La recherche translationnelle

A l'Institut des maladies du métabolisme et du cardiovasculaire
INSERM UMR 1048, RF Lab

- L'exemple de la Néphrologie -

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Equipe de Néphrologie Médecine Interne Rhumatologie Pédiatrique,
CDR SORARE
Hôpital des Enfants

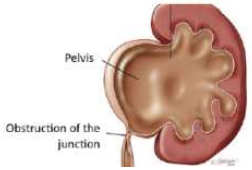
Centre de référence Sud Ouest
SORARE
Maladies RénAles RarEs

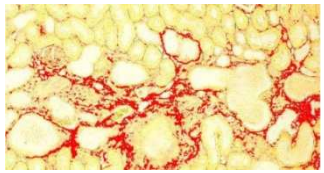
Hôpitaux de Toulouse

R F lab

Une pathologie - 2 équipes (2003)

Inserm

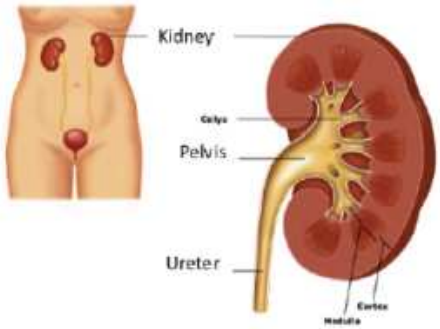




Fibrose

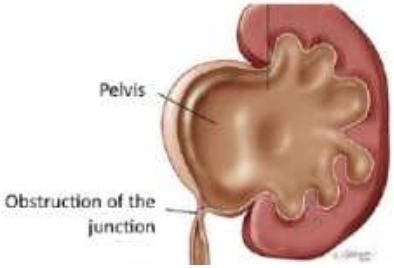
bradykinine, chémokine, arginase

Syndrome de la jonction pyéloureterale



Obstructed Kidney

Pathological dilatation (urine accumulation)



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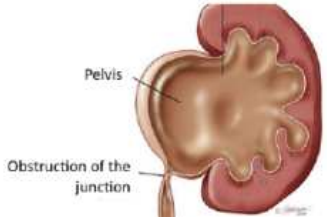
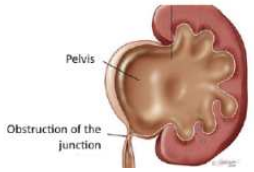
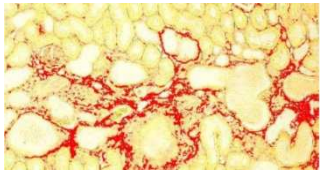


Progression?

Une pathologie - 2 équipes (2003)

Inserm





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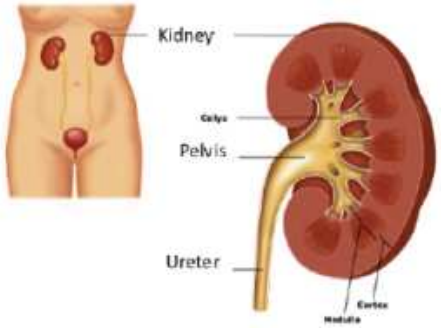

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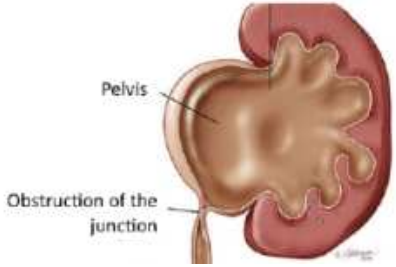

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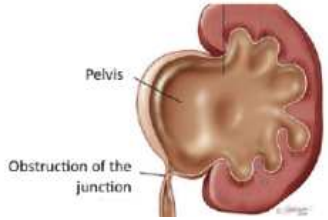
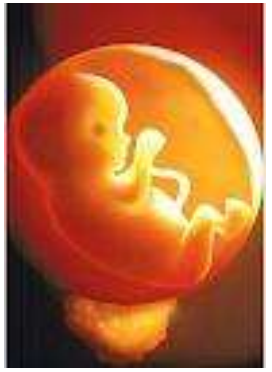


Progression?

Rares Diseases

Congenital anomalies of the kidney and the urinary tract (CAKUT)



- Accounts for >50% of chronic kidney disease (CKD) in children! (< 0.5% in adults)

- Hyperechogenic Kidneys

- Obstructive nephropathies (the most common cause of CAKUT).

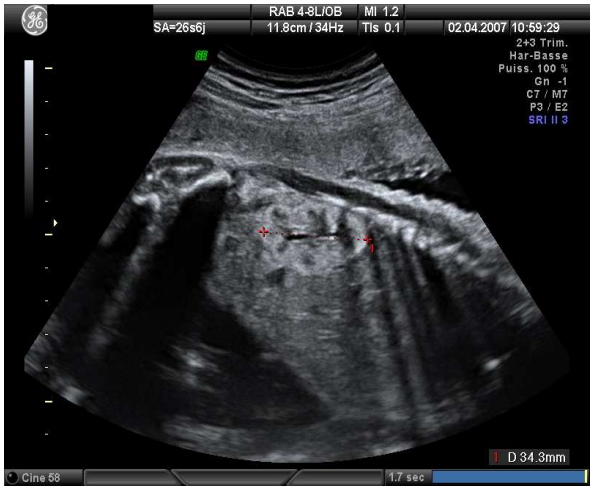
- Consultation structurée : # 500

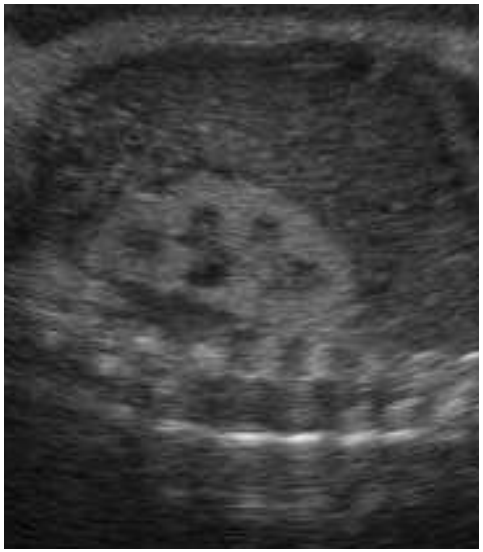
- Projet ADARAN 

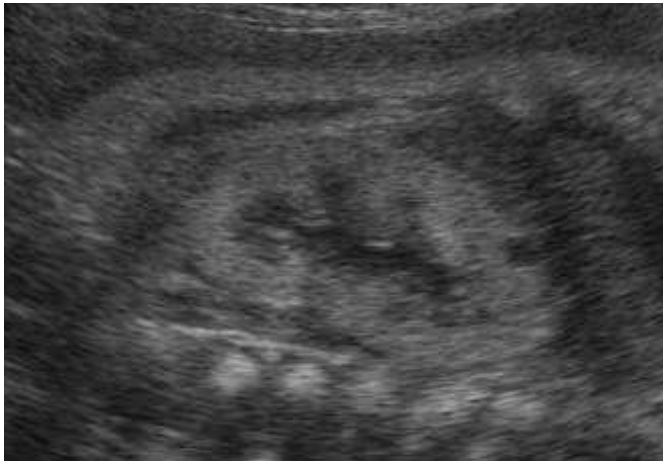
Annonce Diagnostique d'une Anomalie Rénale Anté Natale

Fondation Maladies Rares *2013*

Reins Hyperéchogènes







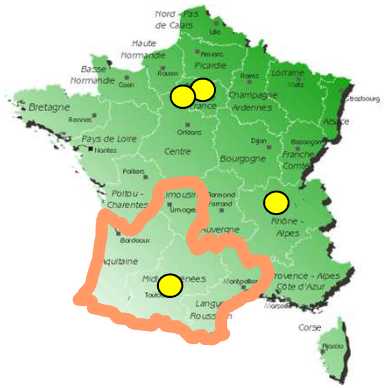
- **377** patients avec une anomalie du développement rénal

  - Mutation de *HNF1B* : 75 patients (19.9%)

  - Les anomalies de *HNF1B* sont la 1^{e} cause de reins fœtaux hyperéchogènes de taille normale ou discrètement augmentée, 34/55 (62%)

