

Necker- Enfants Malades Hospital

A. Edelman, T. Nguyen-Khoa, INSERM U845, Paris
aleksander.edelman@inserm.fr
thao.nguyen-khoa@nck.aphp.fr



Necker- Enfants Malades Hospital

A. Edelman, T. Nguyen-Khoa, INSERM U845, Paris



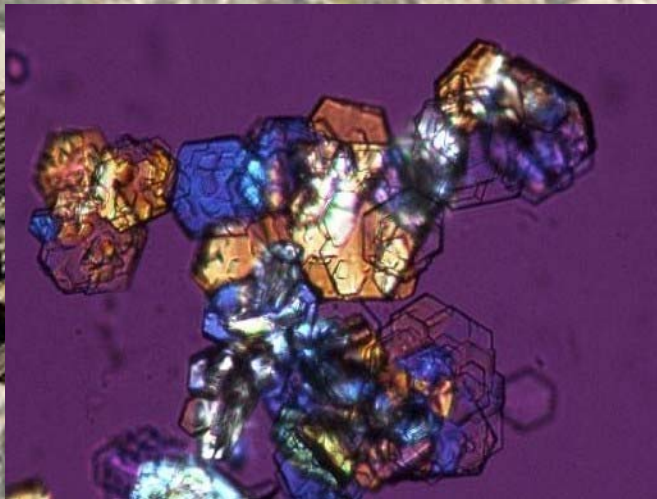
Jean Hamburger

A. Edelman

T. Nguyen-Khoa

Urinary proteomics of cystinuria, a nephrolithiasis disease: new project

Nguyen-Khoa T, Lacour B, Daudon M.
Knebelmann B, Bollée G.
Guerrera C, Edelman A



Nephrolithiasis disease

Prevalence of 10%, in constant progression.

Sex ratio : 2H/1F.

Recurrence of renal stone formation

50% of cases.

Long-term outcome leads to end-stage renal disease.

2 to 5% of ESRD.

Cystinuria : 1-2% of adult nephrolithiasis 5-10% of children

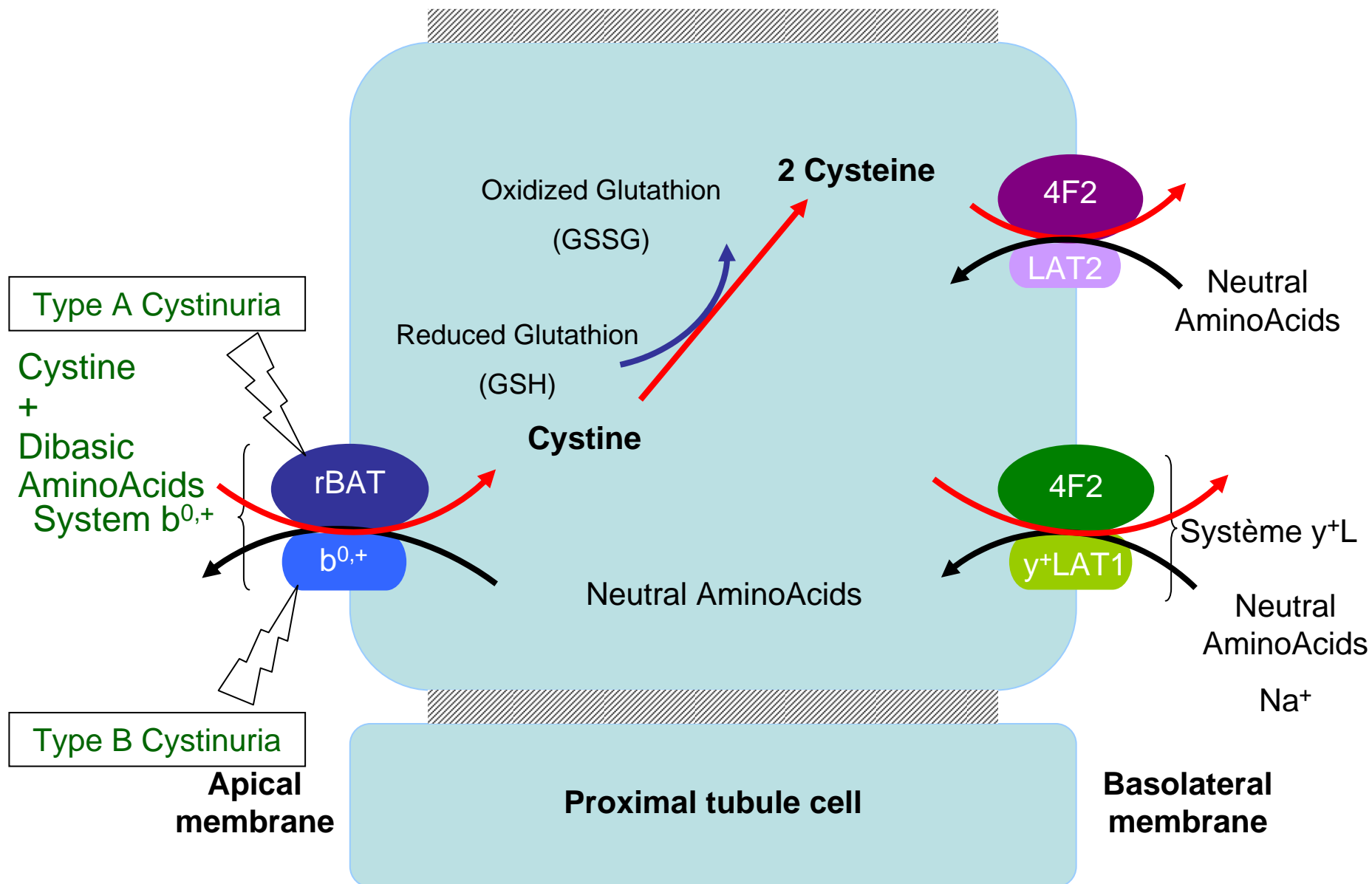
Genetic disease, autosomal recessive disorder : an abnormal transport of cystine and dibasic aminoacids (lysine, arginine, ornithine) in the proximal renal tubule.

