urinary calcium ↑
Ototoxicity	Deafness

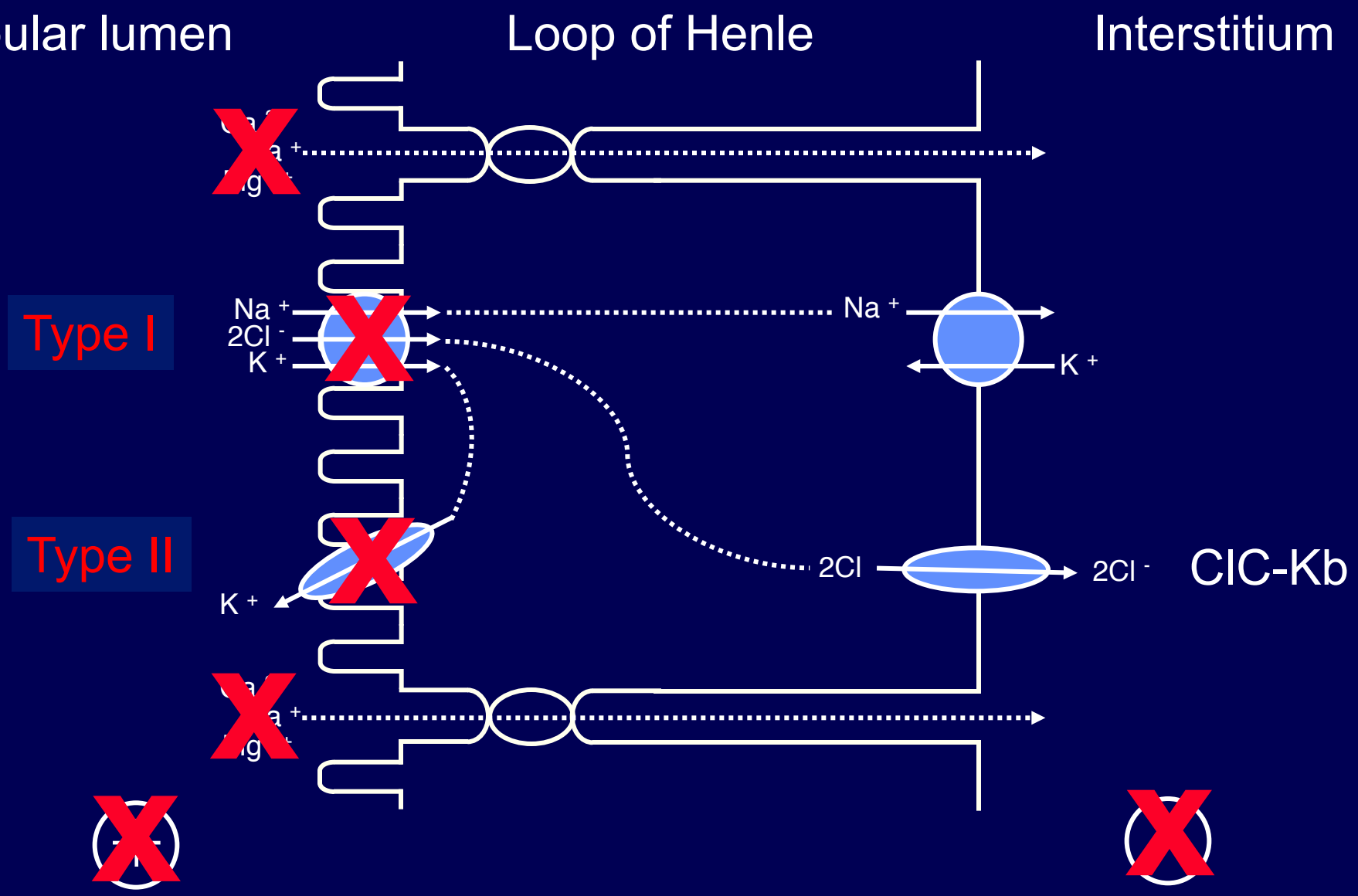
(*Landau et al*. 1995, *Madrigal et al*. 1997)

Chloride excretion after furosemide



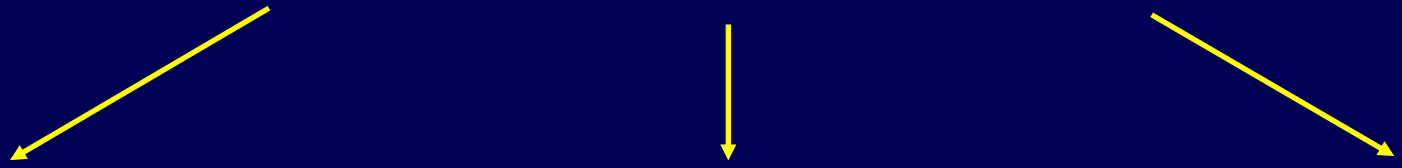
Köckerling et al, J Pediatr 1996

Pathophysiology of antenatal Bartter syndrome



„Bartter-like syndromes“

Set of inherited tubular disorders with hypokalemic metabolic alkalosis



antenatal Bartter-syndrome

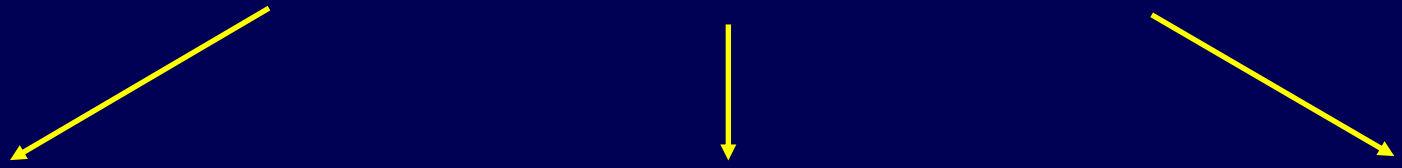
NKCC2
ROMK

classic Bartter- syndrome

Gitelman syndrome

„Bartter-like syndromes“

Set of inherited tubular disorders with hypokalemic metabolic alkalosis



antenatal Bartter-syndrome

NKCC2
ROMK

classic Bartter- syndrome

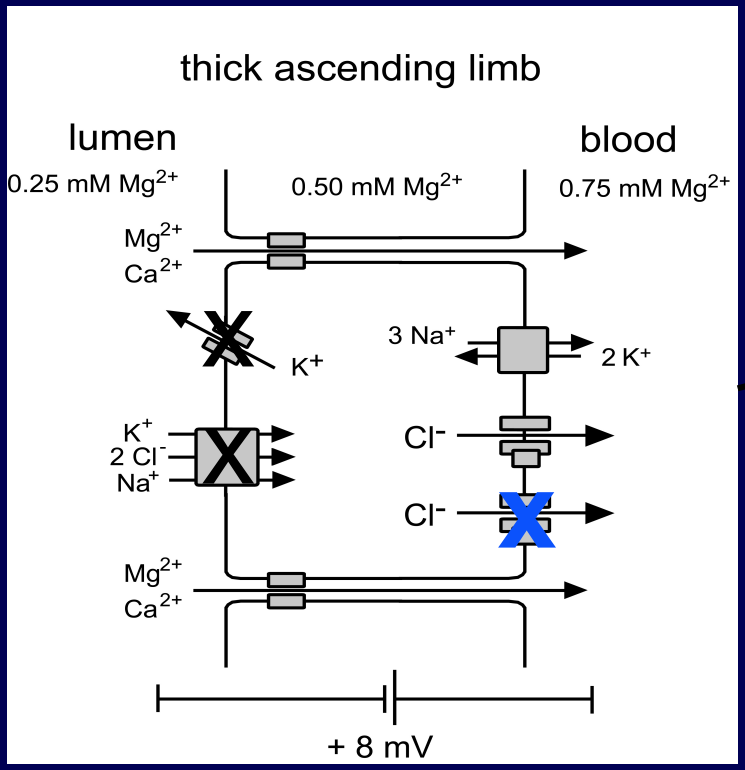
Gitelman syndrome

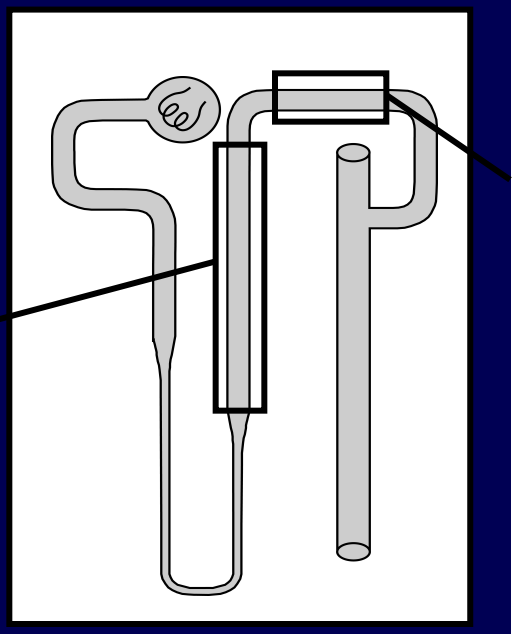
NCCT

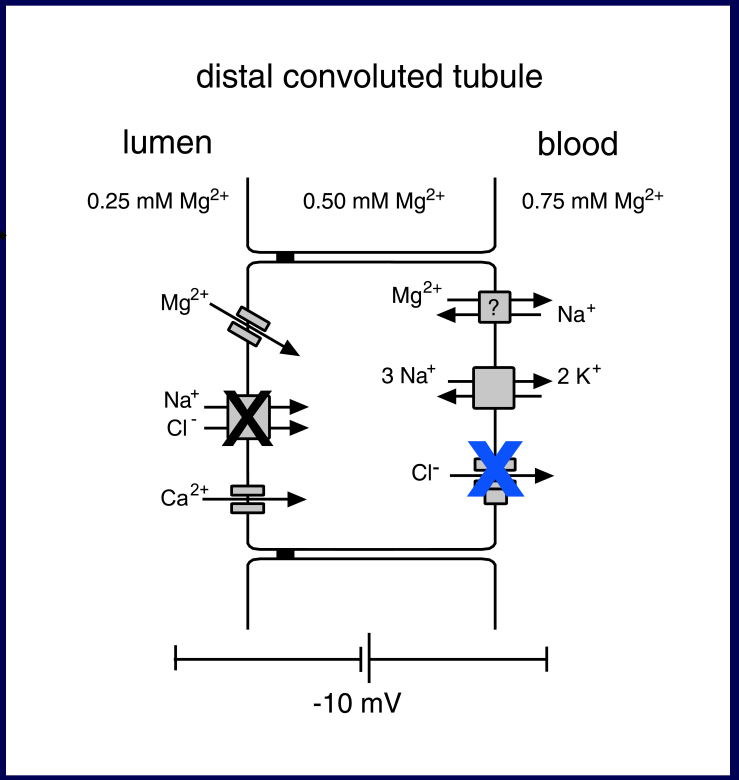
Classic Bartter Syndrome

- Polyuria, failure to thrive in the first 2 yrs of life
- Rarely polyhydramnios, prematurity
- Rarely hypercalciuria / nephrocalcinosis
- Hypomagnesemia during follow-up is common