- **200** patients avec une anomalie de HNF1β/TCF2

Suivi centralisé au sein du CDR



Études fondamentales
D Chauveau, S Faguer
JL Bascands

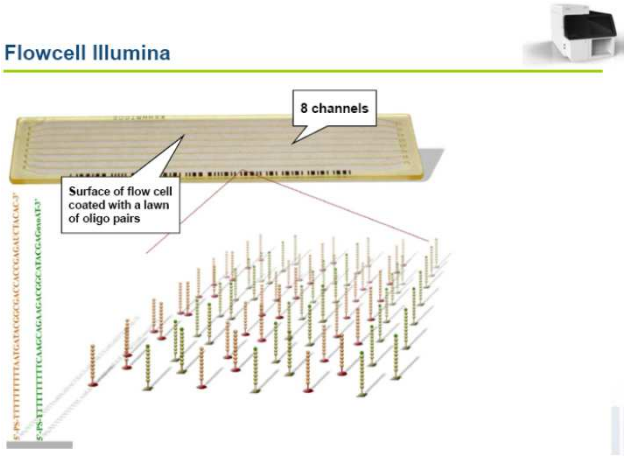
Cohorte RaDiCo

Decramer S et al J Am Soc Nephrol. 2007; 18:923-33

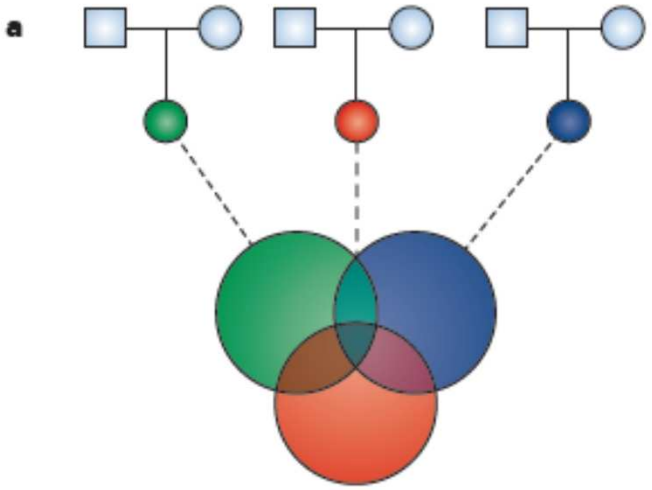
L Heidet et S Decramer. CJASN, 2010

Les reins hyperéchogènes non HNF1β

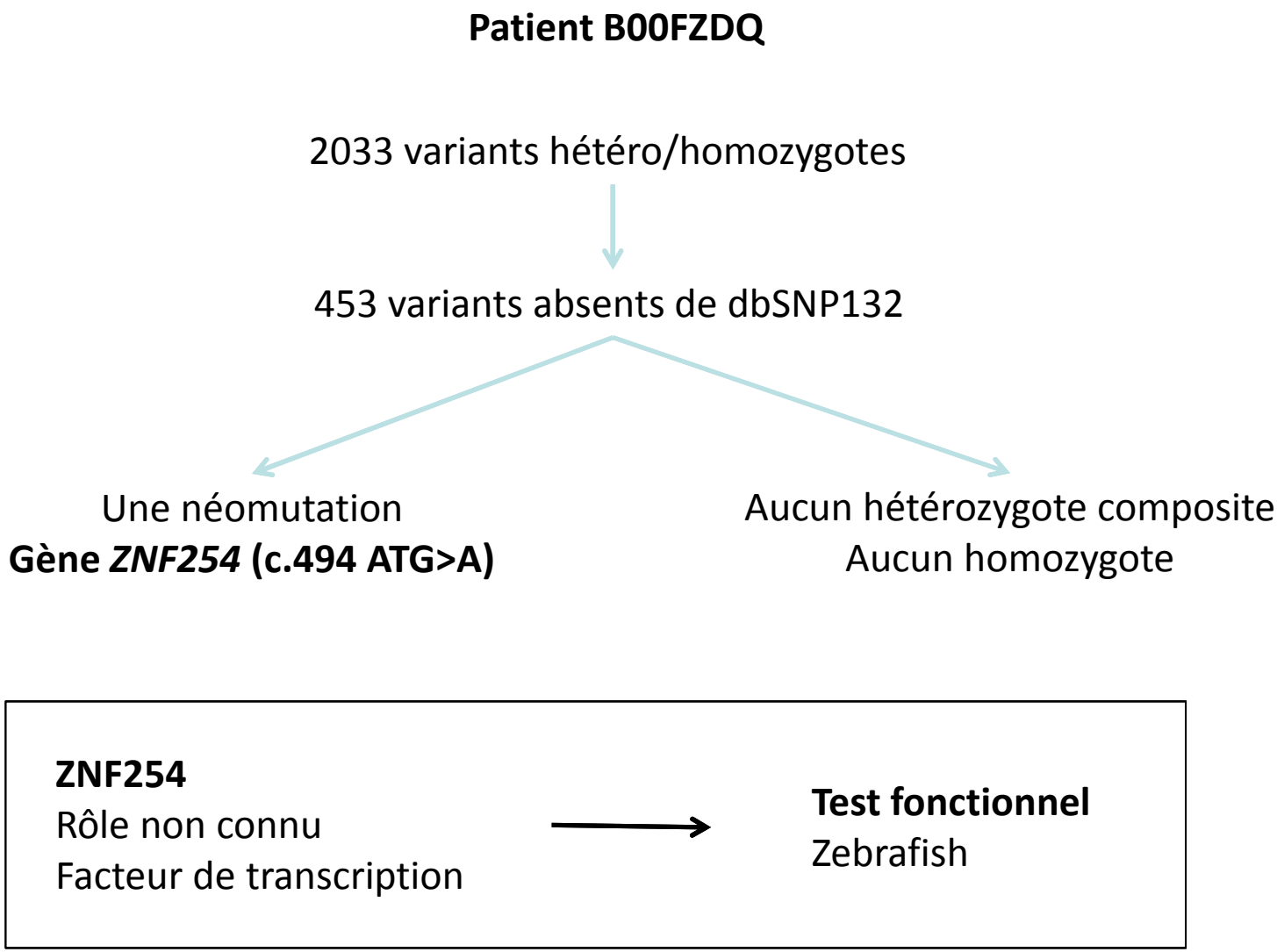
Séquençage haut débit
Ex / Seq



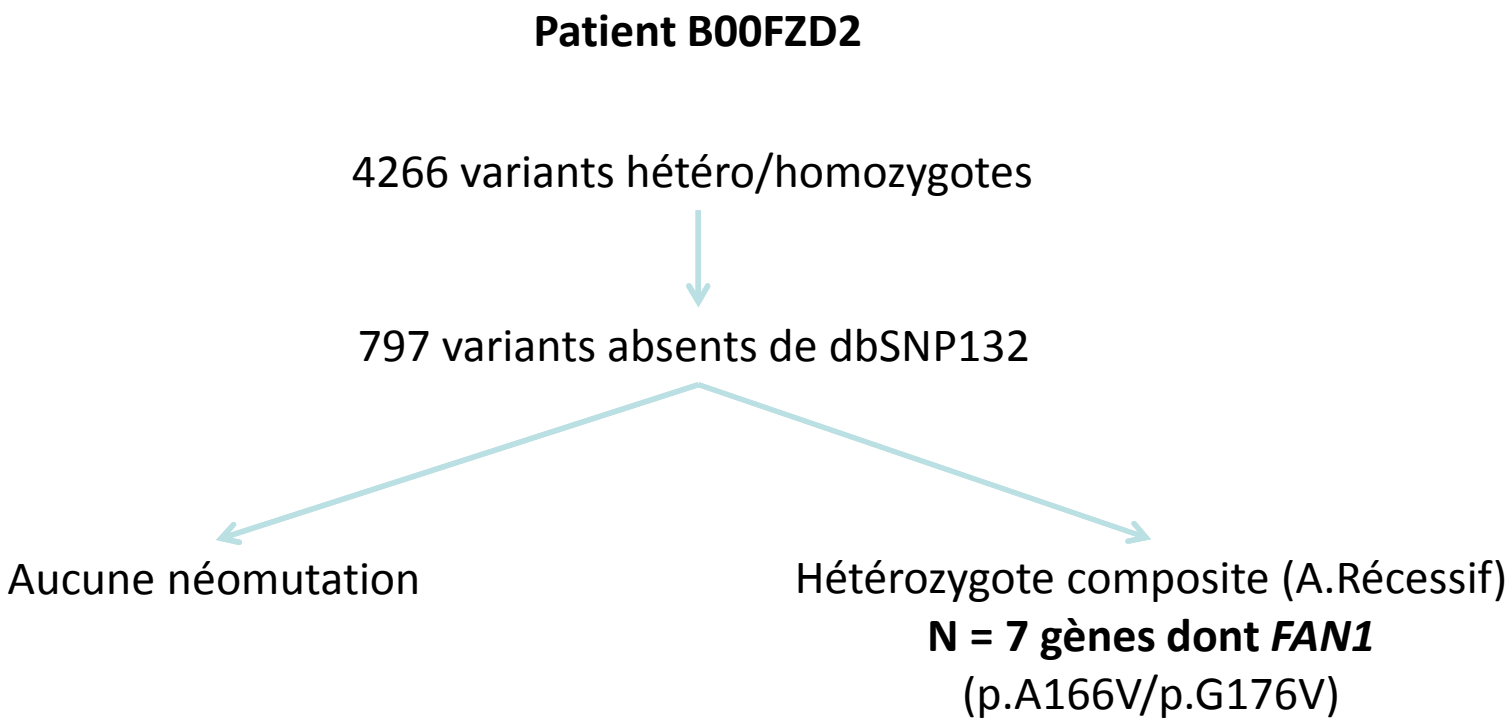
15 trios
Fondation Maladies Rares 2012



Trio 1


Patient B00FZDQ
2033 variants hétéro/homozygotes
453 variants absents de dbSNP132
Une néomutation
Gène ZNF254 (c.494 ATG>A)
Aucun hétérozygote composite
Aucun homozygote
ZNF254
Rôle non connu
Facteur de transcription
Test fonctionnel
Zebrafish

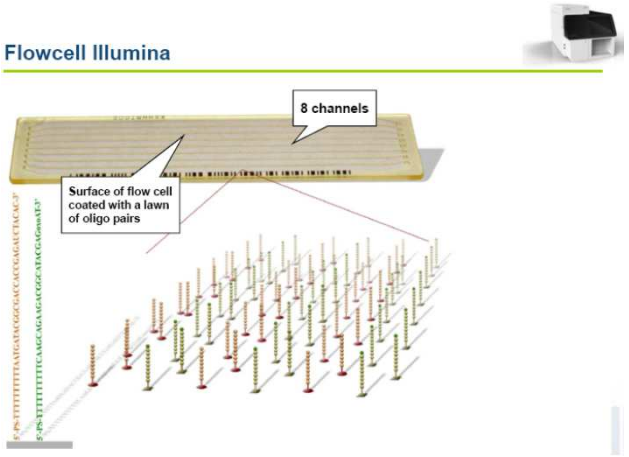
Trio 2



Nat Genet. 2012 Jul 8;44(8):910-5.
FAN1 mutations cause karyomegalic interstitial nephritis, linking chronic kidney failure to defective DNA damage repair.

Les reins hyperéchogènes non HNF1β

Séquençage haut débit
Ex / Seq



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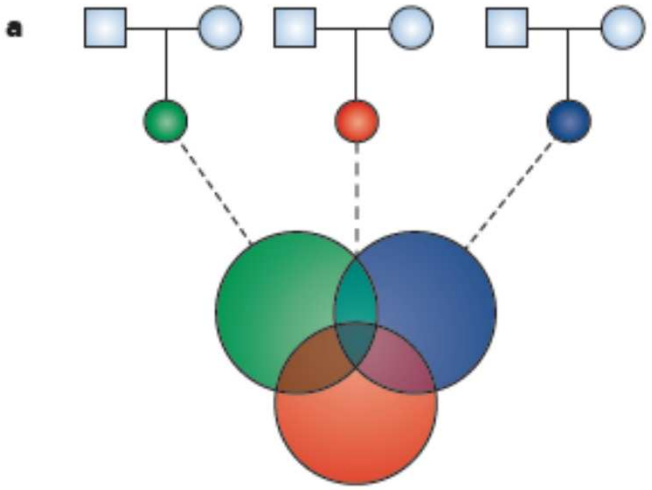
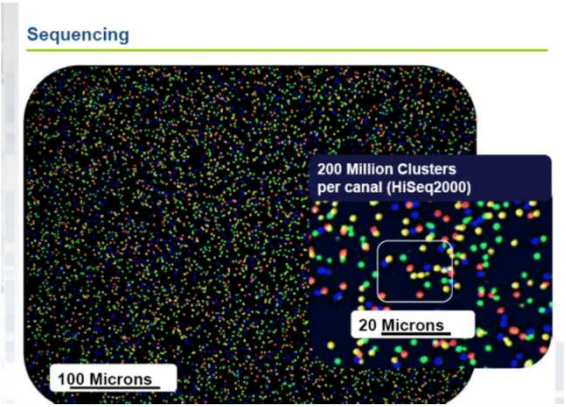
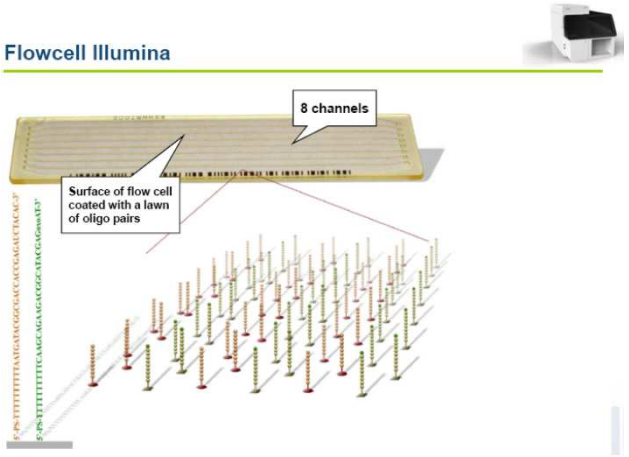


Validation cohorte 200 patients
séquençage ciblé (puce 150 gènes)
Sce Génétique Pr P Calvas, N Chassaing



Les reins hyperéchogènes non HNF1β

Séquençage haut débit
Ex / Seq



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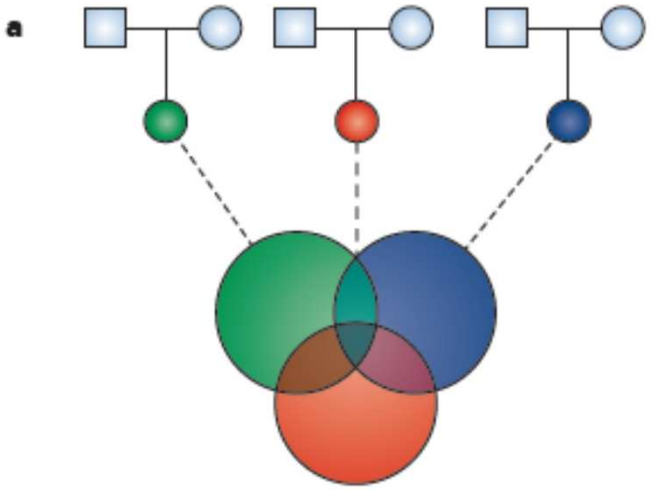
Fondation Maladies Rares 2012



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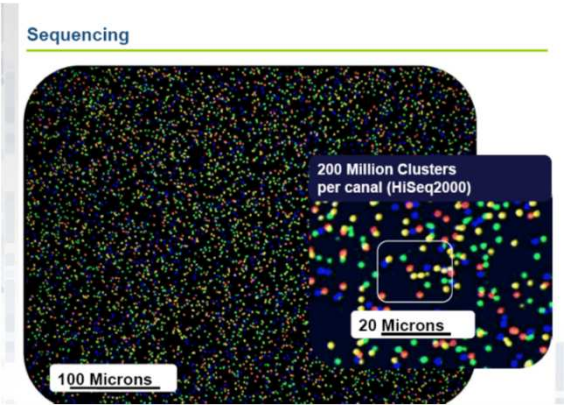
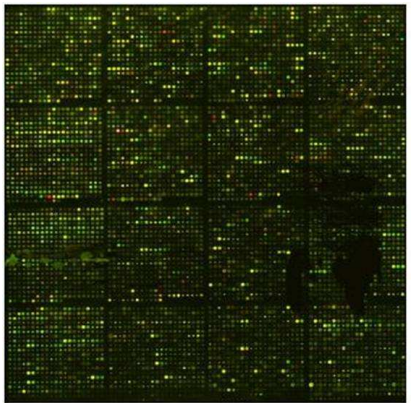


« CAKUTOME »
150 gènes
en routine



Pourquoi identifier de nouveaux gènes ?