Recurrent cystine nephrolithiasis is the main clinical feature.

Long-term outcome is poor and leads to renal insufficiency.



Physiopathology



Risk factors of stone formation

1. Biochemical factors

Metabolic risk factors

- hypercalciuria
- hypocitraturia
- hyperoxaluria
- hyperuricosuria
- abnormally low urinary pH

Dietary risk factors

- low urine volume
- high urinary sodium

Others risk factors

- high cystinuria
- alkaline urine from an infection with urea-splitting organisms

2. Modulators of intrarenal crystallization

Inhibitory compounds

- **Glycoproteins:** nephrocalcin, Tamm-Horsfall protein, osteopontin, urinary prothrombin fragment 1, bikunin)
- **Glycosaminoglycans:** chondroitin sulfate, heparan sulfate, hyaluronic acid

Promoters of crystal formation

- Inflammatory molecules such as monocyte chemoattractant protein-1 (MCP-1)

Aims of the study

- **To find the prognostic markers:**
 - nephrolithiasis is the only clinical feature
 - severity is unpredictable and leads to renal insufficiency
 - **To identify the modulators of crystal formation**
- In fine, to understand the frequency of crystal occurrence in some patients.**

Necker's department of nephrology

- **Cohorte**

- 110 patients had a nephrology consultation within the last 5 years
- mean age of 42 ± 17 years (range 11 to 88 years)

- **Convocation**

- recall by letter for information
- letter of consentment for mutation investigation
- collection of blood and urine before nephrology visit

Strategy

Selection of patients in our cohort

GRF > 60 ml/min/1.73 m²
matched in sex and age
Consentment for the study

Healthy subjects

N = 30

Cystinuric patients Mild form

- Low frequency of stone expulsion
- Crystal volume in urine < 3000 $\mu\text{m}^3/\text{mm}^3$

N = 30

Cystinuric patients Severe form

- High frequency of stone expulsion (> 2/ year)
- Crystal volume in urine > 3000 $\mu\text{m}^3/\text{mm}^3$

N = 30

Strategy

Healthy subjects
N = 10

Cystinuric patients
Mild form
N = 10

Cystinuric patients
Severe form
N = 10

Blood collection

- Genotype
- Biochemical parameters : creatinine, sodium, potassium, calcium, phosphates, urates, proteins, etc

Urinary collection

- **24h-urinary collection:** creatinine, sodium, potassium, calcium, phosphates, urates, proteins, cystine
- **First morning urines:** crystal number and crystal volume determination, sulfates, citrates, oxalates, calcium, phosphates, urates, pH, density
- **Sample collection with antiprotease:** mass-spectrometry analysis

Strategy

Urinary collection

50 ml urinary collection with antiprotease: mass-spectrometry analysis

HKUPP standard procedure

(<http://intramural.niddk.nih.gov/research/UroProt/collection-storage.shtml>)

1. **First 17 000 g spin step** to remove whole cells, large membrane fragments and other debris
2. **200 000 g spin step** with isolation solution (triethanolamine 10 mM, sucrose 250 mM, pH 7.6) to pellet the exosomes