Transepithelial Chloride Transport in TAL and DCT







Antenatal Bartter Syndrome, ROMK
Antenatal Bartter Syndrome, NKCC2

Gitelman Syndrome, NCCT

Classic Bartter Syndrome, ClC-Kb

Seyberth & Schlingmann, 2011

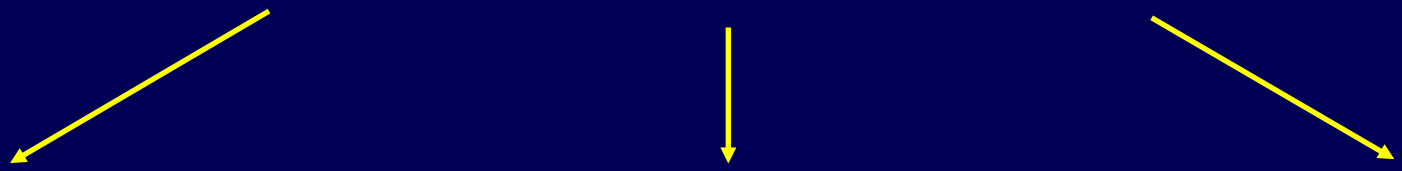
Clinical Characterization

	ROMK / NKCC2 (n=34)	ClC-Kb (n=35)	NCCT (n=20)
Polyhydr/Hyposthen/NC	100 %		
Polyhydramnios	100 %	35%	
Hypo- / Isosthenuria	100 %	45%	
Nephrocalcinosis	100 %	11%	
Hypocalciuria		10%	90 %
Hypomagnesemia		43%	90 %

Peters et al, Am J Med 2002

„Bartter-like syndromes“

Set of inherited tubular disorders with hypokalemic metabolic alkalosis



antenatal Bartter-syndrome	classic Bartter- syndrome	Gitelman syndrome
NKCC2 ROMK	ClC-Kb	NCCT

„Bartter-like syndromes“

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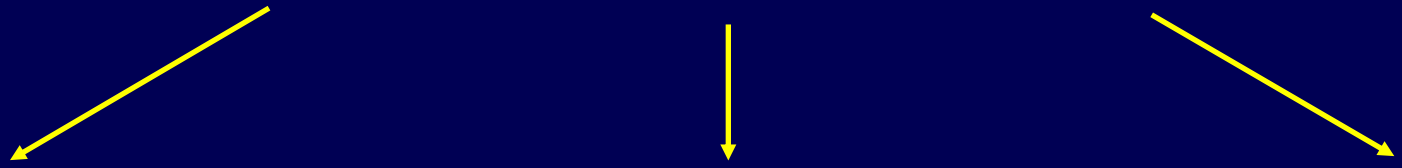


antenatal Bartter-syndrome	classic Bartter- syndrome	Gitelman syndrome
NKCC2 ROMK	ClC-Kb	NCCT
Other ?		

Pathophysiology of antenatal Bartter syndrome

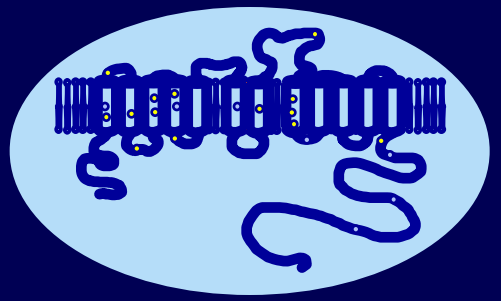
Furosemid	Antenatal BS
mTAL	mTAL
Diuresis ↑	Diuresis ↑
Saliuresis ↑	Saliuresis ↑
Urinary calcium ↑	Urinary calcium ↑
Ototoxicity	Deafness

(*Landau et al*. 1995, *Madrigal et al*. 1997)

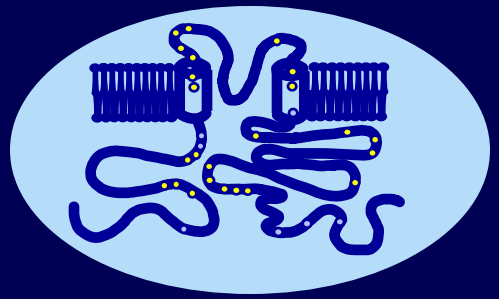
Results of linkage analysis

25
aBS families

8
NKCC2



11
ROMK



6
excluded
?

Clinical characterisation (n=8)

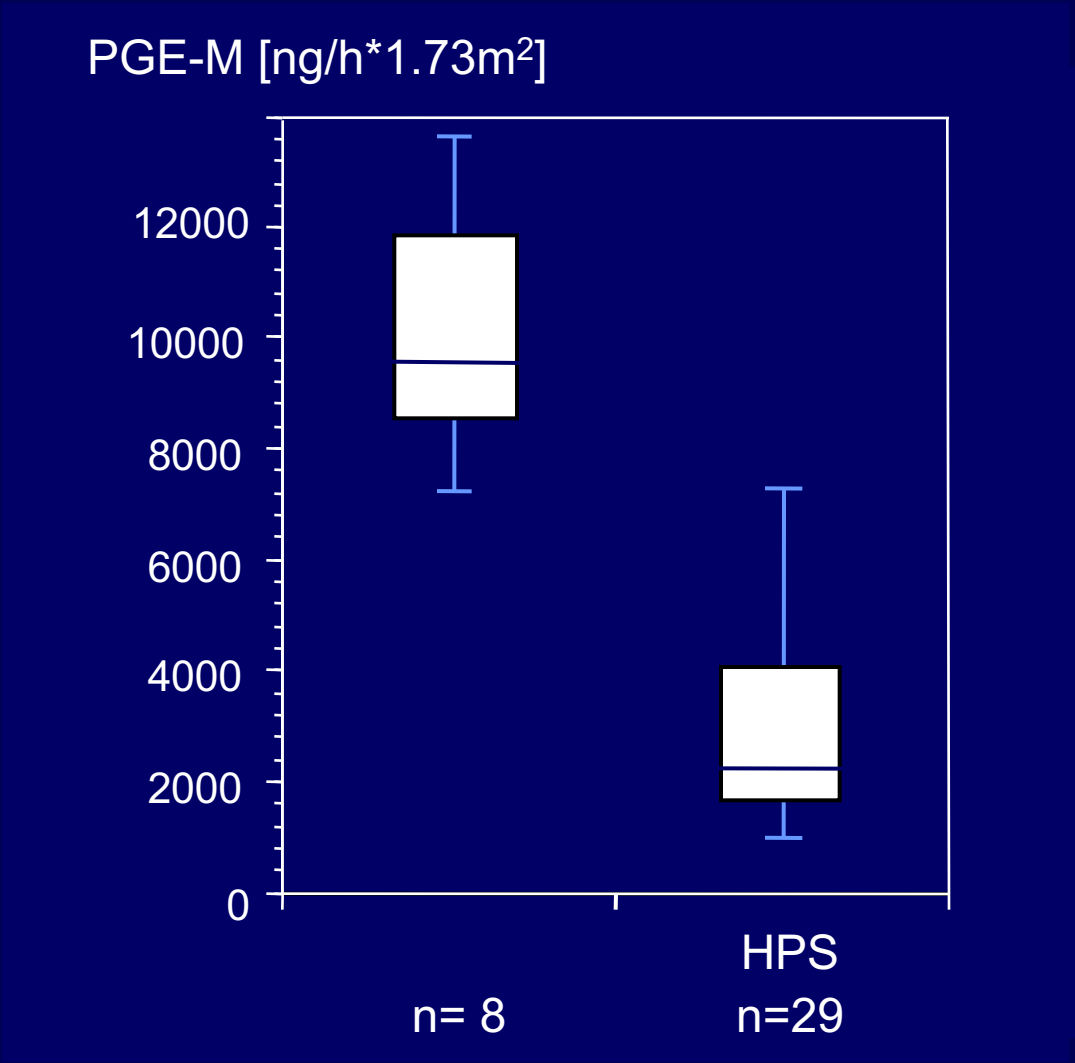
gestational age: 30 weeks (2 weeks ↓)