Anomalie rénale ≈ 90%

Hypoplasie – Dysplasie rénale
Kystes corticaux
Dilatation pyélique; reflux vésico-urétéral
IRC lentement progressive (-2ml/min/an)

Anomalie pancréatique ≈ 45%

Diabète type MODY5
Hypoplasie pancréatique
Insuffisance exocrine

Anomalie hépatique ≈ 30%

Cytolyse
Cholestase

Anomalie génitale ≈ 30%

Utérus bicorne, Agénésie utérine
Absence des canaux déférents

Autres

Cancer du rein chromophobe
Retard mental - Autisme …

Maladie liée à HNF1β/*TCF2*

Autosomique dominante
($TCF2^{+/-}$)

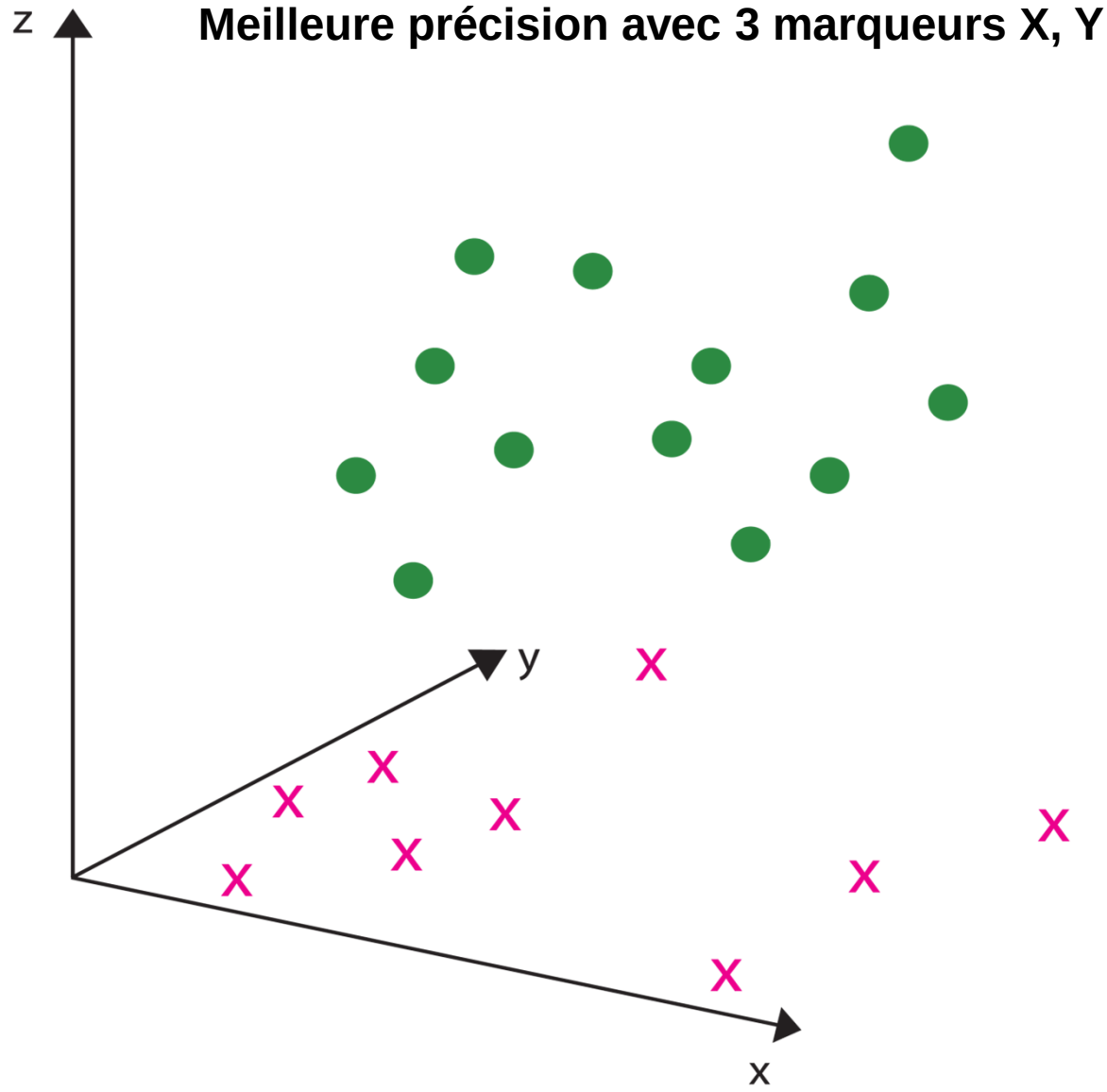
Pour un suivi adapté

Greffes

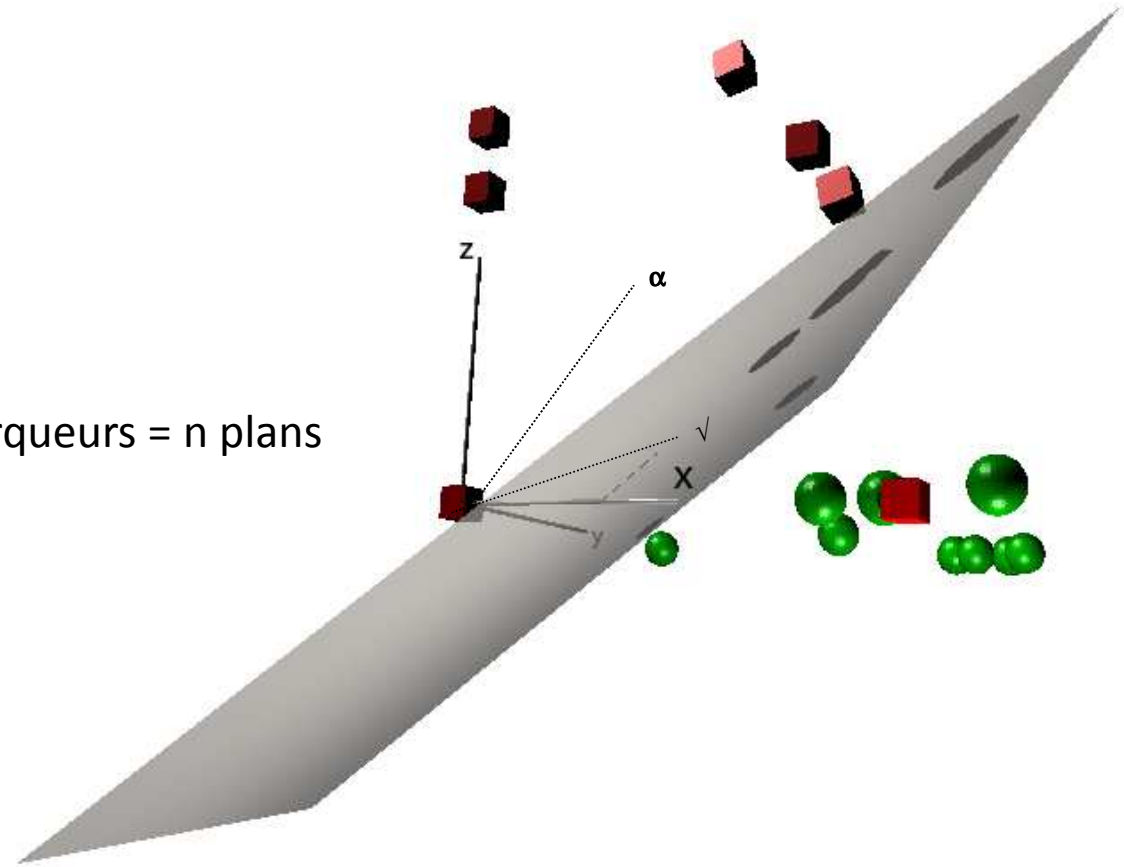
Protocole IS adapté
Greffe rein - pancréas

Peut on prédire *in utero* la fonction rénale postnatale ??

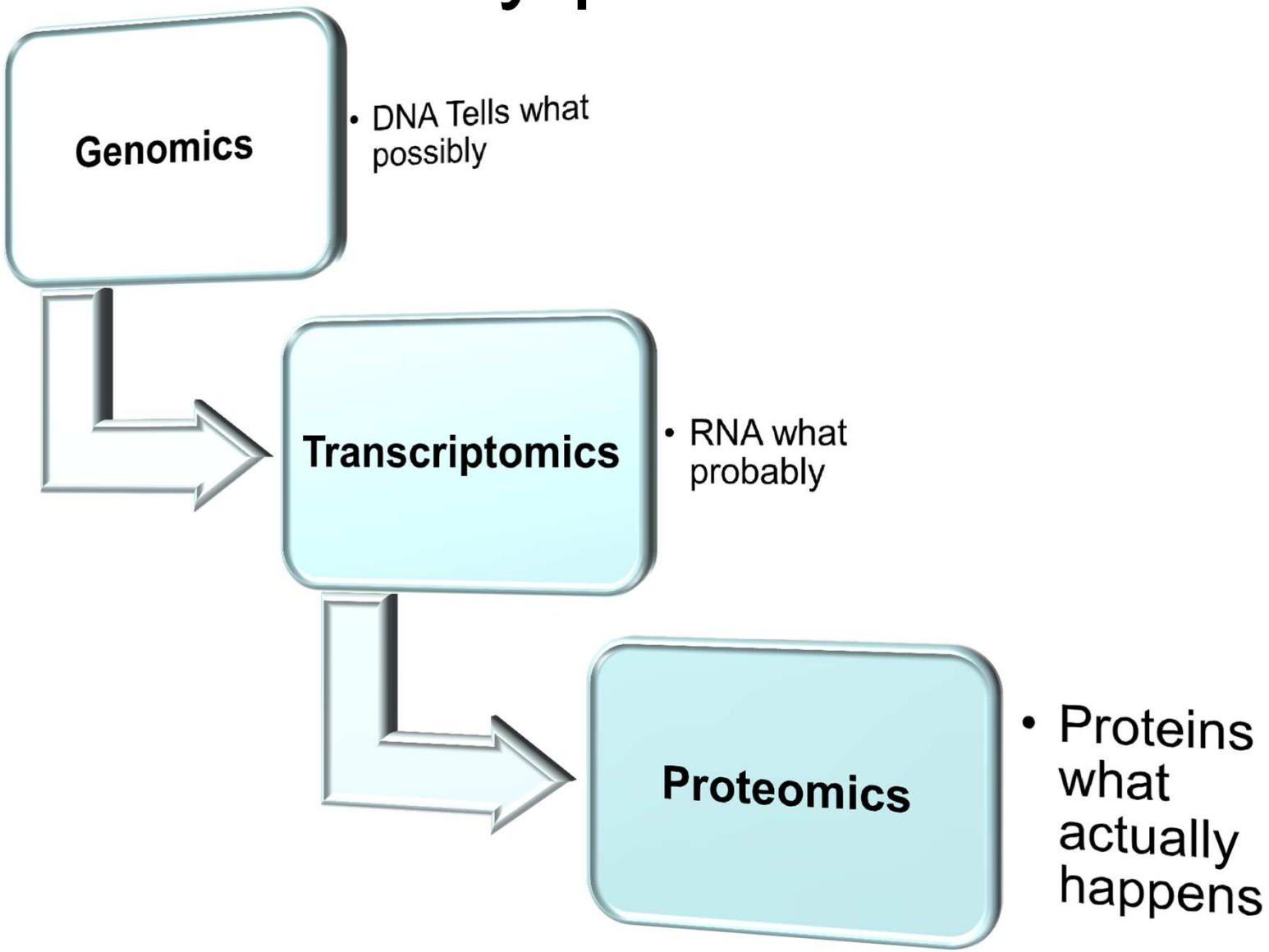
Meilleure précision avec 3 marqueurs X, Y et Z



N biomarqueurs = n plans

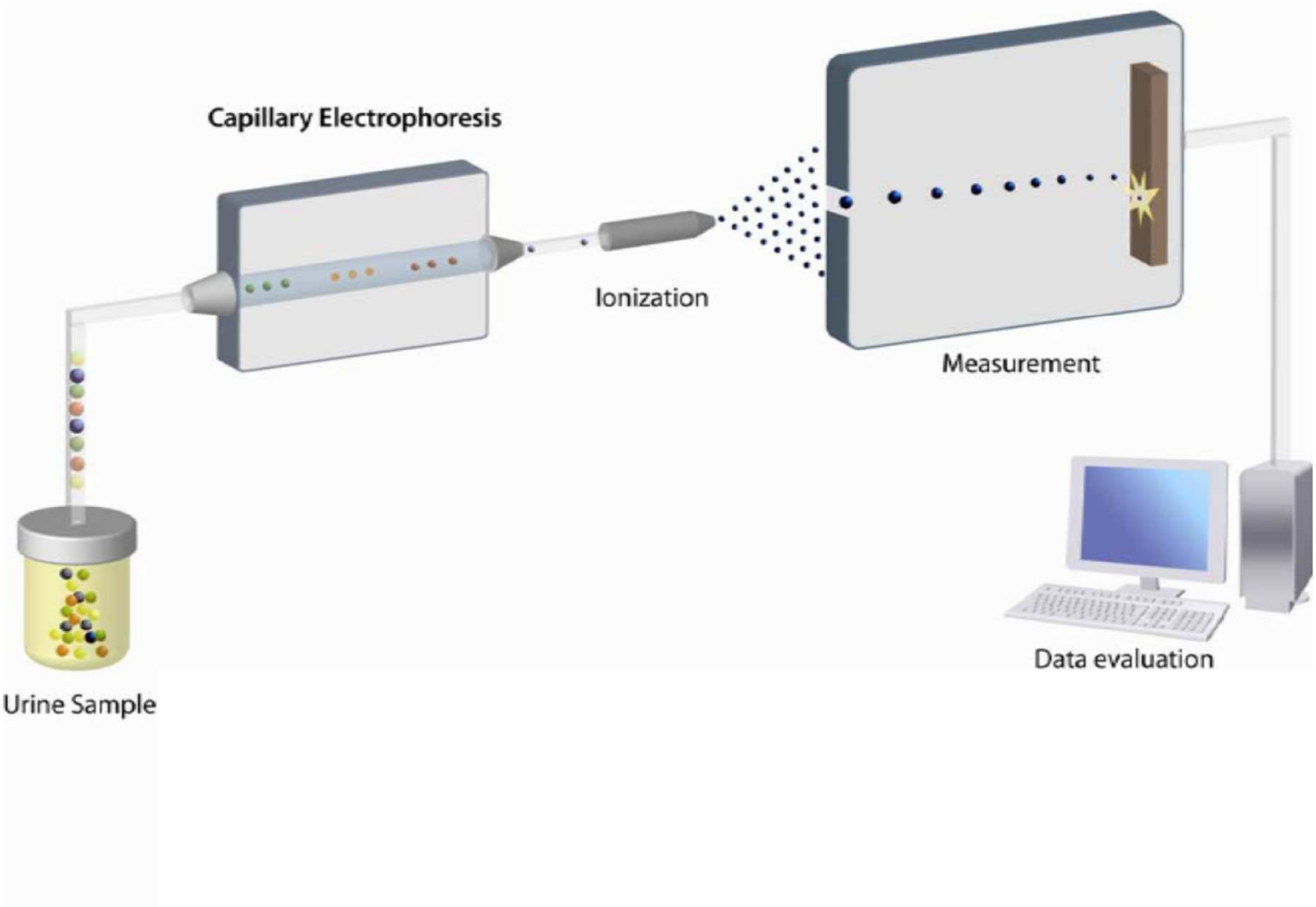


Why proteomics?