Supernatant

Clin Prot® analysis

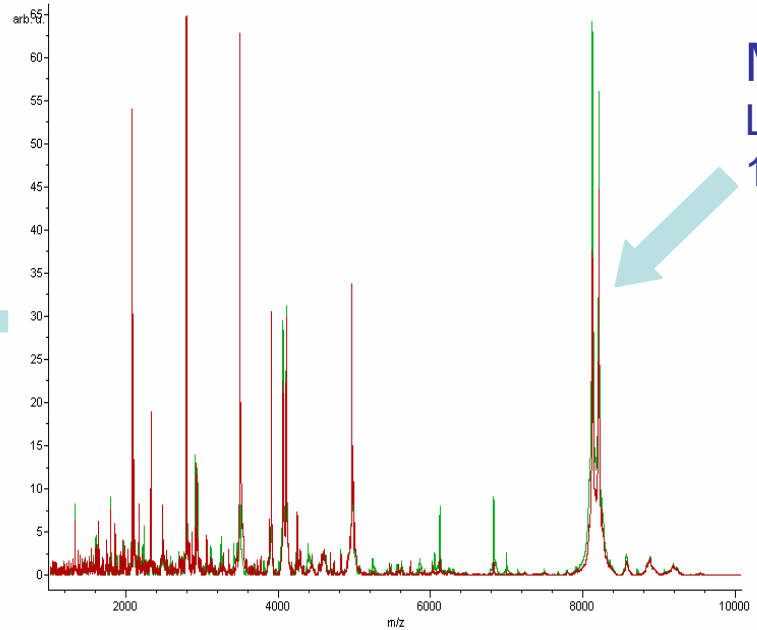
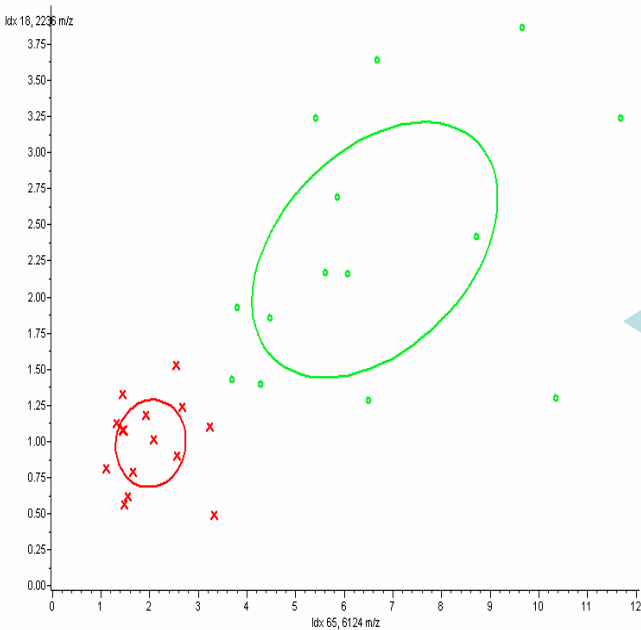
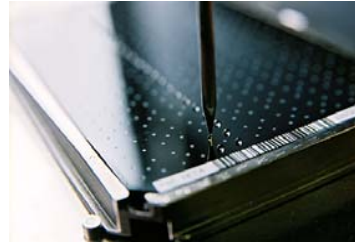
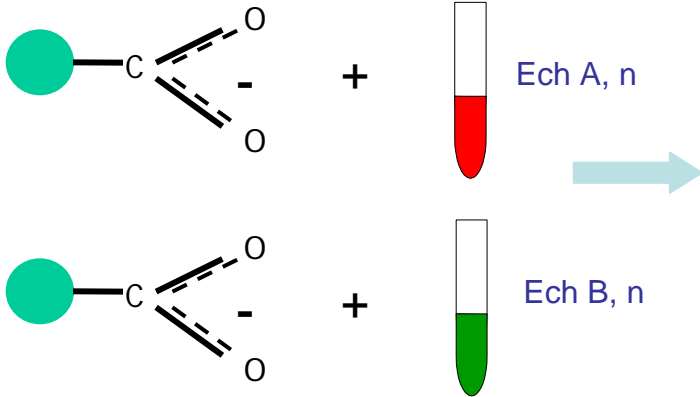
Pellet