potassium wasting 5 x ↑

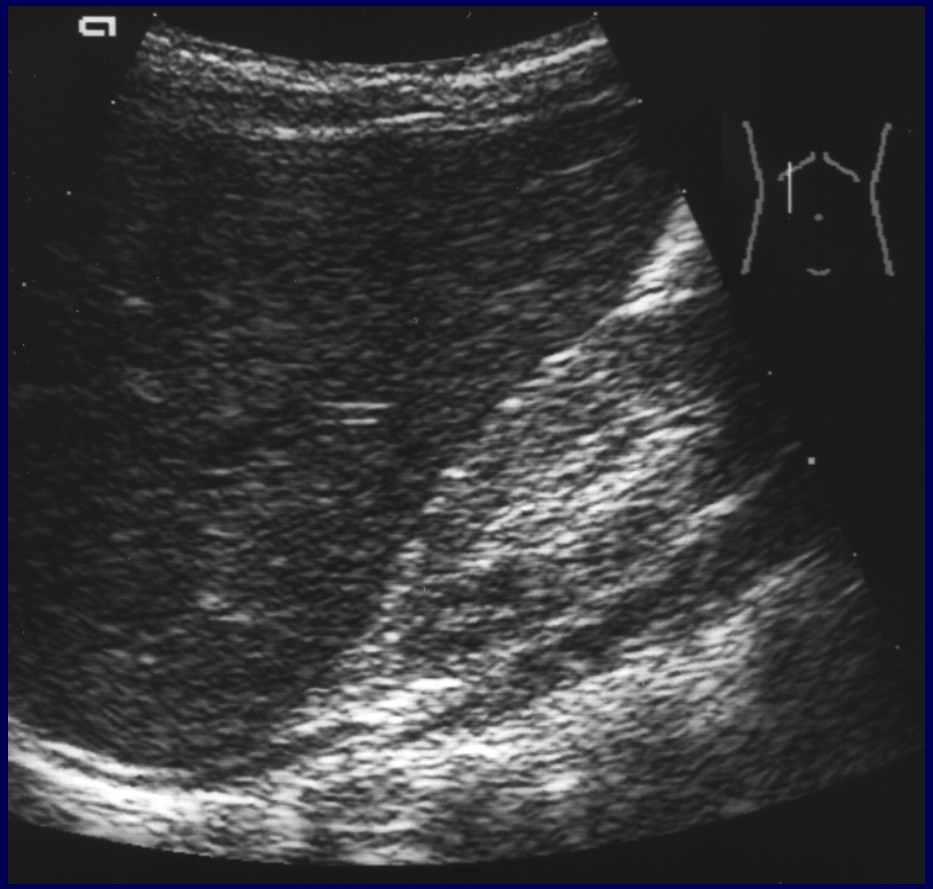
renal PGE2/PGE-M excretion 4 x ↑

inadaequate response to indomethacin

necessitates parenteral fluid supply

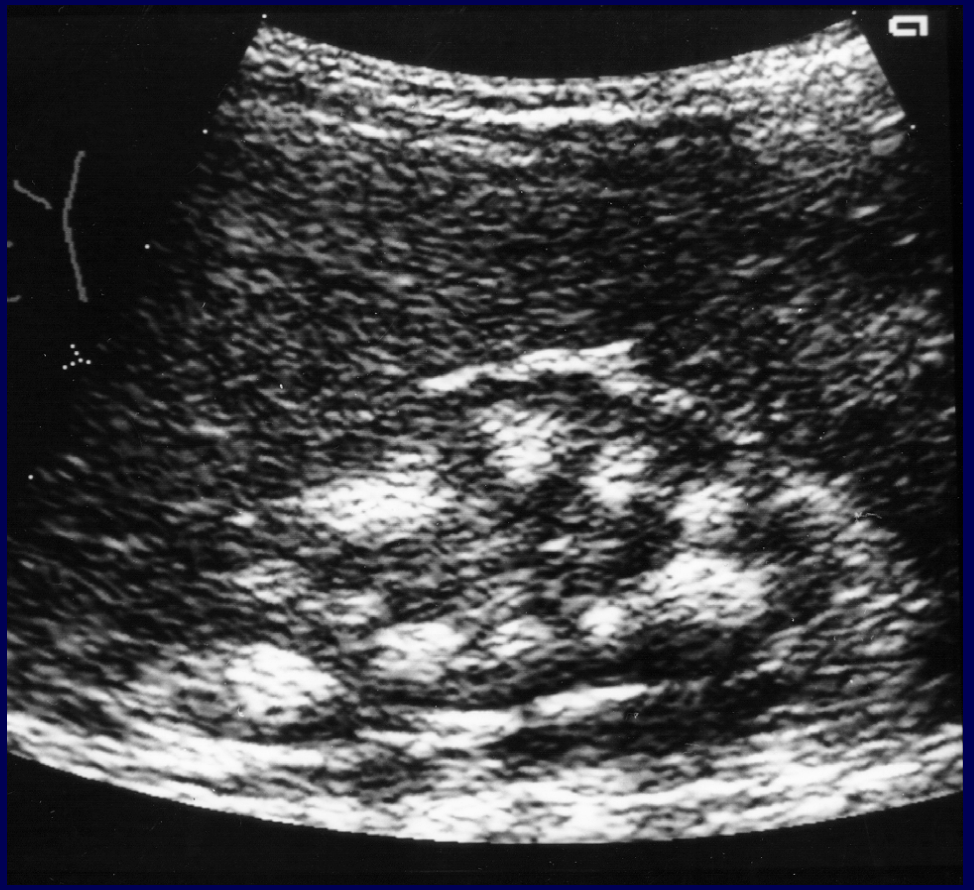


Jeck et al, J Pediatr 2001



1p31

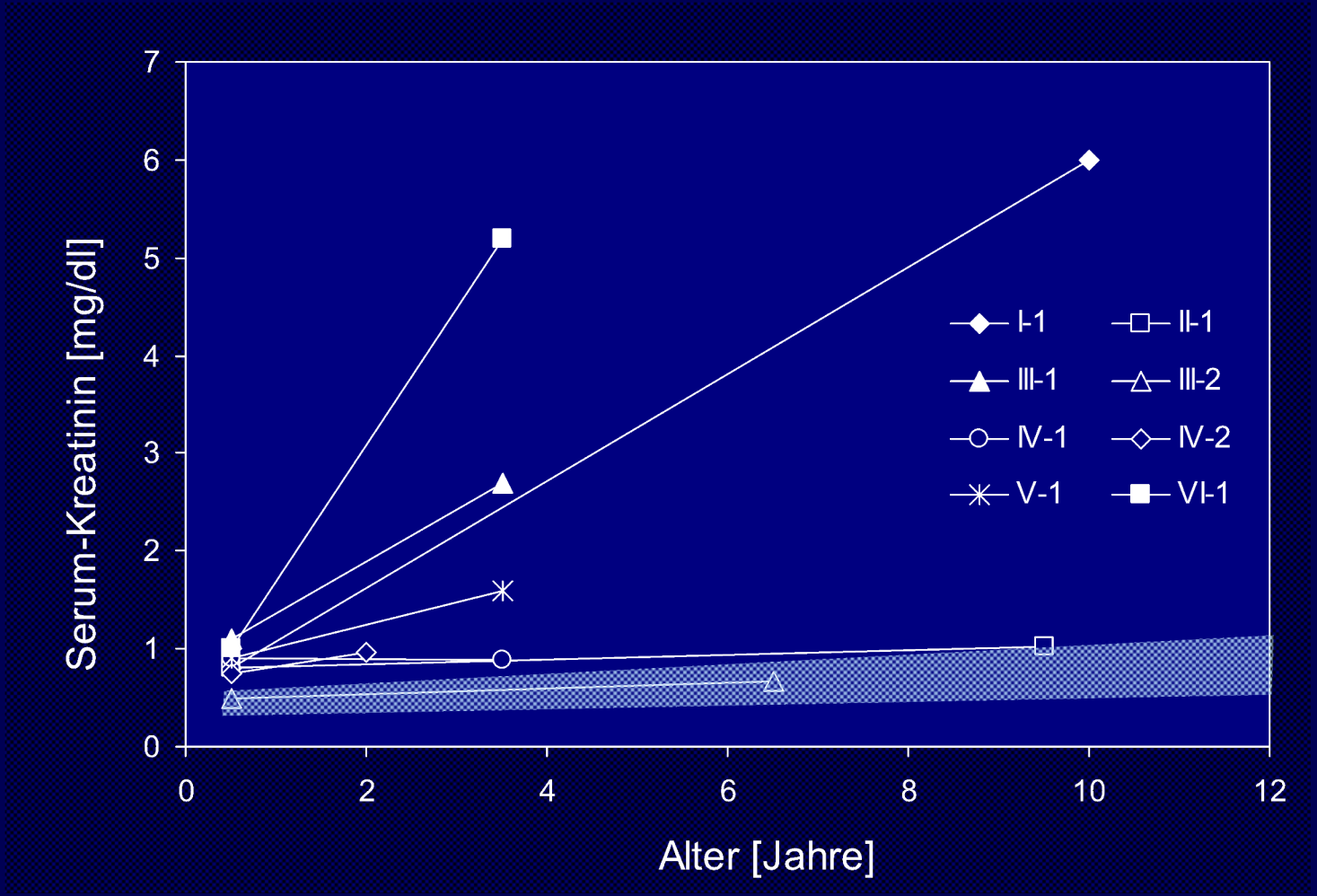
- diffuse hyperechogenicity
- loss of cortico-medullary-differentiation



HPS/aBS

- hyperechogenic pyramids
- nephrocalcinosis type C

Progressive renal failure is common (n=8)



Jeck et al, J Pediatr 2001

Extrarenal Manifestations

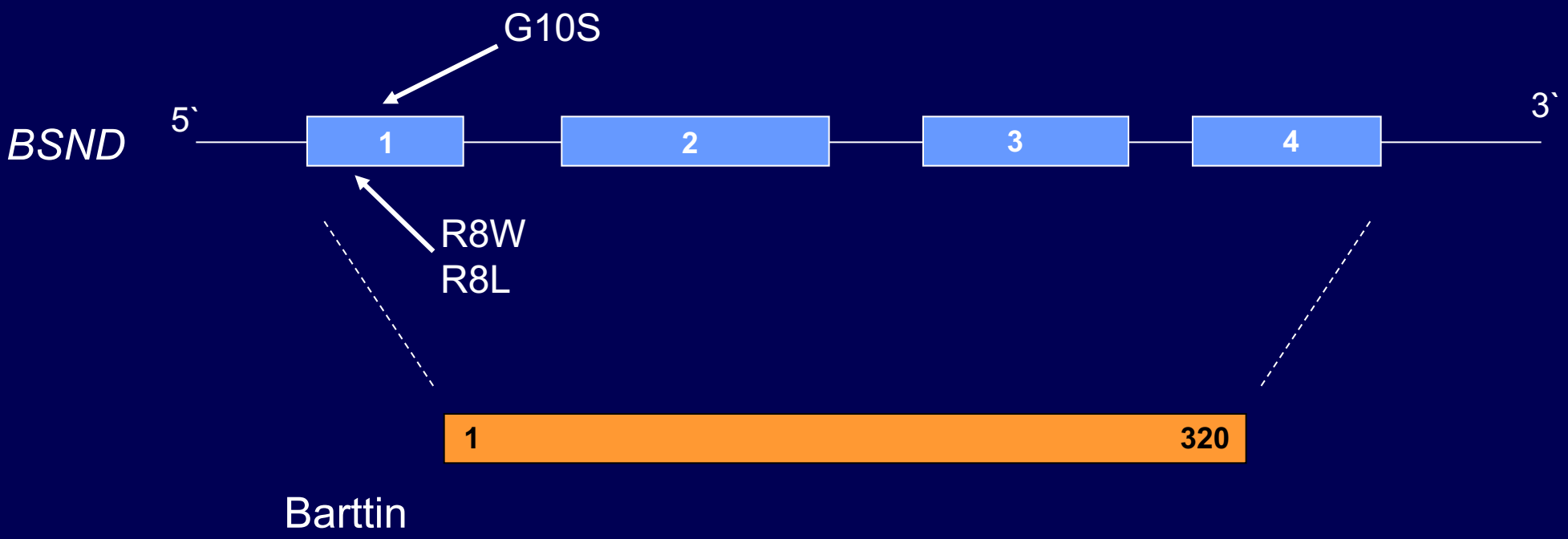
Muscular hypotonia

developmental delay

sensorineural deafness

Jeck et al, J Pediatr 2001

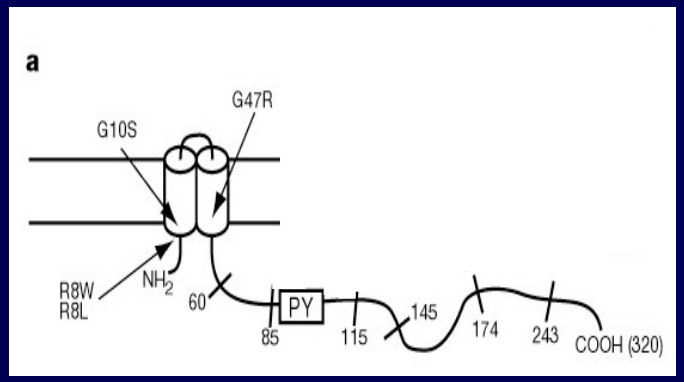
Bartter syndrome with sensorineural deafness is caused by mutations in *BSND* (gene product „Barttin“)