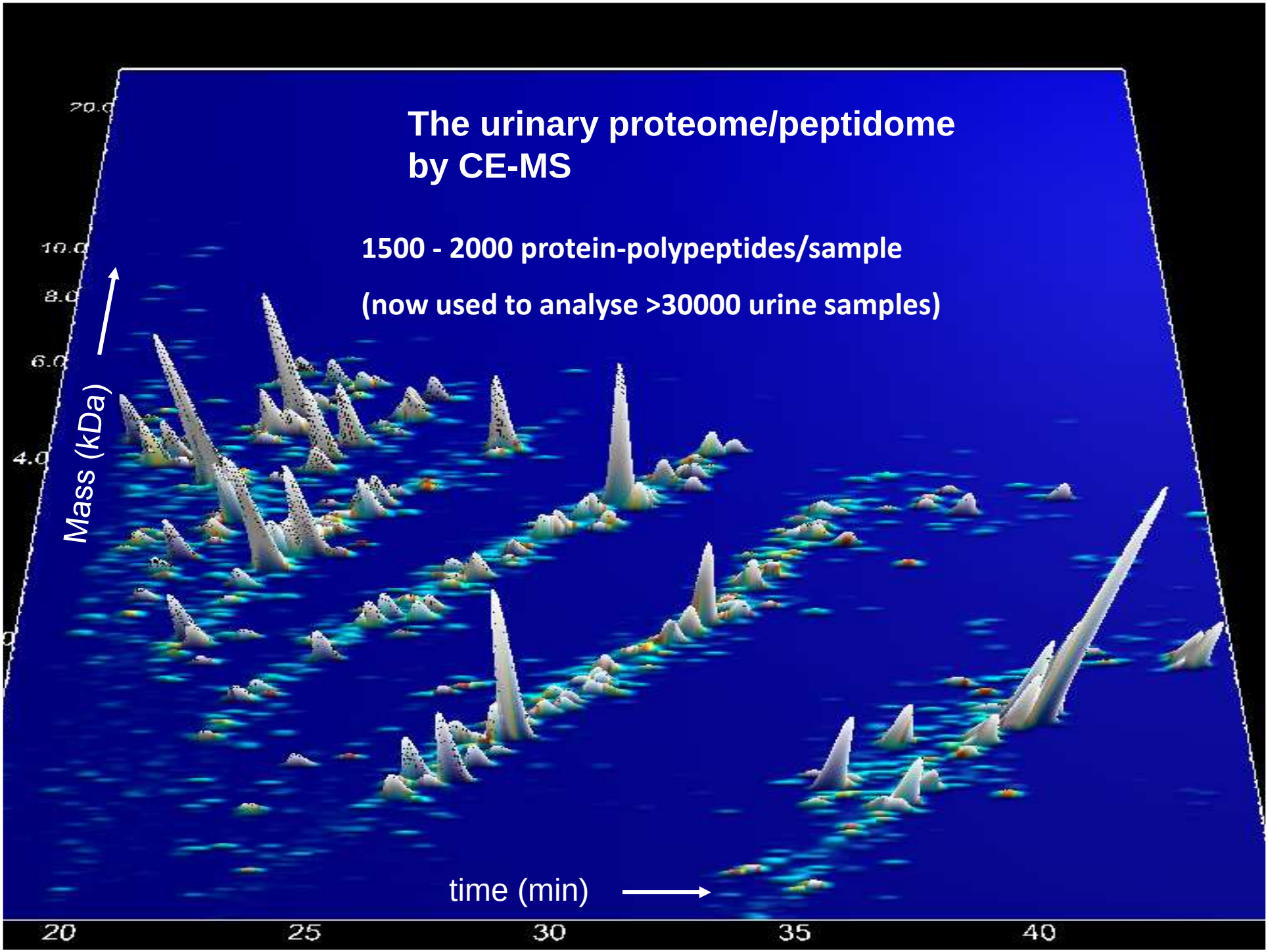


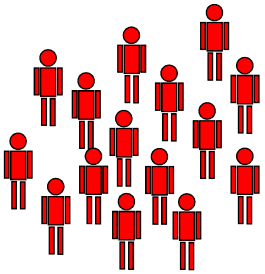
CE-MS

Capillary electrophoresis coupled to mass spectrometry



Kolch et al., 2005, Mass Spectrometry Reviews 24: 959-977


The urinary proteome/peptidome
by CE-MS
1500 - 2000 protein-polypeptides/sample
(now used to analyse >30000 urine samples)
Mass (kDa)
20.0
10.0
8.0
6.0
4.0
time (min)
20
25
30
35
40



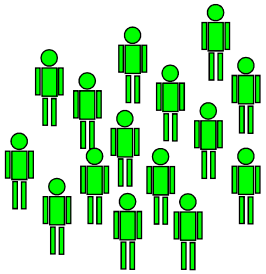
CASE

- *diseased*
- *treated (Drug)*

CE/MS

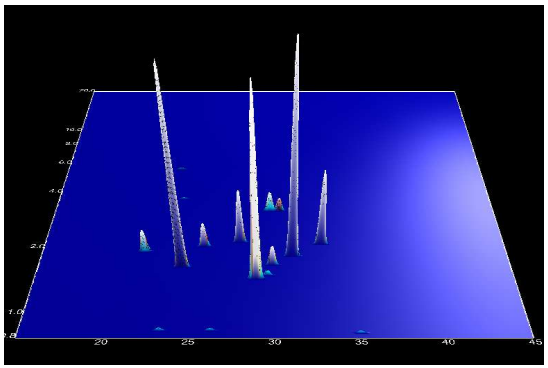
Diagnostic/prognostic Pattern

CE/MS

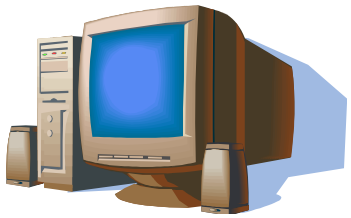


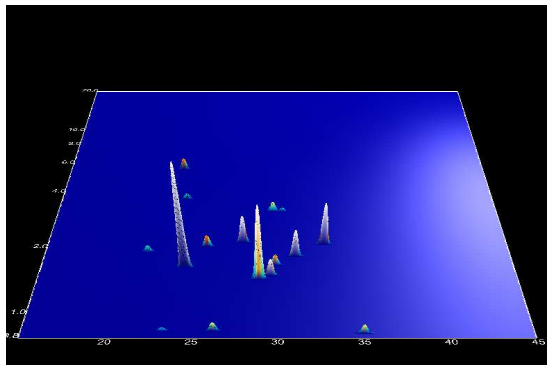
CONTROL

- *healthy*
- *untreated (Placebo)*



Discriminatory Biomarkers





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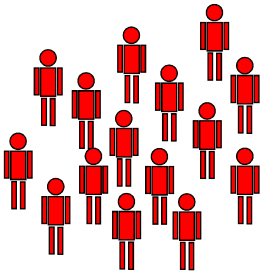

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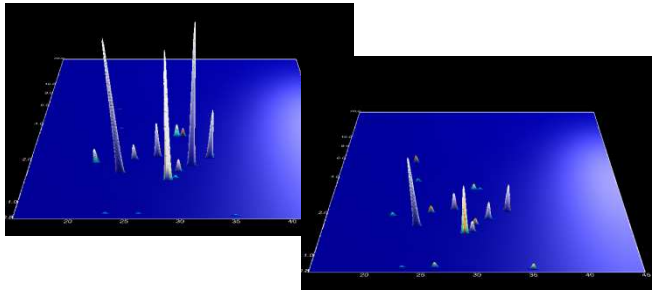

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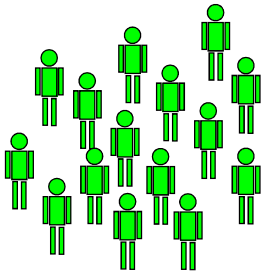

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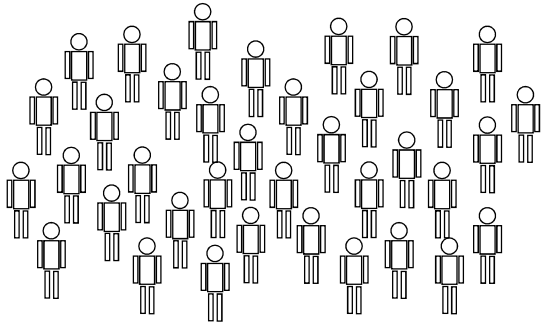
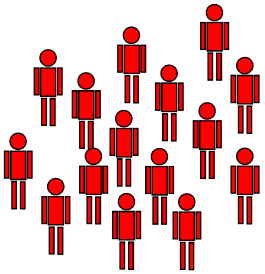


Blinded cohort



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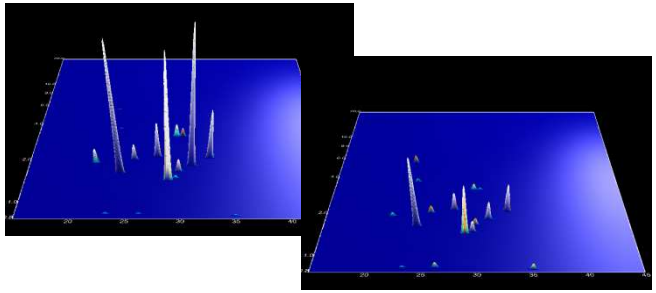
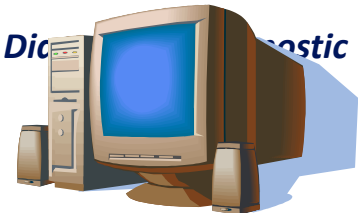
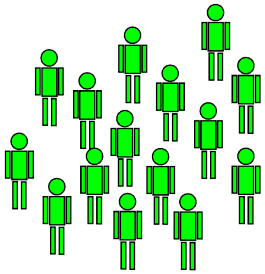




Dic astic



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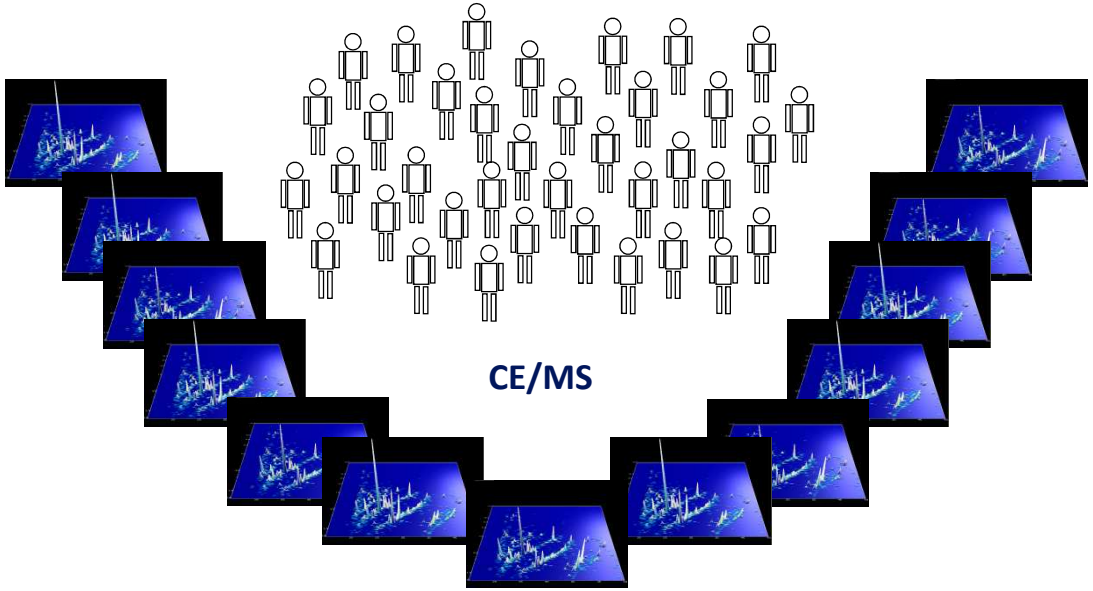

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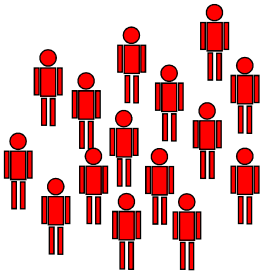

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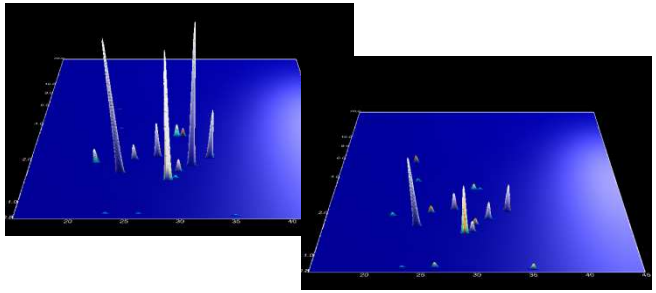
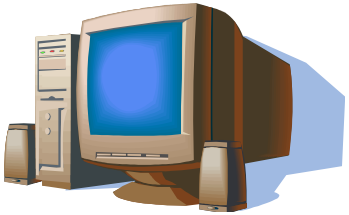
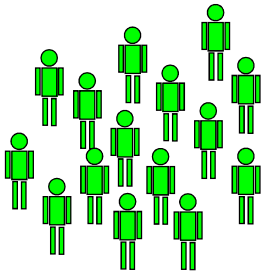






CONTROL

- *healthy*
- *untreated (Placebo)*

Unblinding

Sensitivity and **Specificity**

94% ***89%***

ETUDE BioMAN

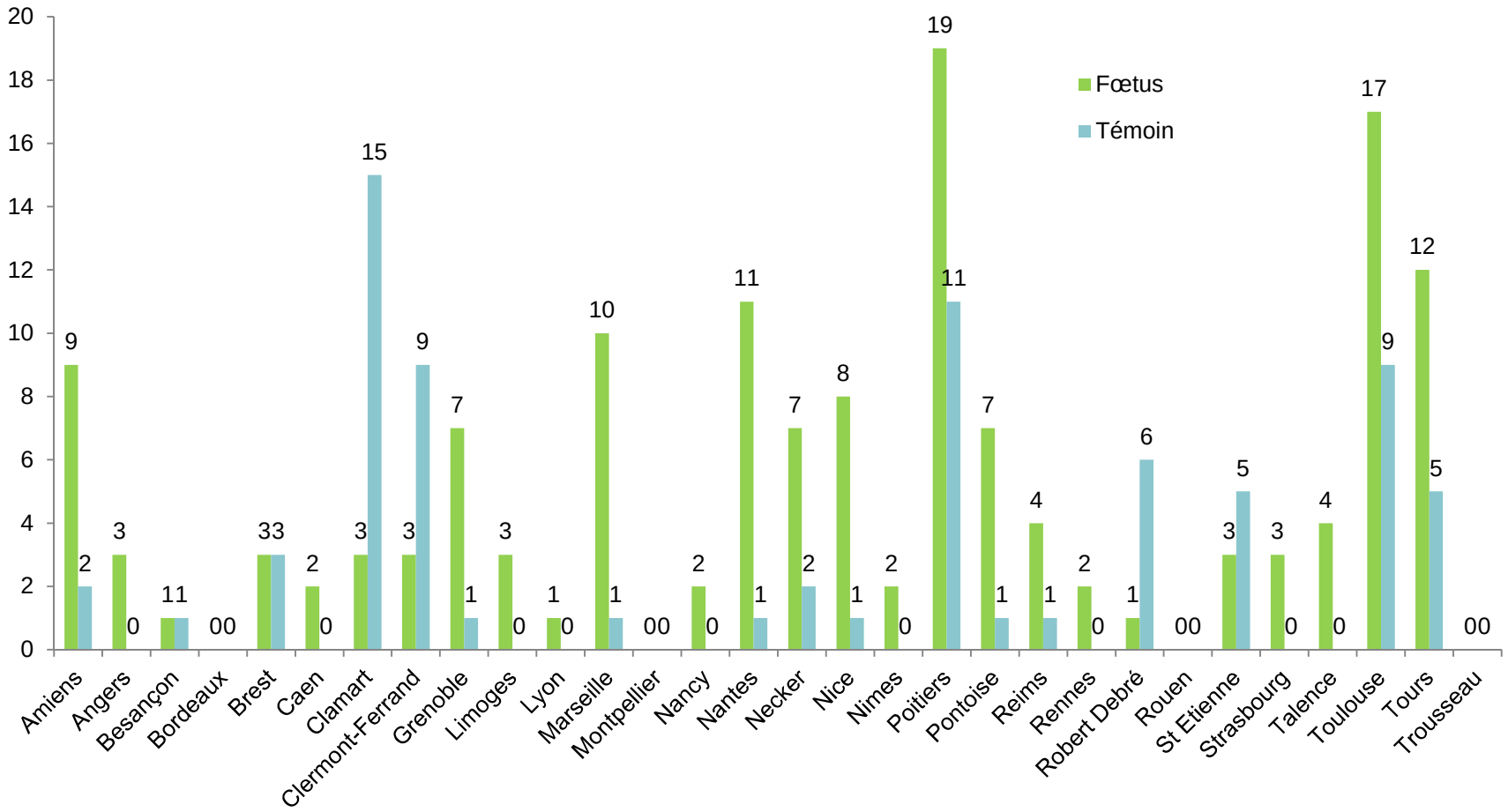
RECHERCHE DE BIOMARQUEURS PREDICTIFS DE LA FONCTION RENALE POSTNATALE DE FŒTUS PORTEURS D'UNE ANOMALIE BILATERALE DU DEVELOPPEMENT

Centre de référence Sud Ouest
Maladies RénAles RarEs

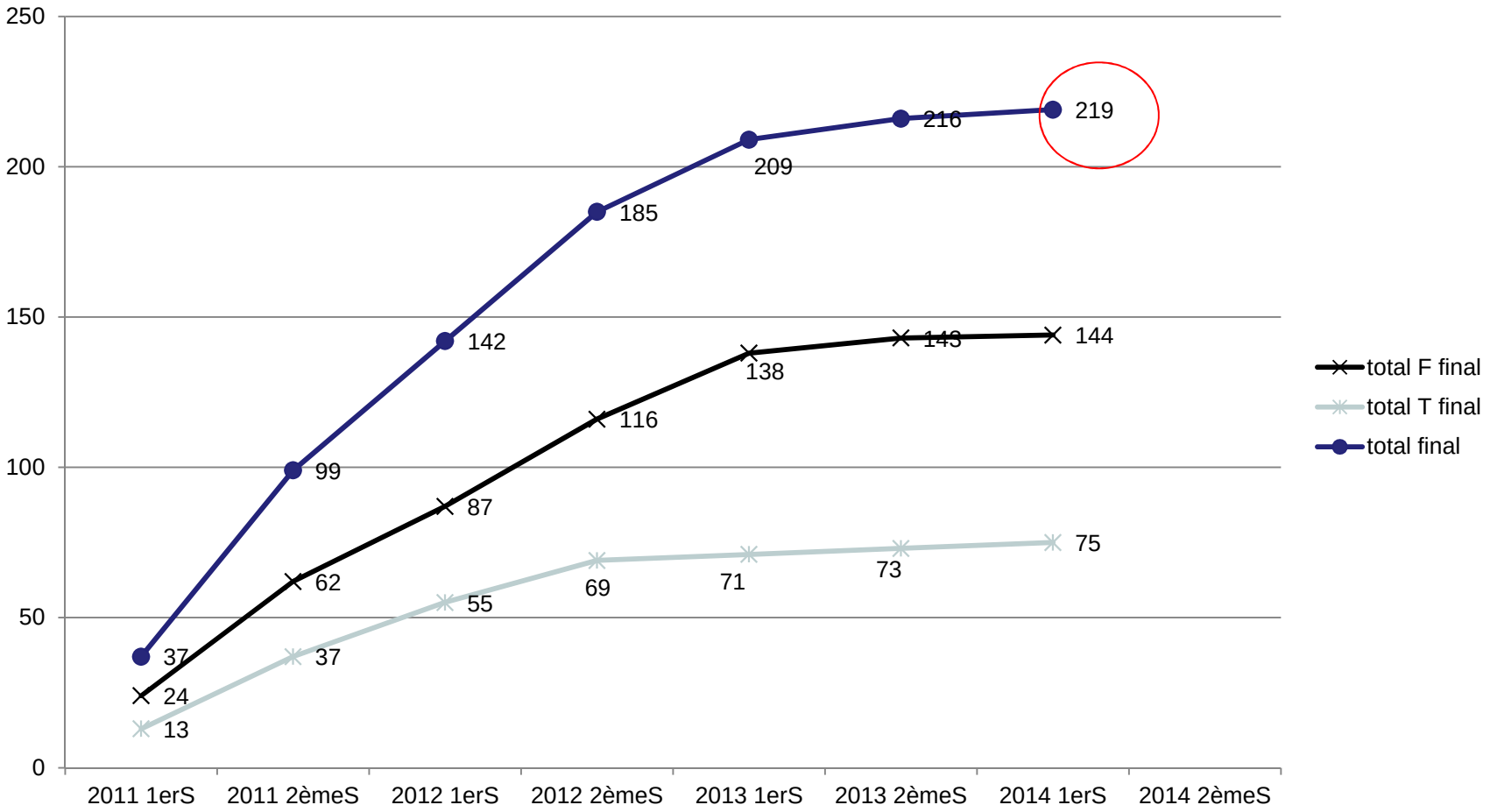
Hôpitaux de Toulouse

R F lab

BILAN DES INCLUSIONS PAR VILLE

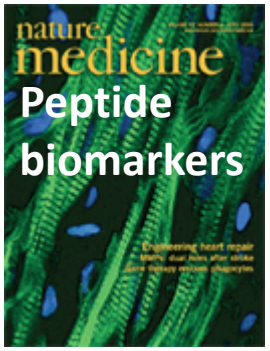


CINETIQUE DES INCLUSIONS

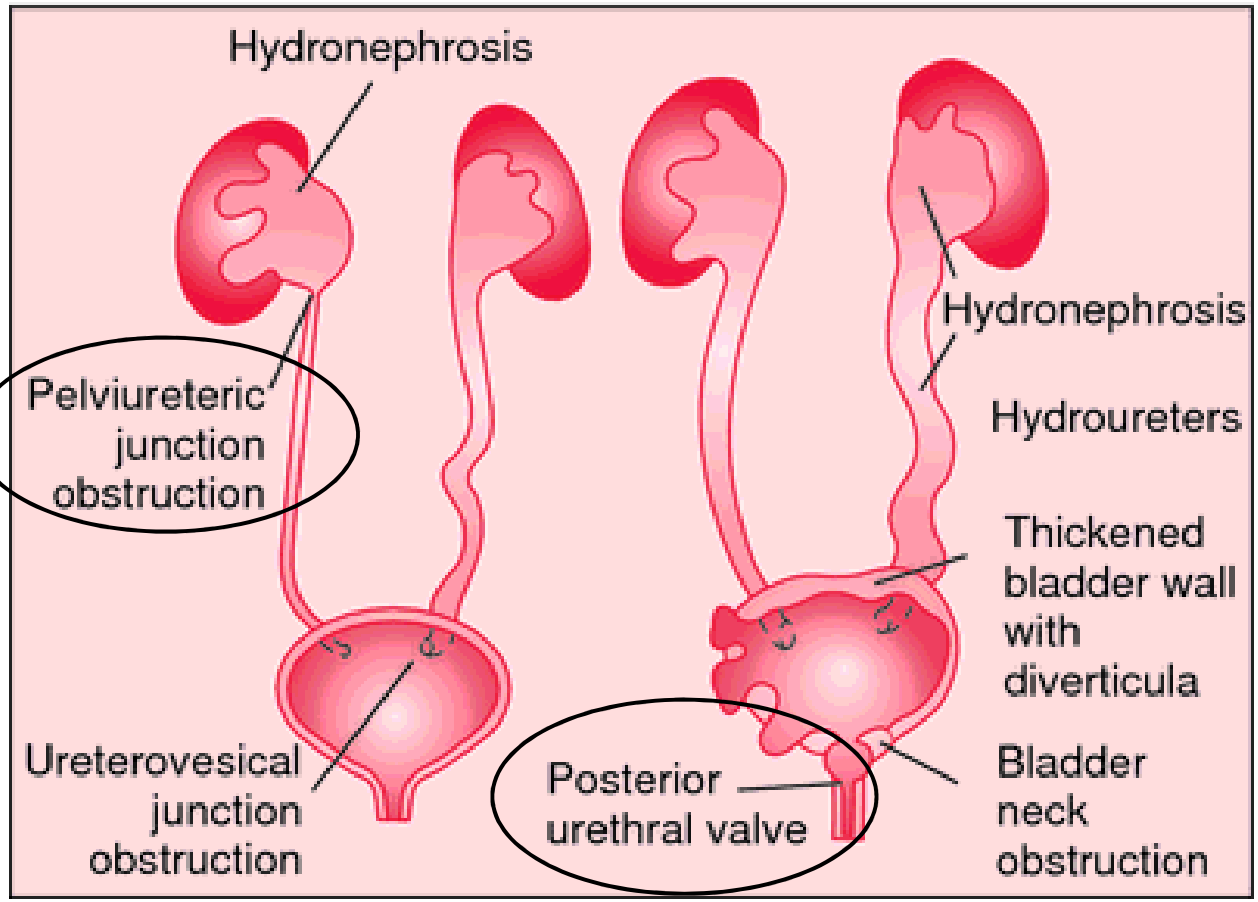


Obstructive nephropathy

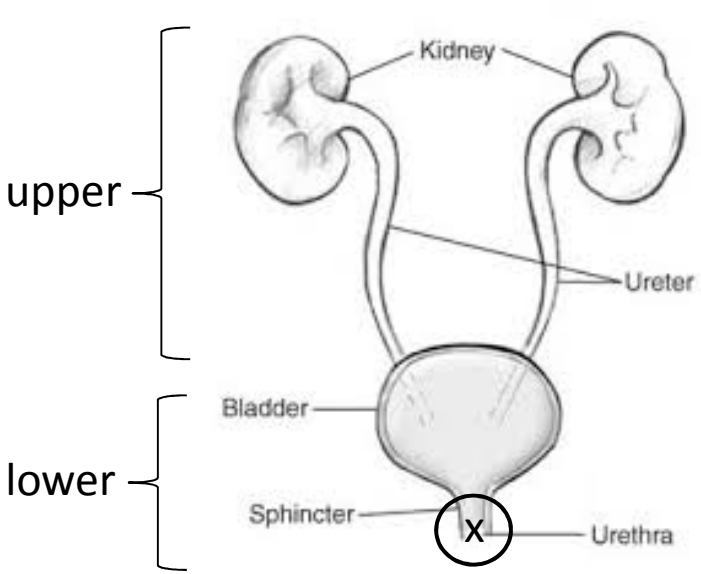
« A plumbing problem ?»