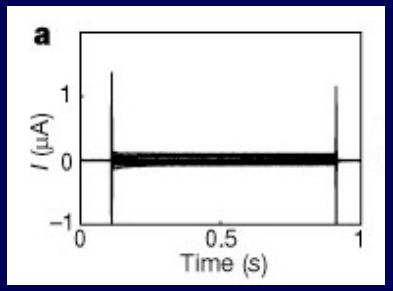
Birkenhäger et al., *Nat Genet* 2001

Barttin is a β-subunit of the kidney chloride channels ClC-Ka and ClC-Kb

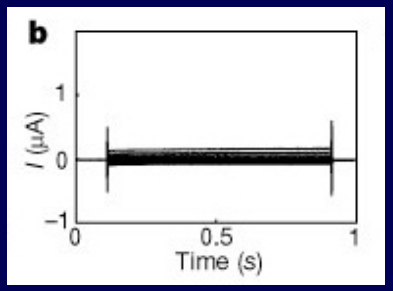
Barttin



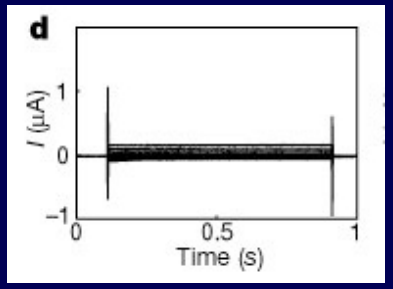
Barttin



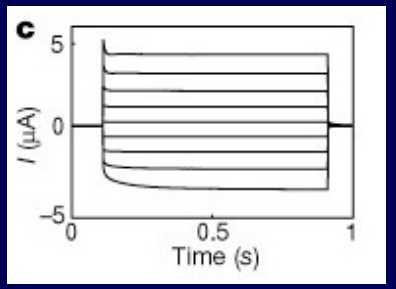
ClC-Ka



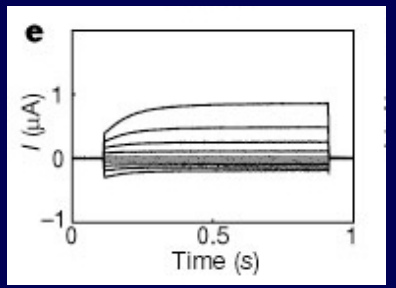
ClC-Kb



ClC-Ka+Barttin



ClC-Kb+Barttin



Estevez et al., *Nature* 2001

Immunolocalisation of Barttin in the distal tubule and the inner ear

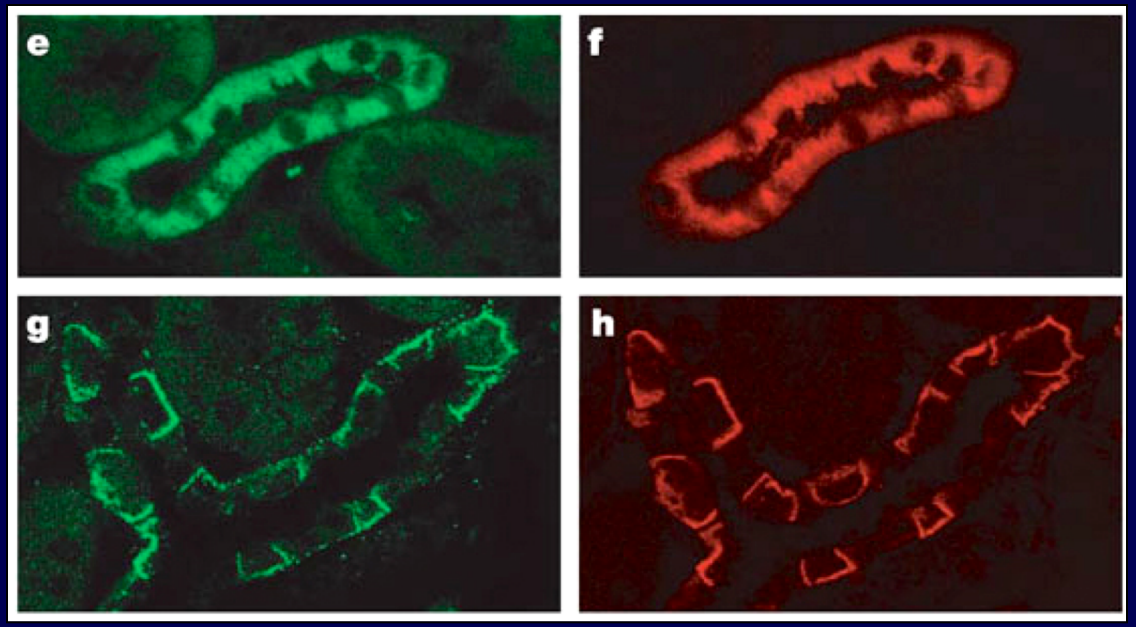
kidney

ClC-Ka

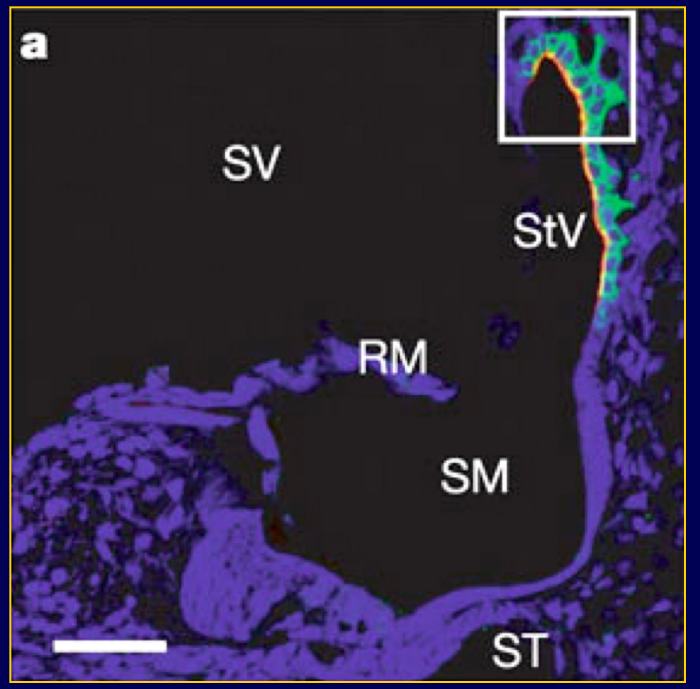
Barttin

mTAL

CCD



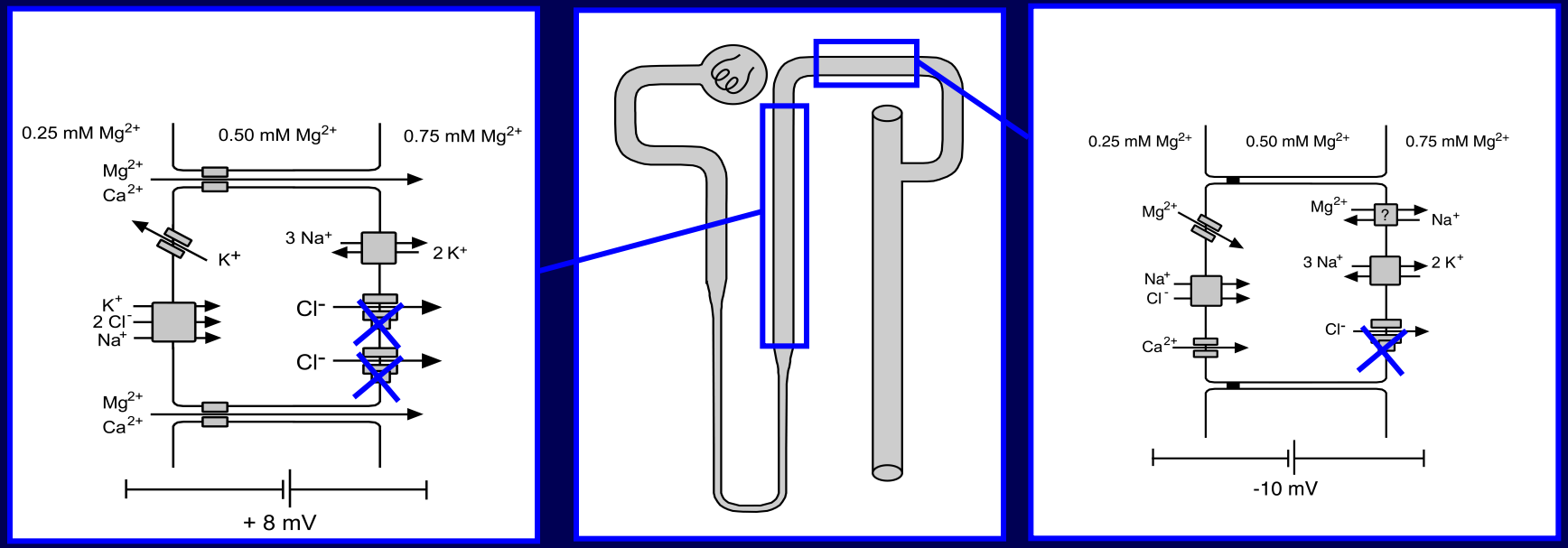
inner ear



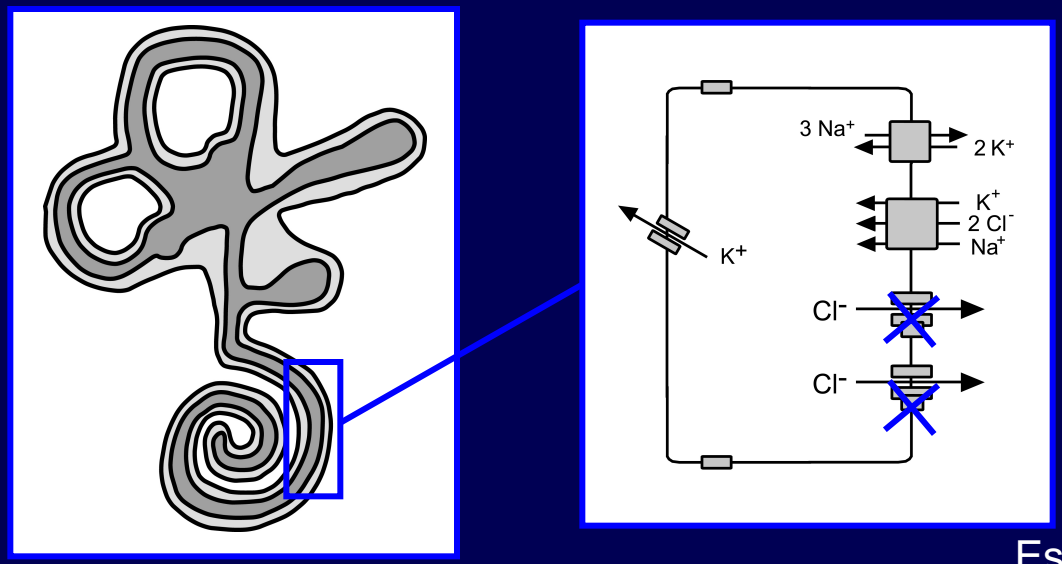
Estevez et al., *Nature* 2001

Pathophysiology of Barttin defects

Kidney



Inner ear



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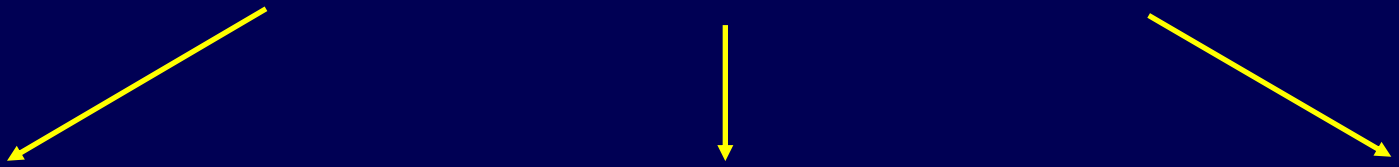
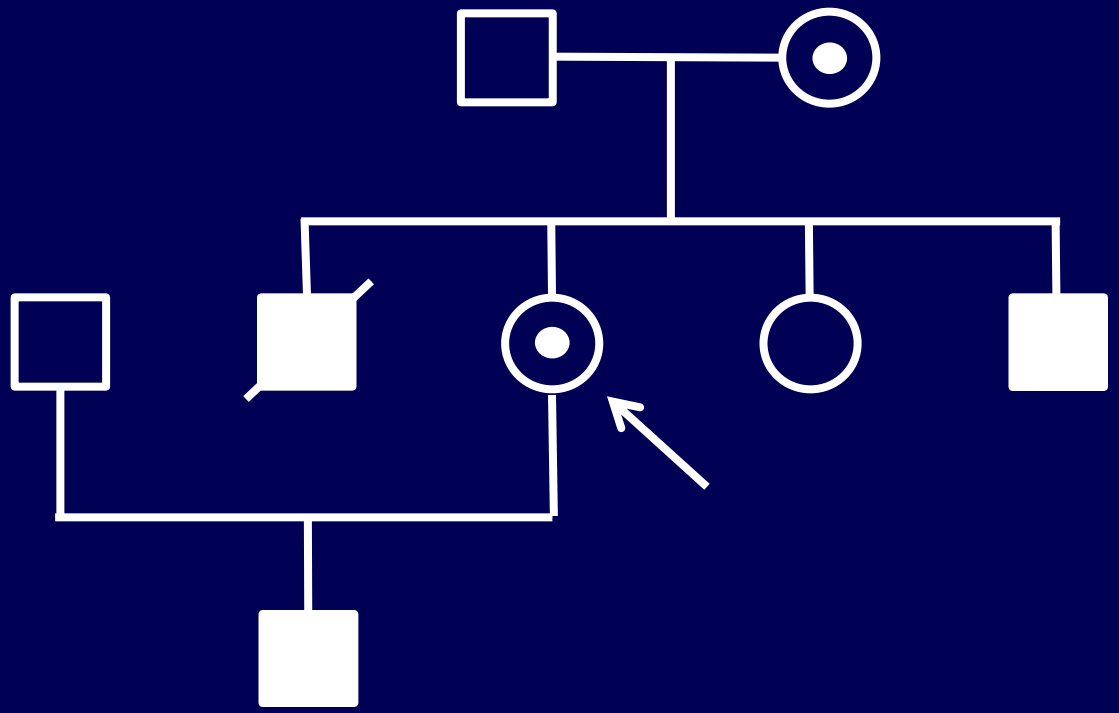
„Bartter-like syndromes“

Set of inherited tubular disorders with hypokalemic metabolic alkalosis



antenatal Bartter-syndrome	classic Bartter- syndrome	Gitelman syndrome
NKCC2 ROMK	ClC-Kb	NCCT
Barttin digenic Ka/Kb defects		
transient aBS ?		

Dutch family with three boys affected by polyhydramnios and polyuria in two



Laghmani et al, NEJM 2016

Whole exome sequencing

premature stop codon (c.1038C→G, p.Y346*)
in *MAGED2* encoding Melanoma associated antigen D2

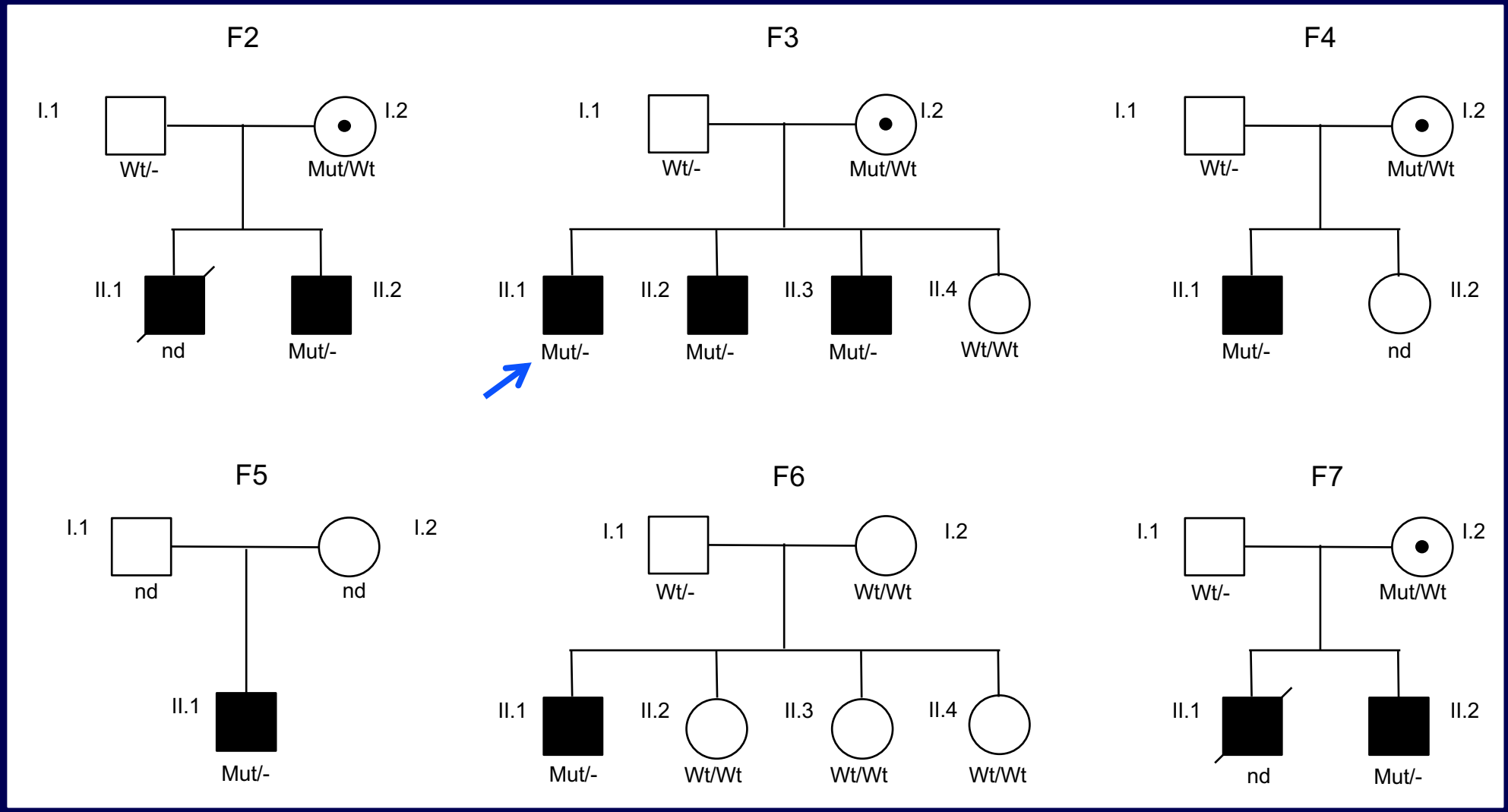
the mutation segregates with the phenotype and female carriers in the index-family

Certain MAGE‘s promote ubiquitination, not shown for MAGED2

Interference with fetal salt and water transport ?

Laghmani et al, NEJM 2016

MAGED2 mutations in six additional families with transient Bartter syndrome



Laghmani et al, NEJM 2016

MAGED2 and acute (recurrent) Polyhydramnios ?

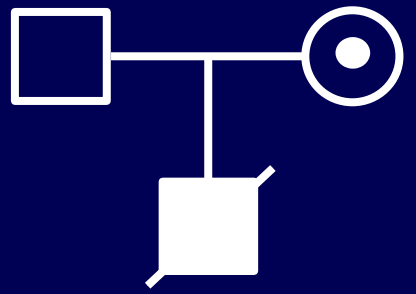
Rare condition affecting male fetuses, first described in 1976 in 3males (Pitkin 1976).

Polyhydramnios in 4 males (Weissman 1987).

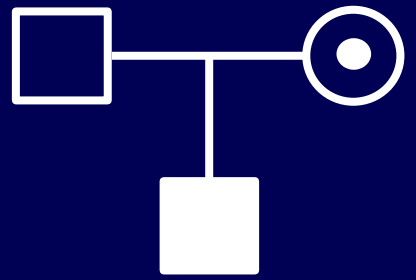
Repeated amniocentesis with NSAIDs may be curative (Rode et al, 2007).

We studied a cohort of 11 women with acute, idiopathic polyhydramnios and male fetuses.