Decramer et al. Nat Med 2006



Posterior Urethral Valves (PUV)



- 35% TOP
- 40% ESRD
- 40% Bladder dysfunction

Fetal **bilateral** obstructive nephropathy (**rare** disease, 1/10.000 male births)

Nearly always associated to renal lesions:

abnormal cortico and medullar differentiation

hypo/dysplasia

cysts

hyperechogenicity

What is the problem of PUV?

To predict post-natal renal function (often chronic kidney disease (CKD)/ end stage renal disease (ESRD).

Current clinical practice

- Fetal ultrasound –non invasive-

- Fetal urinary biochemistry: b2-microglobulin, Na+, ….-invasive-

These lack either sensitivity or specificity.

- « Current evidence demonstrates that none of the analytes of fetal urine…nor threshold could be shown to be of particular clinical value. » *Morris et al., Prenat Diagn 2007, meta analysis 23 studies.*

- Ultrasound: same conclusion. *Morris et al., BJOG 2009, 13 studies*

Low sensitivity and specificity of current clinical tests

10 proposed, **but refused**, terminations of pregnancies

5 children at the age of 29 months had normal renal function!!

Hogan et al., Ped Nephrol. 2012

Values of classical parameters predicting post-natal renal function (ESRD *versus* non-ESRD) in our PUV cohort

Clinical predictor	**Sensitivity** [95% CI] (%)	**Specificity** [95% CI] (%)
Fetal urine biochemistry		
β2m		
cutoff >2 mM§	100 [83-100]	45 [27-65]
cutoff>13 mM§	31 [13-55]	95 [80-100]
Na		
cutoff>50 mM§	100 [83-100]	27 [13-47]
cutoff>100 mM§	13 [2-34]	91 [74-98]
Ultrasound parameters		
Oligohydramnios	25 [9-48]	64 [44-80]
Absence of amniotic fluid	25 [9-48]	86 [68-96]
Dysplastic multicystic kidneys	31 [13-55]	100 [87-100]
Hyperechogenic kidneys	25 [9-48]	86 [68-96]
Hypoplastic kidneys with cortico medullar thickening	19 [5-42]	77 [58-91]
Absence of normal cortico medullary differentiation	81 [58-95]	59 [40-77]

§ Morris RK Prenat Diagn 27, 2007

Either high sensitivity or specificity → **never both a high sensitivity and specificity !**

Mutations in CAKUT and post-natal renal function

ildebrandt KI 2014.pdf

Mutations in 12 known dominant disease-causing genes clarify many congenital anomalies of the kidney and urinary tract

Daw-Yang Hwang[1,2,12], Gabriel C. Dworschak[1,3,12], Stefan Kohl[1], Pawaree Saisawat[4], Asaf Vivante[1], Alina C. Hilger[3], Heiko M. Reutter[3,5], Neveen A. Soliman[6,7], Radovan Bogdanovic[8], Elijah O. Kehinde[9], Velibor Tasic[10] and Friedhelm Hildebrandt[1,11]

Genetic diagnosis has been hampered by genetic heterogeneity and lack of genotype–phenotype correlation.

Mutations in CAKUT and post-natal renal function

Table 1 | Genotypes and phenotypes of 41 families with mutations in 17 known autosomal dominant CAKUT-causing genes

Gene	Family-individual	Sex	Ethnicity	Renal phenotype	Nucleotide change[a]	Amino-acid change	Conservation *Mm*	*Gg*	*Xt*	*Dr*	EVS alleles[b]	SIFT[c]	Mutation-taster[d]	PP-2[e]	References
BMP7	A3068-21			VJO	c.[illegible]		E	E	E	E	0/13,006	T	DC	0.192	
	A3068-22			HD											
CDC5L	A4171-11			RA	c.2[illegible]		P	P	P	/	0/13,006	T	DC	0.393	
	A4171-21			RA											
CHD7L	A3051-21	M	WE	R MCDK, L UVJO	c.998C>G	p.P333R	P	P	/	P	0/13,006	D	DC	0.953	
CHD7L	A549-21	F	Asi	B kidney malrota-tion	c.1199A>G	p.E400G	E	E	/	E	0/13,006	D	DC	0.997	
CHD7L	A3902-21[f]	M	Ind	PUV	c.1551A>G	p.I517M	I	I	/	I	0/13,006	D	DC	0.505	
CHD7L	A3925-21	F	Ind	R RD	c.1551A>G	p.I517M	I	I	/	I	0/13,006	D	DC	0.505	
CHD7L	A3219-21	M	Ind	Horseshoe kidneys, R DS	c.1551A>G	p.I517M	I	I	/	I	0/13,006	D	DC	0.505	
EYA1	A1522-21[g]	M	Ara	R UPJO	c.647C>T	p.P216L	P	P	P	P	0/13,006	D	DC	0.079	45*
EYA1	F1438-21[h]	[illegible]		[illegible], B RHD	c.966+1G>A	NA					0/13,006				
EYA1	A1542-21			UPJO	c.1733C		S	S	S	S	0/13,006	D	DC	0.984	
GATA3	A4733-21			VUR	c.766C		R	R	R	R	0/12,988	D	DC	0.404	
GATA3	A1319-21			VUR	c.869C		Q	Q	Q	Q	0/13,006	D	DC	0.439	
HNF1B	A3067-21			UR, NB	c.234G		E	E	E	E	0/13,004	D	DC	0.992	
HNF1B	A2921-21	M	EE	L RHD, R MCDK	c.477delT	p.M160*					0/13,006				46
	A2921-12	F		Unspecified CAKUT											
HNF1B	A3069-21	F	EE	L VUR	c.499G>A	p.A167T	A	A	A	A	0/13,006	D	DC	0.999	
HNF1B	A3840-21	M	Ind	VUR, PUV	c.542G>A	p.R181Q	R	R	R	R	0/13,006	D	DC	0.888	
HNF1B	A2326-21	M	WE	L UPJO, subcapsu-lar cysts	c.823C>T	p.Q275*					0/13,006				
	A2326-11	M		subcapsular cysts											
HNF1B	A4672-21[i]	F	EE	R RHD, cystinuria	c.1024T>C	p.S342P	S	S	S	S	0/13,006	D	DC	0.767	
PAX2	A3148-21	M	WE	B RHD, BCT	c.76dup	p.V26Gfs*28					0/12,980				47
PAX2	A2334-21[k]	F	WE	B RHD	c.211A>G	p.R71G	R	R	R	R	0/12,958	D	DC	0.888	
PAX2	A1087-21	M	EE	B UVJO	c.320C>T	p.P107L	P	P	P	P	0/13,006	D	DC	0.999	
PAX2	A3872-21	M	Ind	R RHD	c.343C>T	p.R115X					0/13,006				
PAX2	A1743-12	[illegible]		BCT	[illegible]						0/13,006				
	A1743-21	[illegible]		BCT											
RET	A3836-21	[illegible]		RHD	c.6[illegible]		V	V	V	V	0/12,958	D	DC	0.642	
RET	A1077-21	[illegible]		R UPJO	c.2[illegible]		V	V	V	V	0/13,006	T	DC	0.901	48*
RET	A1518-21	[illegible]		UR, ureter-scele	c.3[illegible]		L	L	L	L	0/13,006	D	DC	0.996	
ROBO2	A1288-21	[illegible]		JO, stone	c.3[illegible]		G	G	G	G	0/12,438	D	DC	1	
ROBO2	A3839-21	M	Ind	PUV	c.724A>G	p.I242V	T	T	T	T	0/11,902	D	DC	0.224	
ROBO2	A3372-21	M	EE	R MCDK	c.808C>G	p.P270A	P	P	P	P	0/11,930	D	DC	0.988	
ROBO2	A521-11	M	EE	R [illegible]	c.3712G>A	p.D1238N	D	D	D	D	0/12,130	D	DC	0.251	
SALL1	A3935-21	M	Ind	PUV	c.220G>A	p.V74I	V	V	V	V	0/12,996	D	DC	0.007	
SALL1	A2333-21	M	WE	B VUR, MCDK	c.548C>G	p.T183R	T	T	T	T	0/12,996	D	DC	0.296	
SALL1	A2898-21	F	EE	L UPJO	c.602A>G	p.Q201R	Q	Q	Q	Q	0/12,996	D	DC	0.968	
SALL1	A617-21	F	EE	B VUR gr III, Rt [illegible]	c.703G>A	p.A235T	A	A	A	A	0/12,996	D	DC	0.782	
SALL1	A3070-21	[illegible]		UPJO											
SALL1	A4448-21	[illegible]		VUR	c.1[illegible]		I	I	I	I	0/12,996	D	DC	0.035	
SALL1	A5006-21	[illegible]		VUR	c.1[illegible]		I	I	I	I	0/12,996	D	DC	0.035	
SALL1	A3607-12[m]	F	EE	L DS	c.2582C>A	p.S861*					0/12,996				
	A3687-21	M		R RHD											
SALL1	F1434-21[n]	M	WE	R RA, L VUR	c.3006_3009de-l	p.C1003Tfs*41					0/12,996				
SIX2	A3904-21	M	Ind	PUV	c.859G>A	p.V287M	V	V	V	V	0/13,006	D	DC	0.987	
SIX5	A959-21	M	EE	R DS, VU, L UVJO	c.1817C>T	p.P606L	P	/	-	P	0/12,946	D	DC	0.994	

CHD1L — PUV

HNF1B — PUV

ROBO2 — PUV

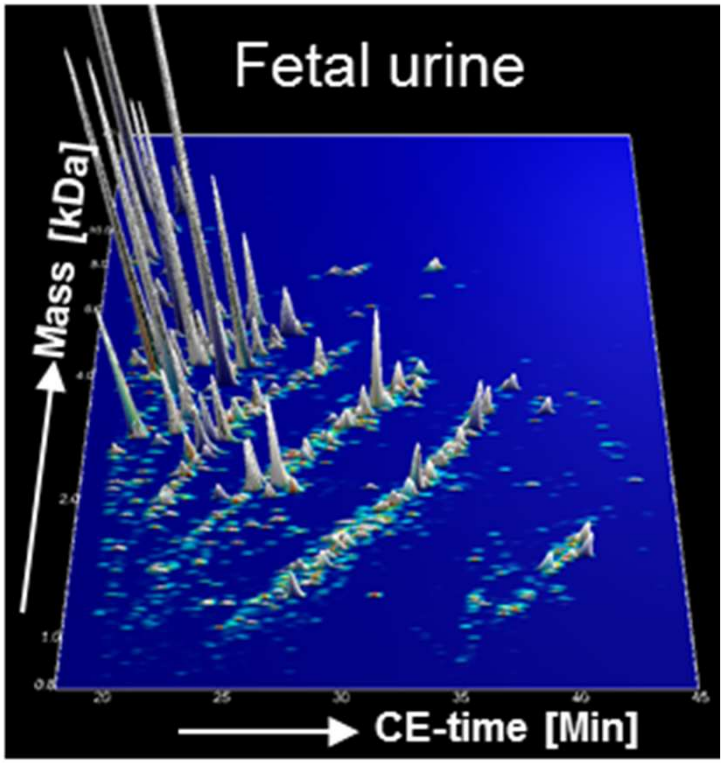
SALL1 — PUV

SIX2 — PUV

genotype ⇢ phenotype ⇢ progression

Biomarkers to predict post-natal renal function

- Fetal urine
- Fetal urinary peptidome analysis



>4000 peptides

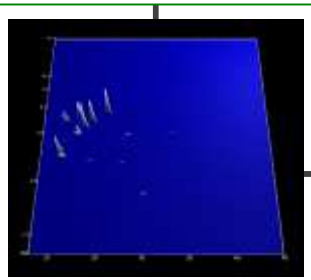
Discovery phase

« no-ESRD »

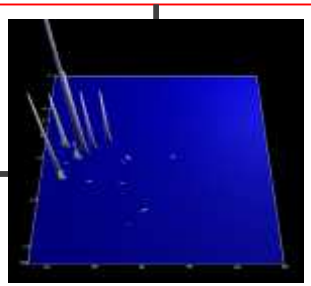
15 PUV
with normal/acceptable
renal function up to 2 years old

« ESRD »

13 PUV
confirmed with autopsy
with TOP or neonatal death



Peptidome analysis



26 differentially secreted peptides (FDR<0.05)