F8

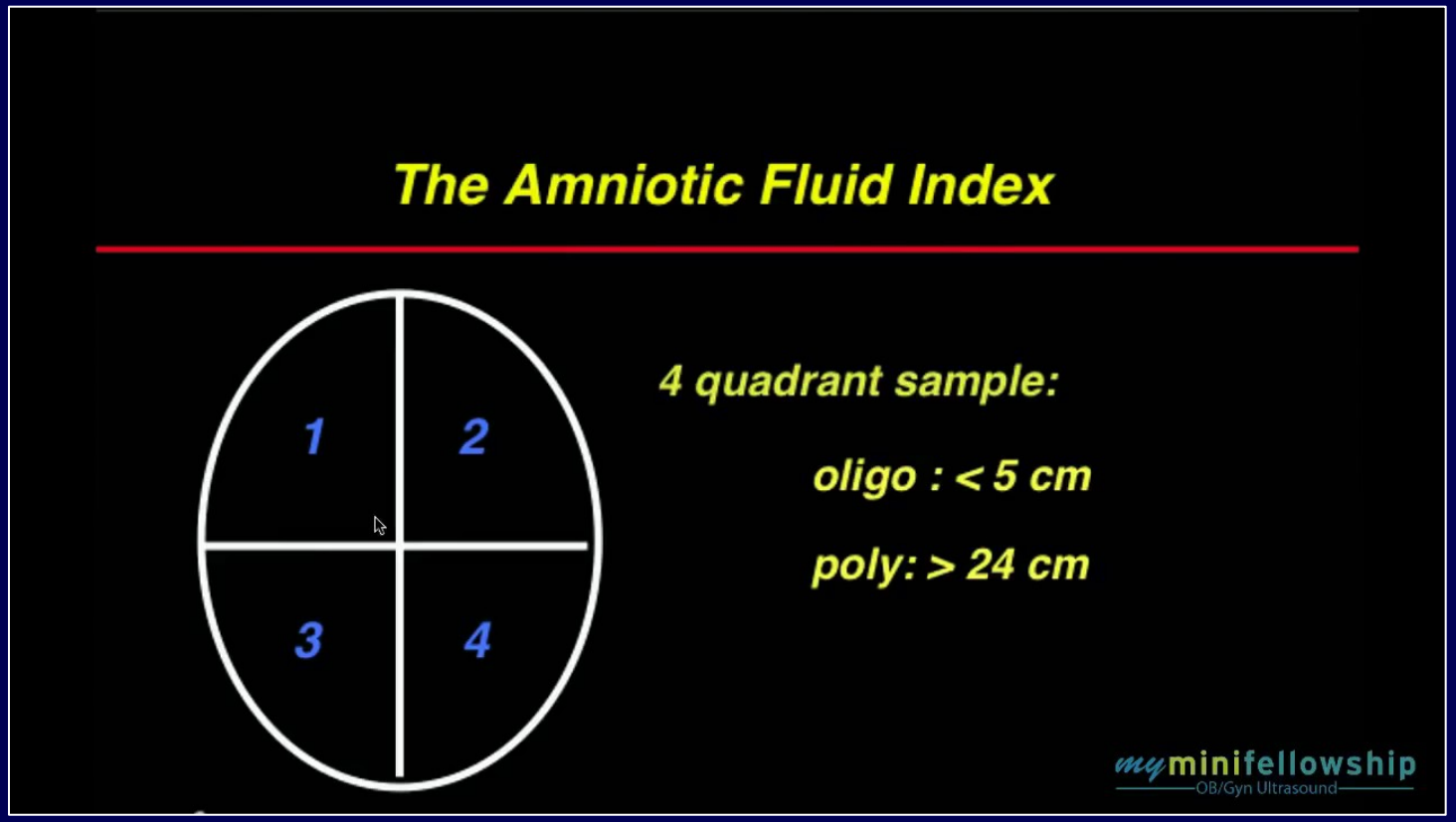


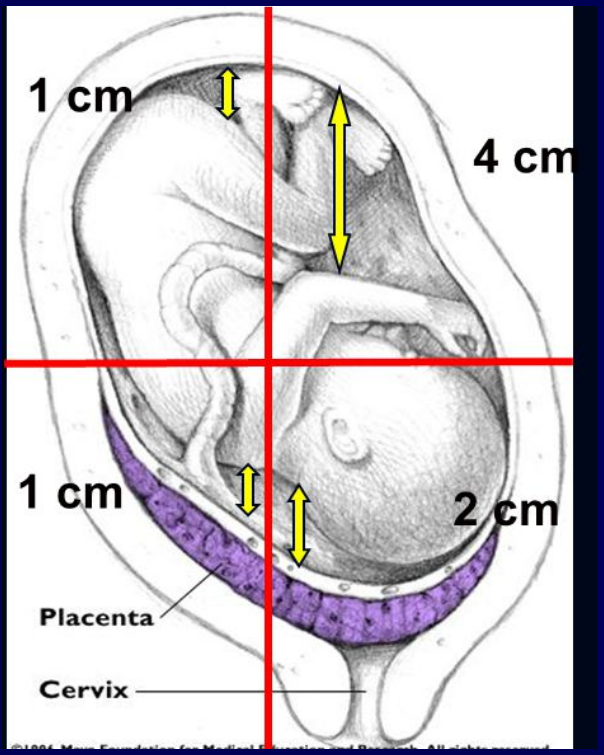
F9



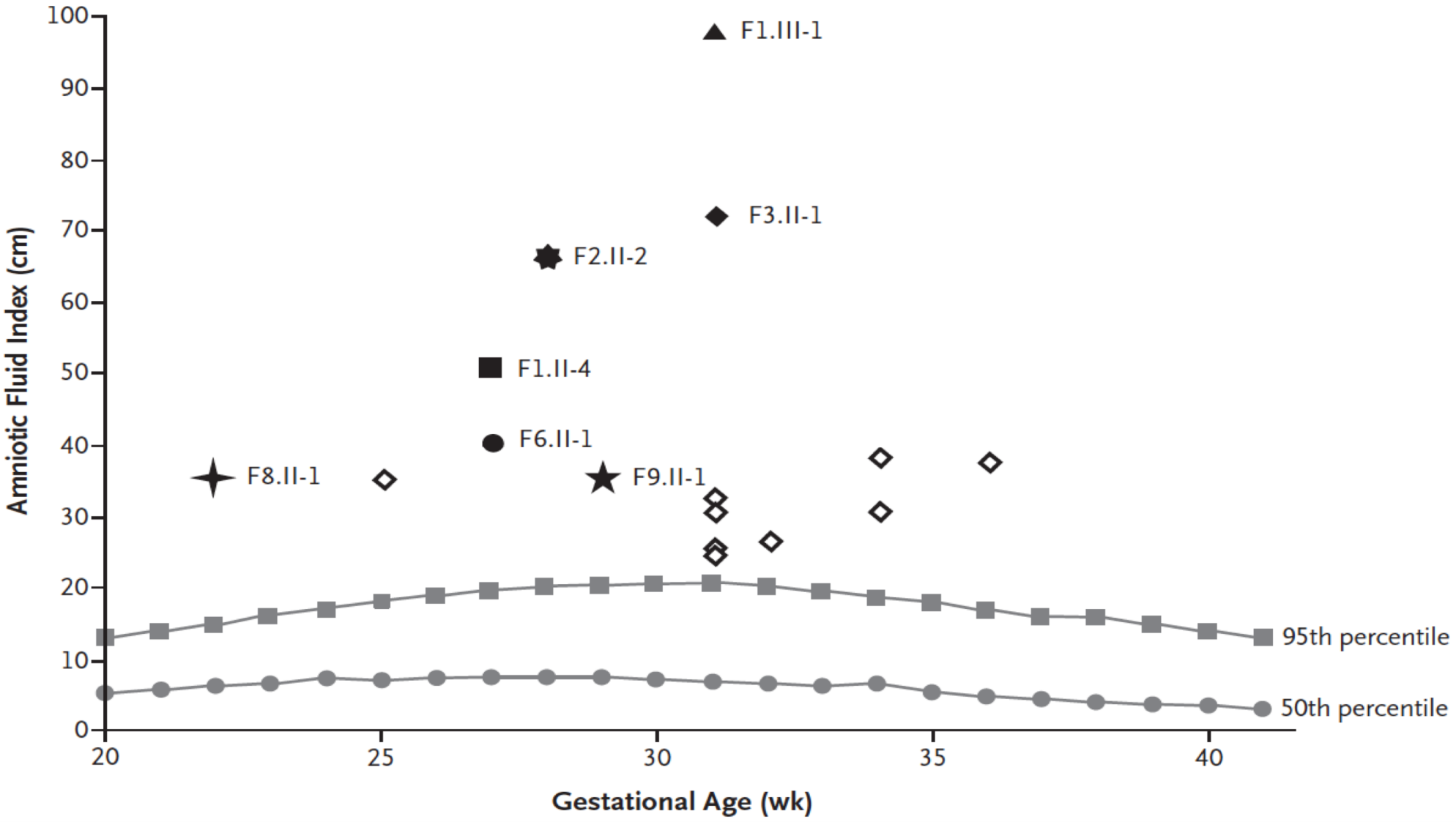
Laghmani et al, NEJM 2016

What is a polyhydramnios by AFI measurement ?