Support Vector Machine Model

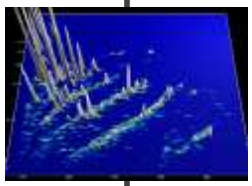
12PUV model

Classification between the 2 groups: 100%

Validation phase

« ? »

38 PUV
Independent cohort
Blind analysis



Peptidome analysis

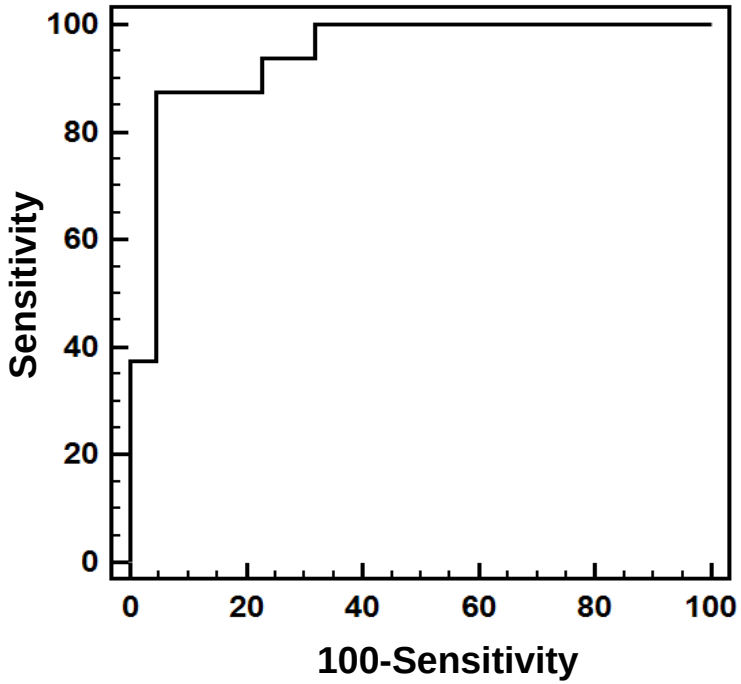
12PUV model

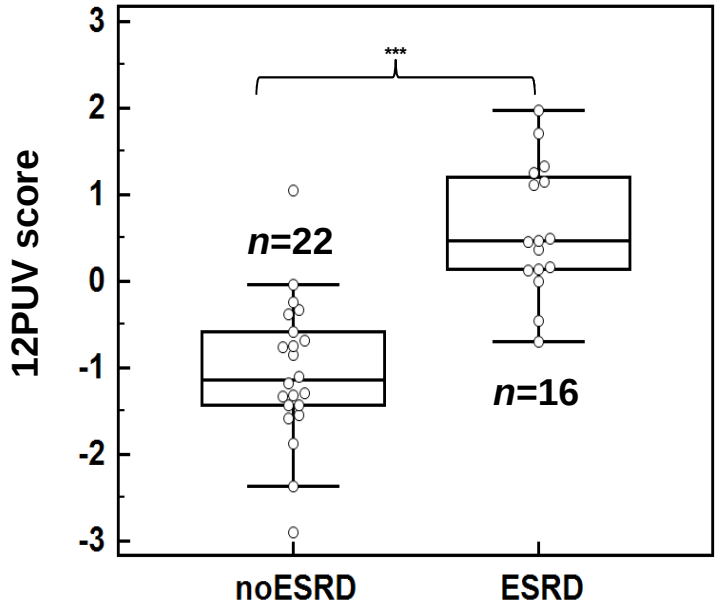
« no-ESRD»

« ESRD»

Comparison of prediction with renal function at 2 years

Prediction of ESRD using the 12PUV model in fetal urine in the blinded cohort (n=38)





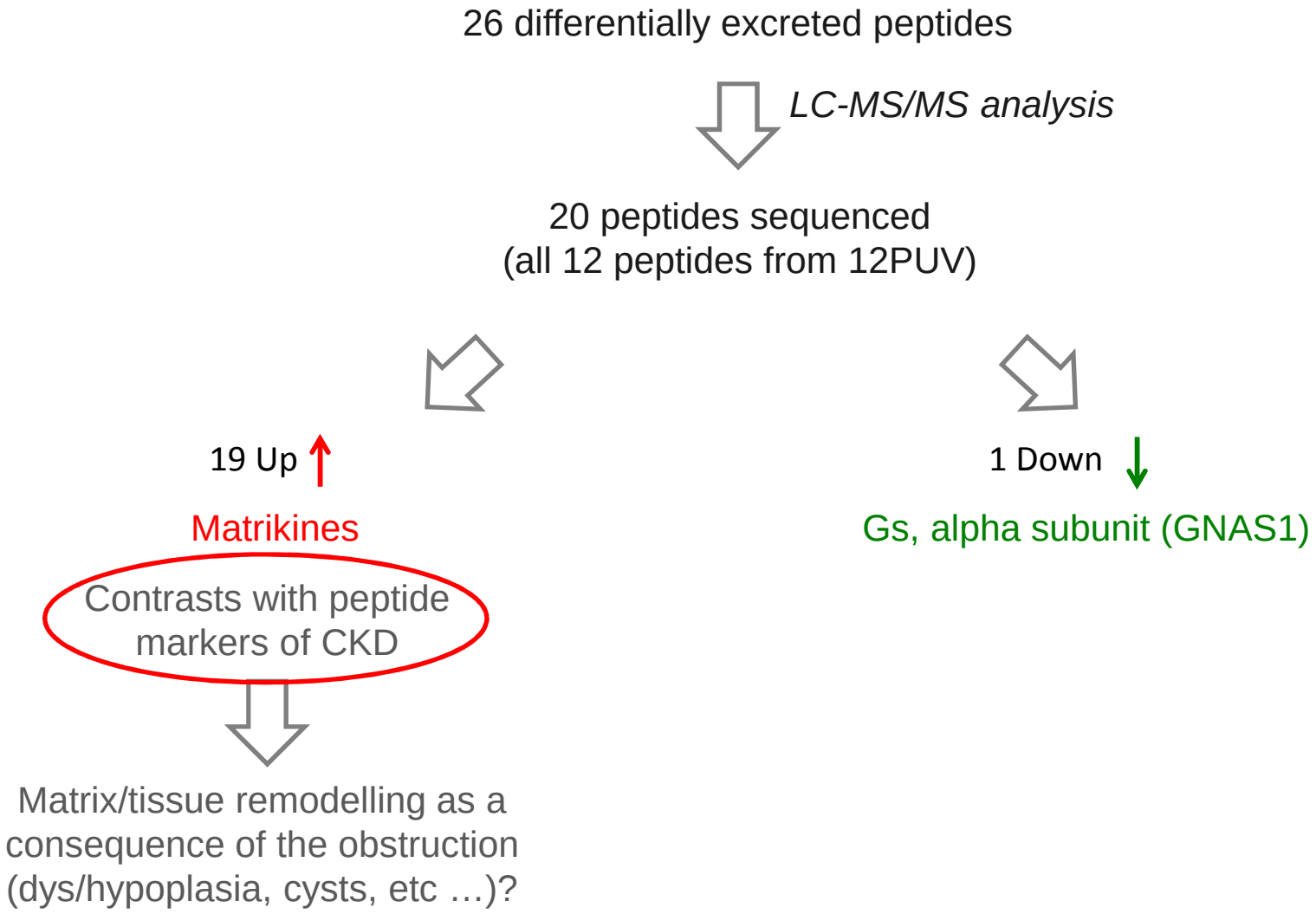
AUC 0.94 [95% CI: 0.82-0.99]

Sensitivity 88% - Specificity 95%

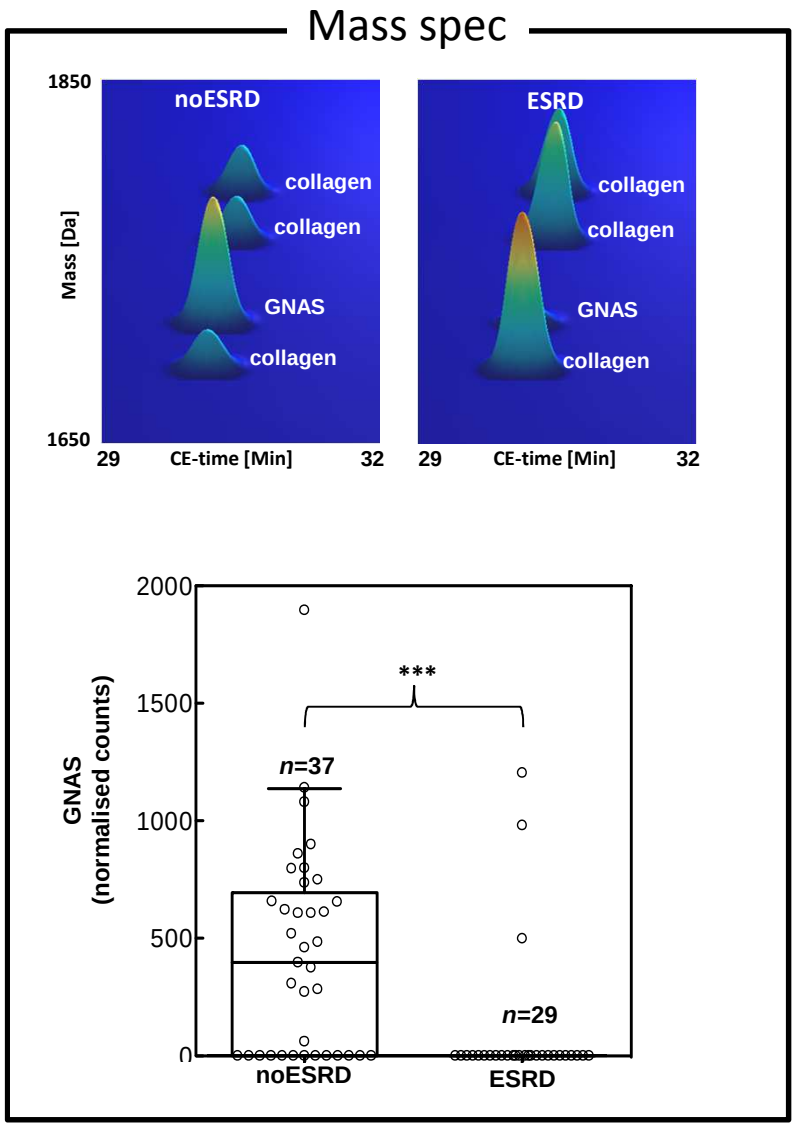
Both high sensitivity and specificity !

Klein et al., Science Translational Medicine 2013

Peptide identification

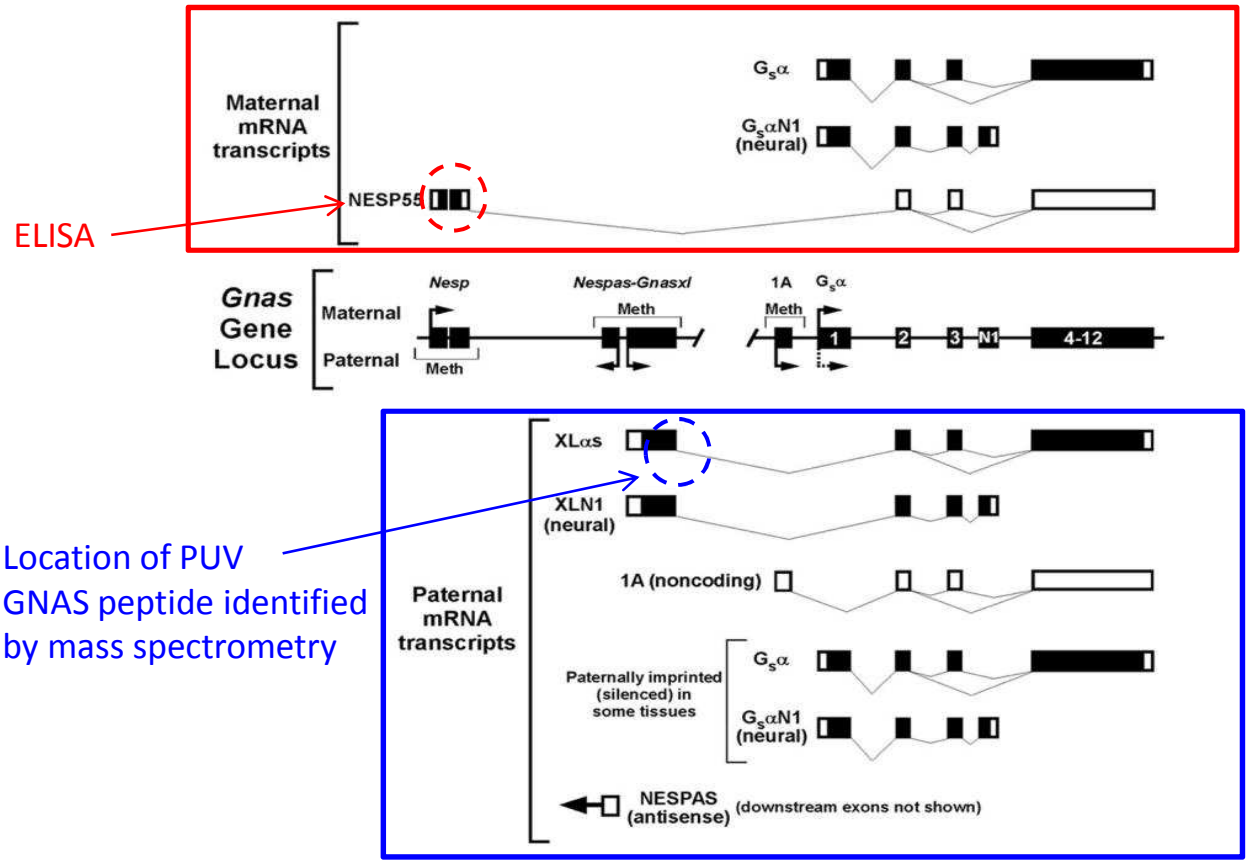


Peptide identification: GNAS



GNAS is an imprinted gene

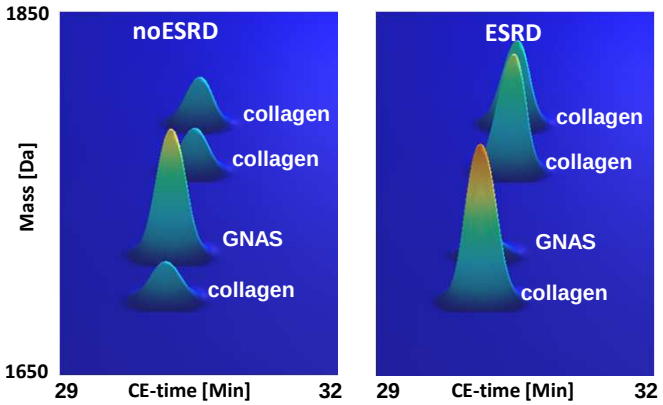
- Specific transcripts when it is maternally or paternally transmitted.

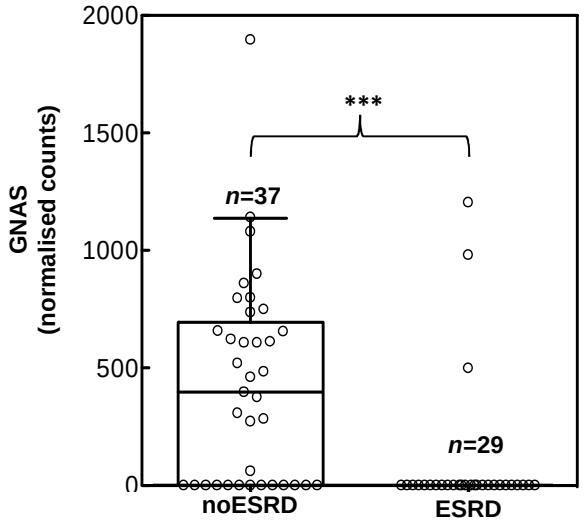


- Involved developmental diseases.
- Imprinted in the proximal renal tubule…

Peptide identification: GNAS

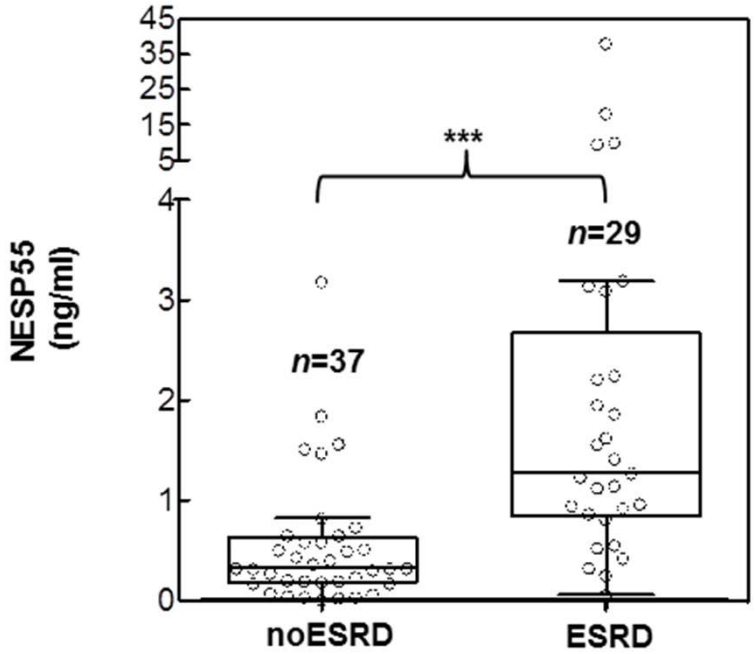
Mass spec





Paternally transmitted

ELISA



Maternally transmitted

Peptide identification

26 differentially excreted peptides

LC-MS/MS analysis

20 peptides sequenced
(all 12 peptides from 12PUV)

19 Up ↑

Matrikines

Contrasts with peptide markers of CKD

Matrix/tissue remodelling as a consequence of the obstruction?

1 Down ↓

Gs, alpha subunit (GNAS1)

Causative/aggravating factor or a bystander?

Sequencing of GNAS locus in PUV patients
(H Jueppner, Boston)

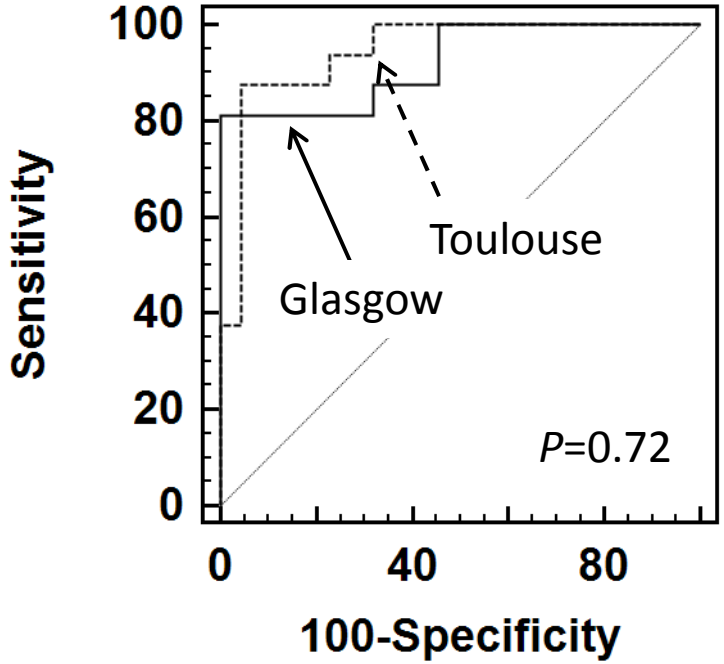
Implementation

What about the “portability” of the analysis?

(*i.e.* can we do the analysis “anywhere” and still compare the results?)

Toulouse (France)

Glasgow (Scotland)



Implementation - PUV case (October 2013)

Mail Tuesday, October 15, 2013 from Dr Elena Levtchenko (Leuven, Belgium)
Fetus with PUV, oligohydramnios, and dense renal parenchyma .

"She is considering pregnancy termination and we had a very difficult discussion. Is it possible to send you a sample to your 12PUV score? It might help to make a decision."

Sequence of events

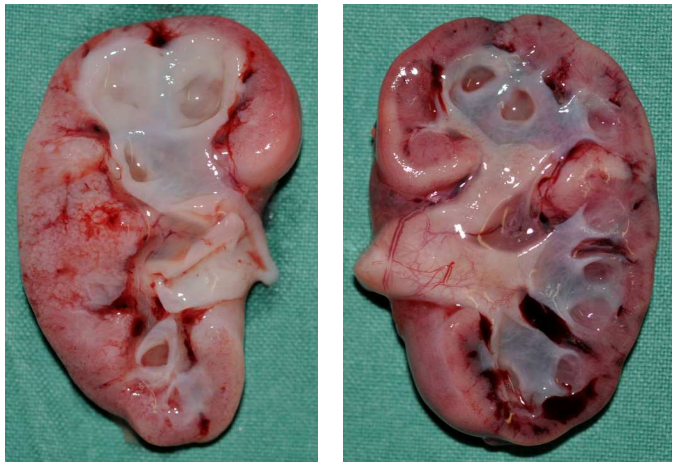
- Fetal urine sampling on Thursday that week (October 17).
- Sample arrived in Toulouse on Friday (October 18) on dry-ice (DHL).
- Sample prepared on Monday (October 21) and analyzed on Tuesday (October 22).
- Data uploaded to the German lab (Wednesday October 23).
- Database matching and scoring using 12PUV (Thursday October 24).
- Results send to physician (Friday October 25).

One week

"We have analyzed and scored the sample. Unfortunately, it scores positive for ESRD (a score of 1.75, which is very high) while a control sample (i.e. noESRD) that we included from de validation cohort clearly scored negative."

Fetal autopsy

"The results of fetal autopsy have confirmed the diagnosis of urethral valves and severe bilateral renal dysplasia with cortical cysts. Furthermore, the fetus had significantly delayed lung maturation."



Conclusion

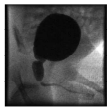
The 12 PUV classifier is the first tool to have both high sensitivity and specificity to predict the post-natal renal outcome (CKD or ESRD).

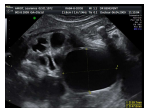
- Truly informed prenatal counselling.
- Stratification of patients that will benefit most from prenatal intervention (laser cystoscopy or vesicoamniotic shunt).

➡ Horizon2020 project « PROFET ».

PROFET

European consortium for PROteomics-guided FETal intervention in lower urinary tract obstruction

Mega bladder 

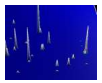
LUTO (PUV) 

Urinary tract anomalies +/-
Dysplastic or hyperechogenic parenchyma

↓

Ultrasound J15

↓



« ESRD » ← | → **« noESRD »**

↓

Intervention? → **no** → **« ESRD »**

(Laser cystoscopy or vesico-amniotic shunt « PLUTON »)

↓

yes

↓

Validation of 12PUV biomarker pattern

↓

Post-natal renal function at D1, D28, 1Y

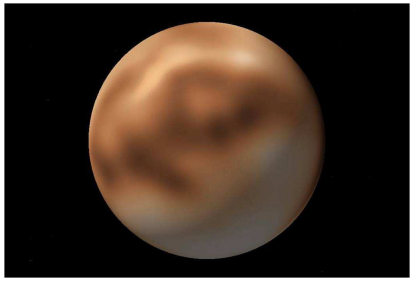
↓

Is the 12PUV biomarker based prediction of post-natal renal function altered by the intervention?

PLUTON

***P**erforming **L**aser in lower **U**rinary **T**ract **O**bstruction*
*to improve **N**eonatal renal function*

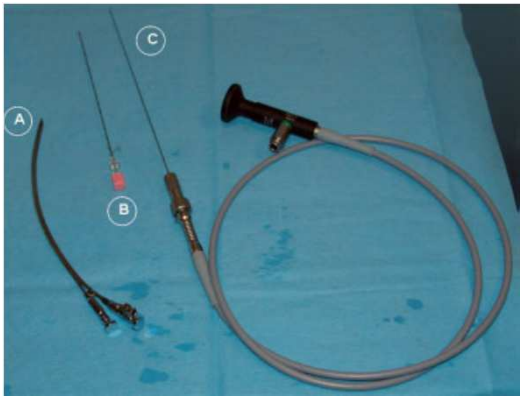
Chaque foetus-bébé est un futur
Chaque planète est un possible
Le possible d'une vie
Ici ou ailleurs

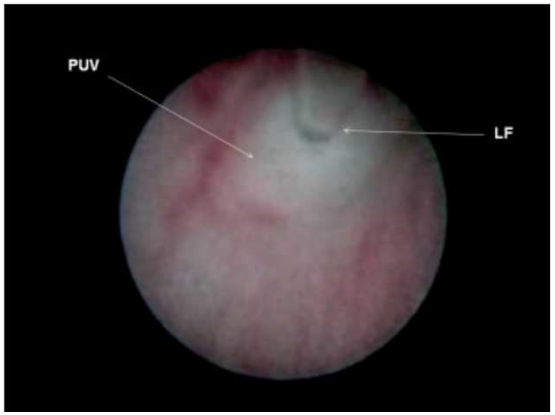


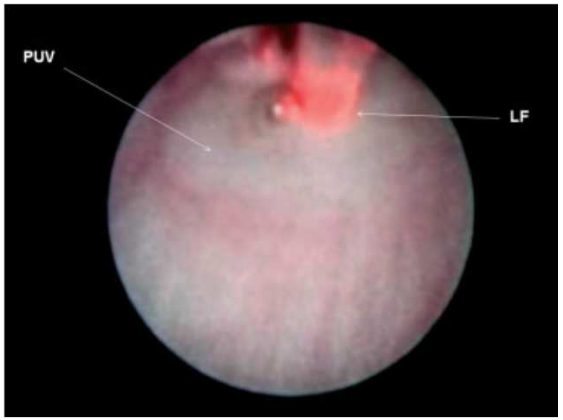
Fetal cystoscopic and directed laser fulguration is effective !!!
fetoscope (diameter of 1.0 mm and length of 20 cm)
600-µm laser fiber + fulguration by shots of pulsed Nd :YAG laser

No randomized controlled studies

« Expert practice » to prevent adverse events







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PROTOCLIN

IMPROVEMENT OF TOOLS AND PORTABILITY OF MASS SPECTROMETRY-BASED CLINICAL PROTEOMICS AS APPLIED TO CHRONIC KIDNEY DISEASE

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