Women with MAGED2 mutations have early & excessive polyhydramnios as shown by the AFI



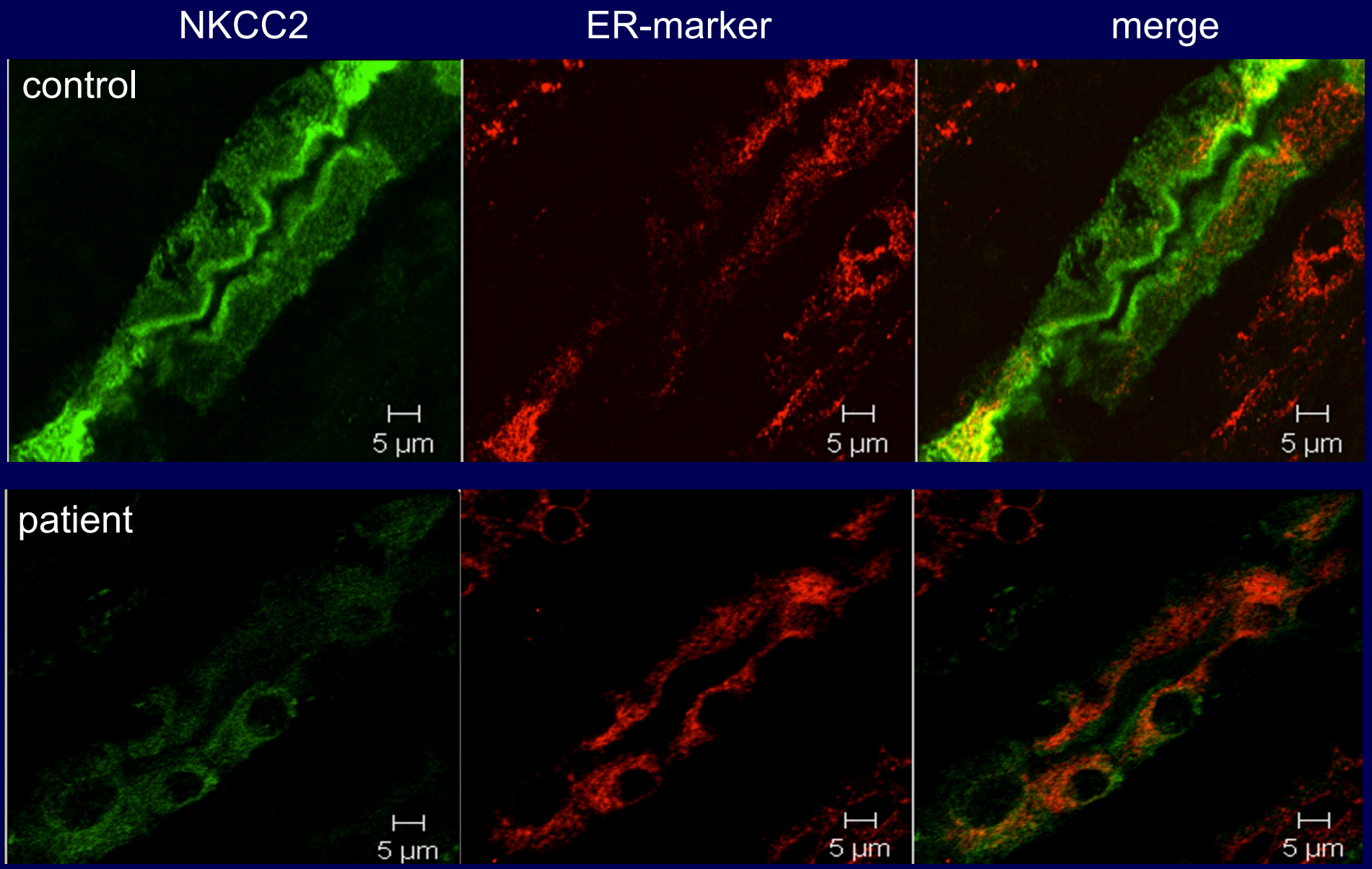
What is the explanation for the phenotype?

MAGE family designated as „tumor antigens“, most data deal with proliferation, apoptosis, etc.

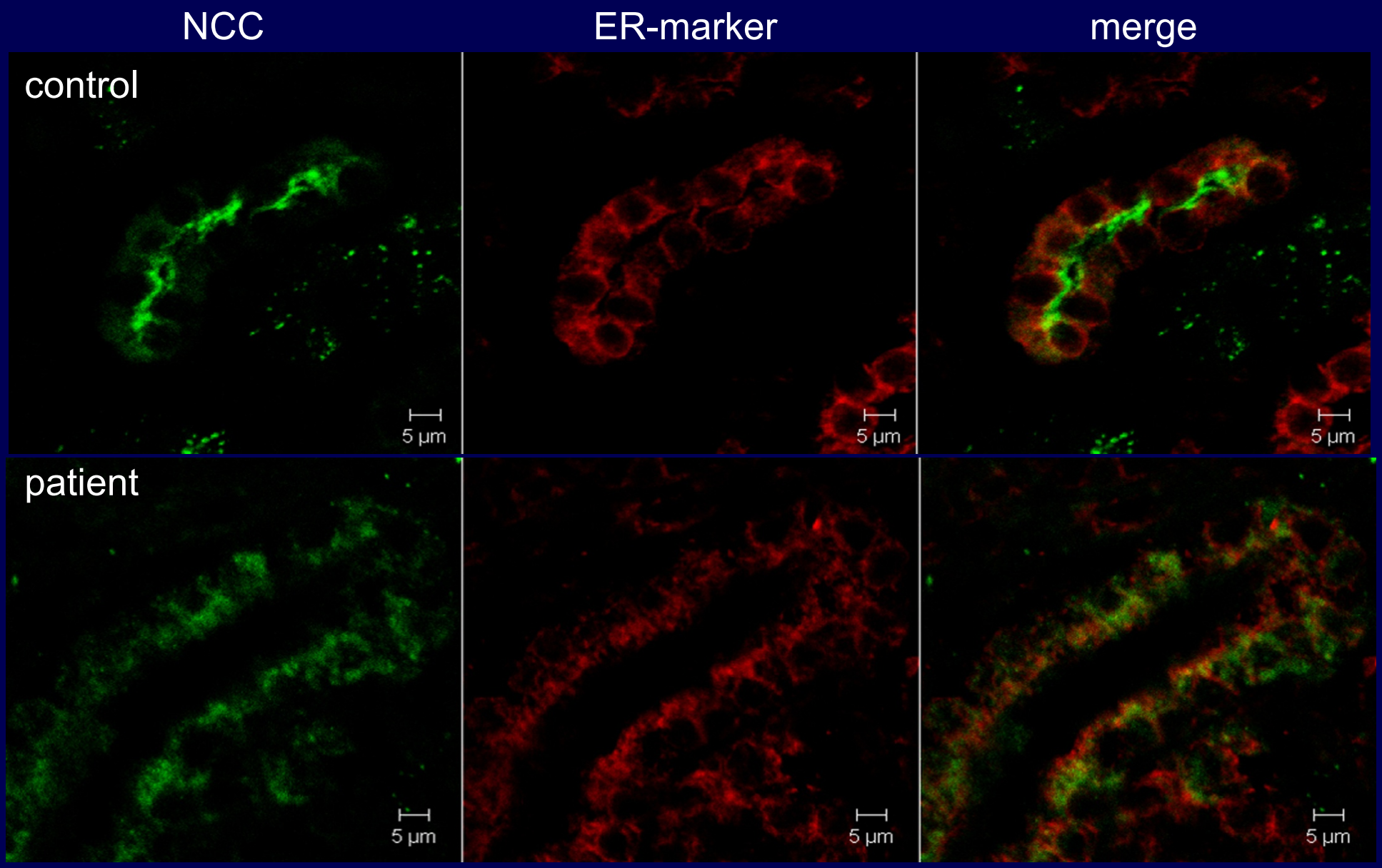
Certain MAGE‘s promote ubiquitination, not shown for MAGED2

Interference with fetal salt and water transport ?

NKCC2 in human fetal control and patient tissue

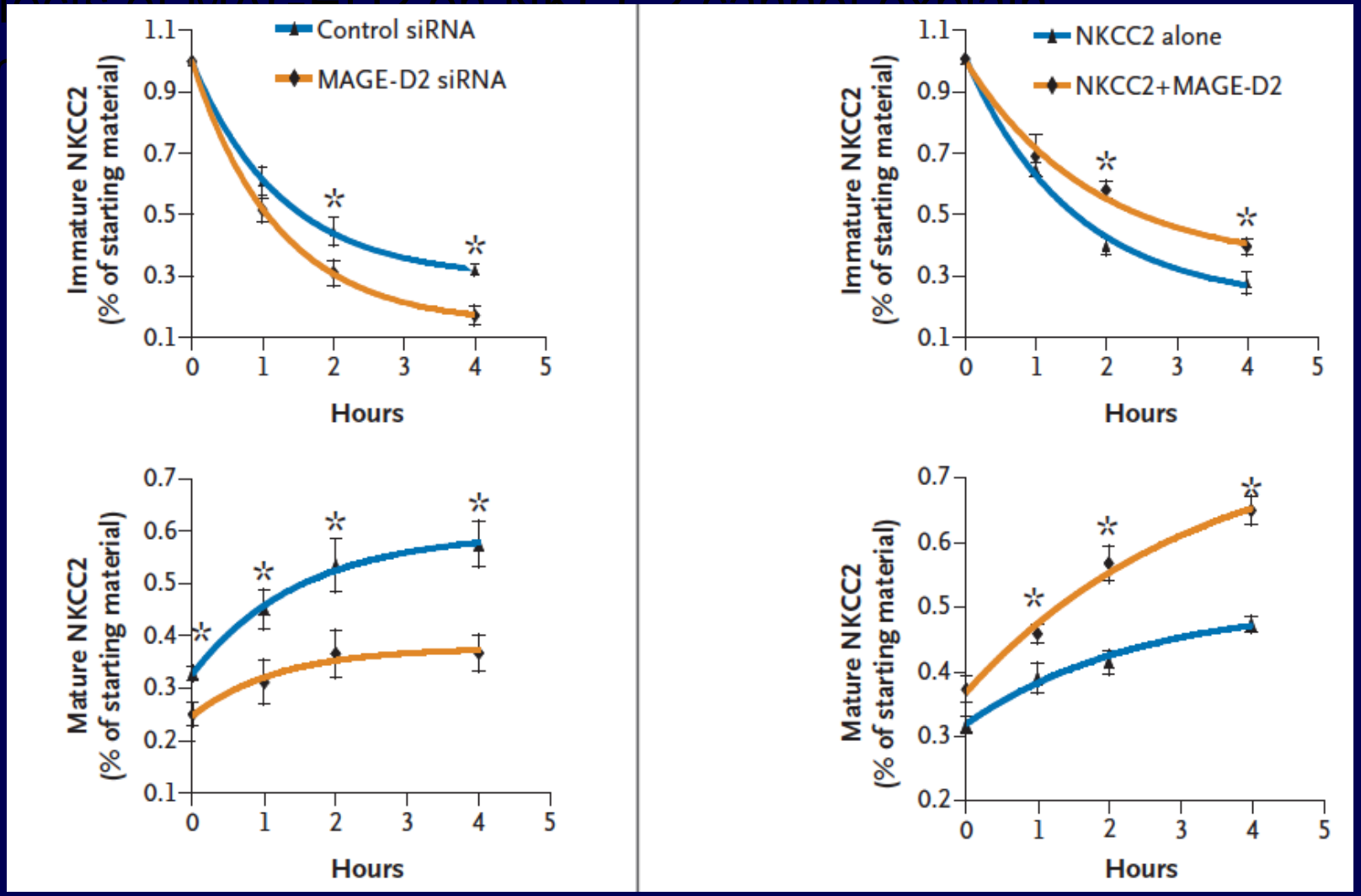


Thiazide-sensitive NCCT in control and patient kidney



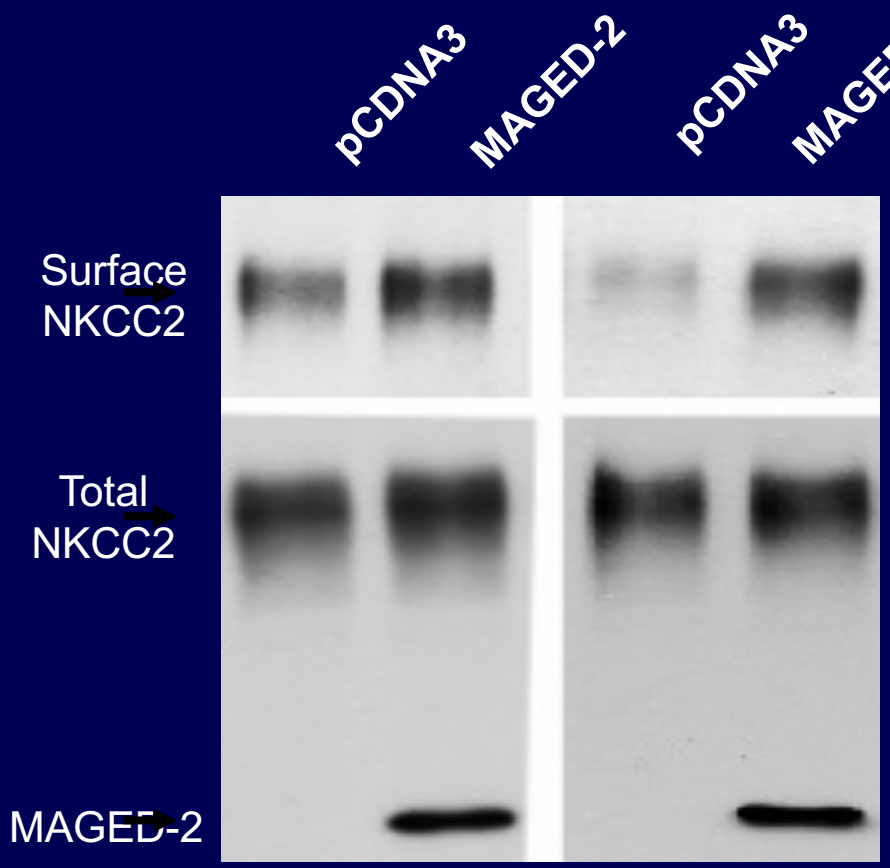
MAGED2 prevents ER-associated degradation of NKCC2

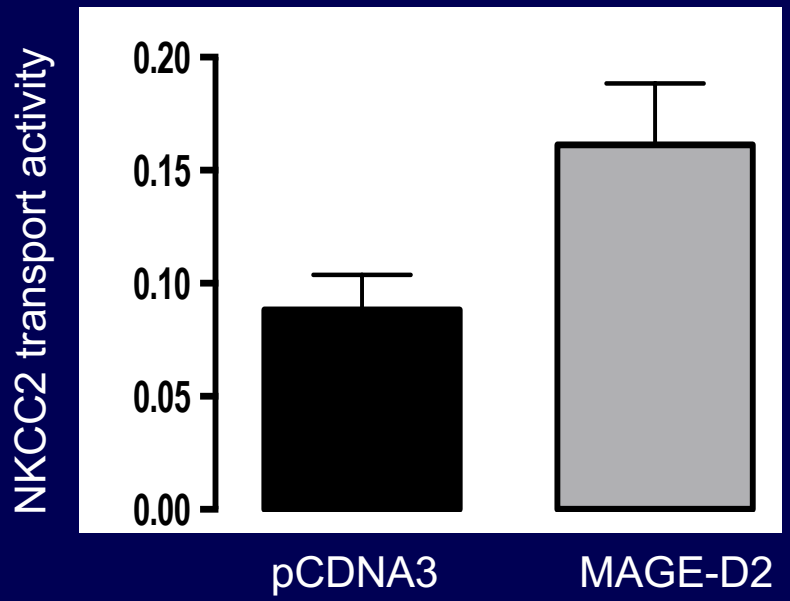
the effects of MAGED2 on NKCC2 cannot explain
the wh



ER stress model: knockdown of MAGED2 impairs the maturation of NKCC2, whereas overexpression accelerated the maturation of NKCC2

MAGED2 upregulates surface expression and activity of NKCC2





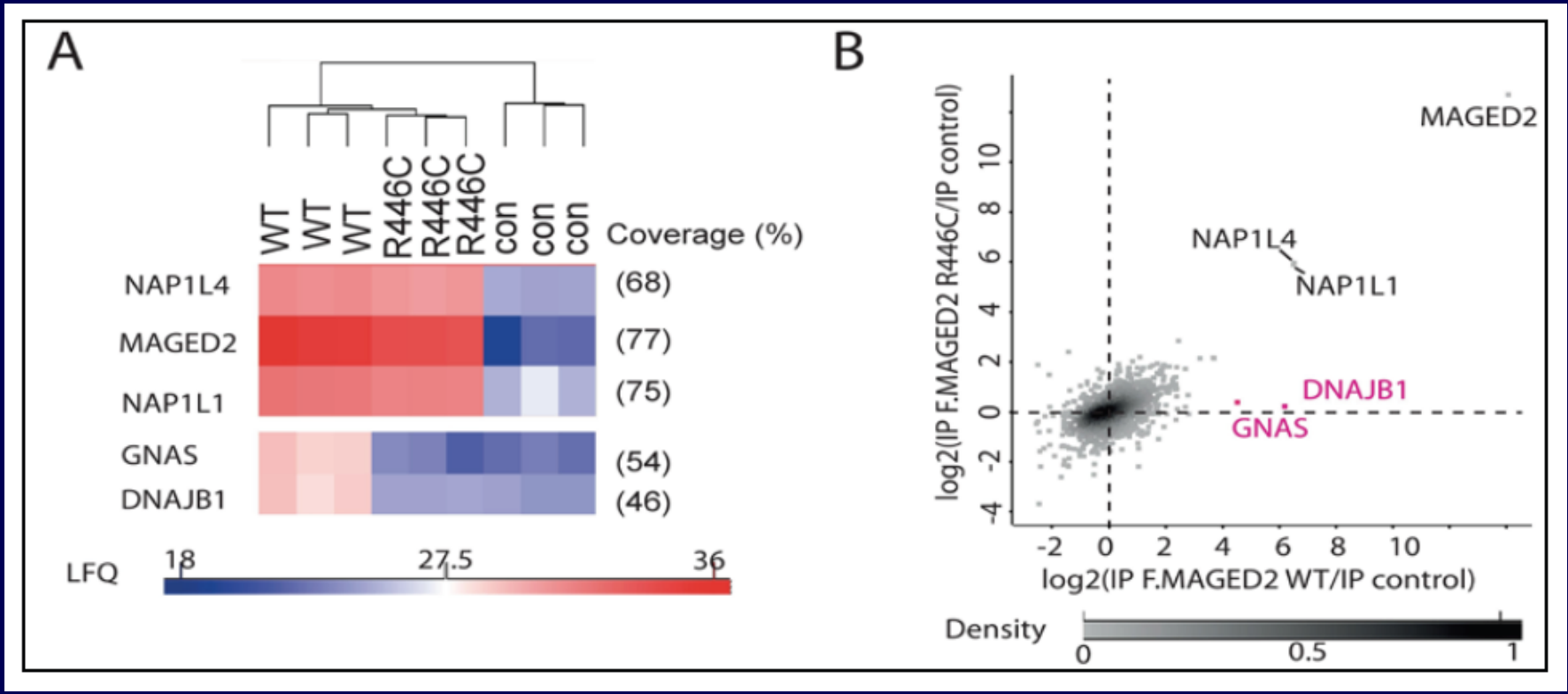
Why is the phenotype transient?

Constitutive renal tubular expression of MAGED2 in fetal and adult tissue cannot explain cessation of symptoms.

Maturation of compensatory mechanisms (reminiscent of transient nature of hyperkalemia in aBS due to ROMK defects?

Anything else?

Determination of the MAGE-D2 Interactome



MAGE-D2 WT vs R446C: Gs-alpha (GNAS) and Hsp40 (DNAJB1), Interaction was confirmed in HEK cells by CoIP.

What is the role for Hsp40 and GNAS?

Hsp40 is a cytoplasmatic chaperone known to interact with NCC (and NKCC2).

Gs-alpha is activated by G protein-coupled receptors and promotes generation of cAMP by activating adenylate cyclase.

Loss of GNAS reduces the expression of NKCC2.

cAMP is a key regulator of NKCC2 surface expression and enhances the activity of NCC.

Why is it a transient phenotype?

The interaction with Hsp40 may prevent NKCC2 and NCCT from stress-induced ER-associated degradation. Stress may result from tissue hypoxia (especially renal cortex) during early pregnancy.

The interaction with Gs-alpha points to developmental changes in the sensitivity of adenylate cyclase to vasopressin (evidence from animal studies).

But, to be honest, we do not know yet.....

New Data in French Bartter Cohort

MAGED2 mutation in 16 / 171 families (9 %).

In 44 % of male patients without mutation in another BS gene.

2 females affected, partly explained by selective X-inactivation.

High birth weight and length, even macrosomia is frequent.

Legrand et al, CJASN 2017

Summary MAGED2 in transient aBS

MAGED2 mutations cause X-linked transient antenatal Bartter syndrome characterized by acute, early and severe polyhydramnios with prematurity and high perinatal mortality.

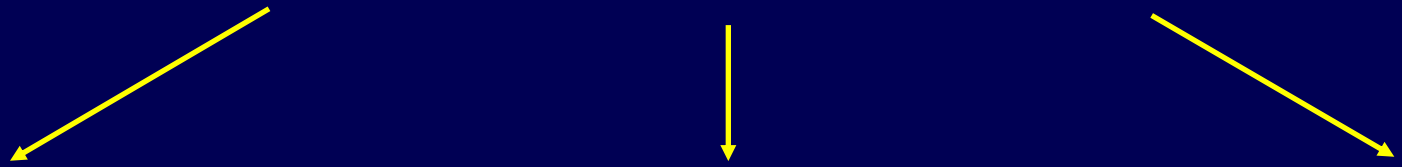
MAGED2 mutations were also detected in acute (recurrent) polyhydramnios.

In tBS, symptoms usually resolve before 35 weeks of gestation.

Even if it is rare, X-linked transient aBS is an important differential diagnosis to avoid unnecessary therapy beyond early infancy and because of the implications for genetic counselling.

„Bartter-like syndromes“

Set of inherited tubular disorders with hypokalemic metabolic alkalosis



antenatal Bartter-syndrome

NKCC2
ROMK
Barttin
combined Ka/Kb

X-linked transient aBS
MAGED2

classic Bartter- syndrome

ClC-Kb

Gitelman syndrome

NCCT

Thank You !