Exosomes

Trypsin
iTRAQ labelling
Clin Prot® analysis

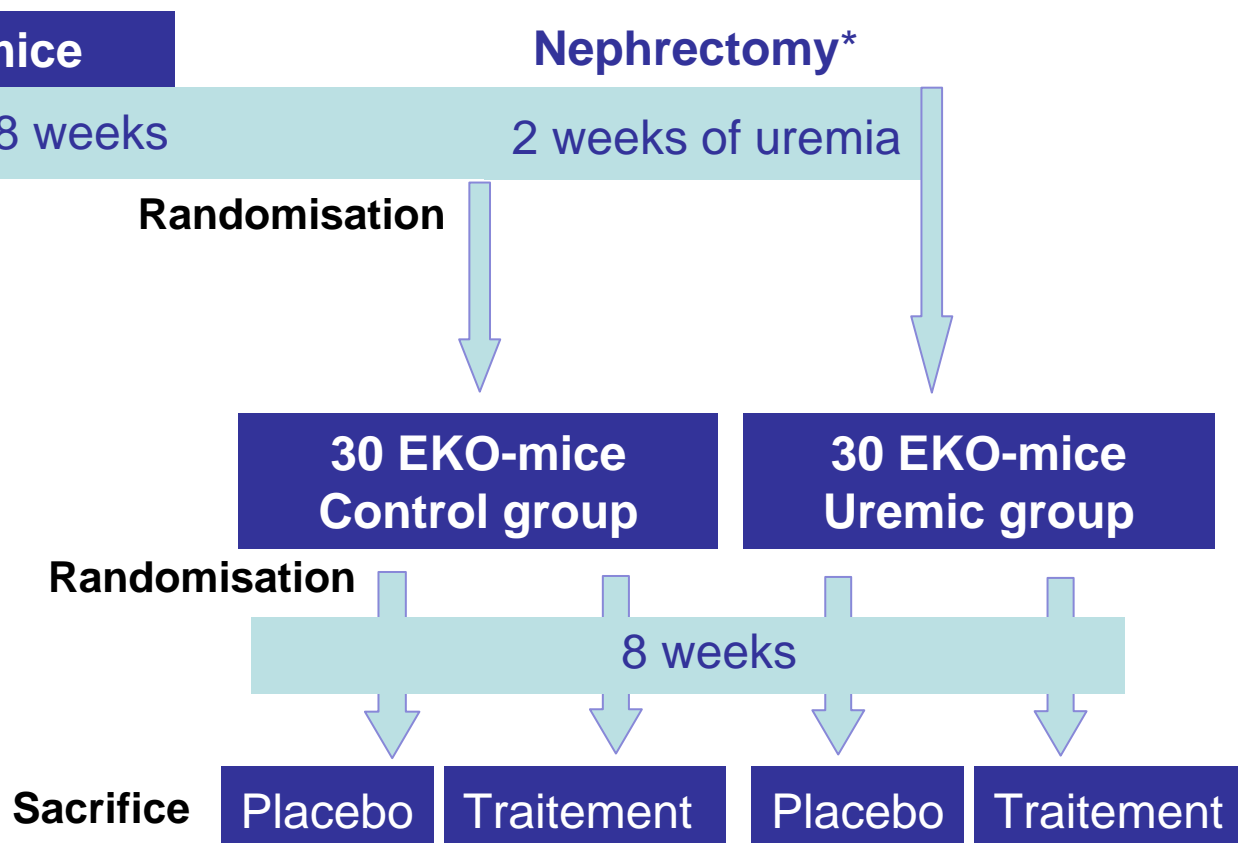
Clin Prot® system

WCX-magnetic beads
Lys, Arg, His



MALDI-TOF-MS
Linear mode
1-20 kDa

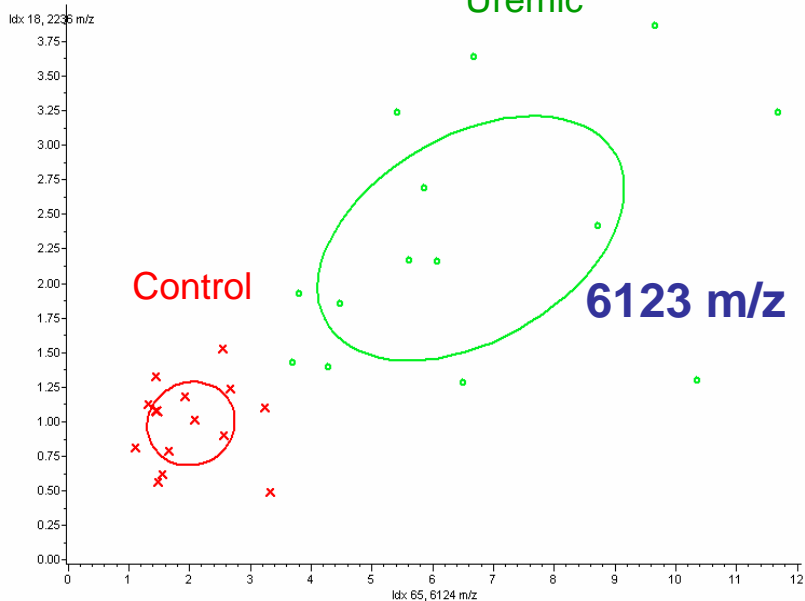
Our experience of ClinProt® proteomic profiling of serum from apolipoprotein E knockout (EKO)-mice



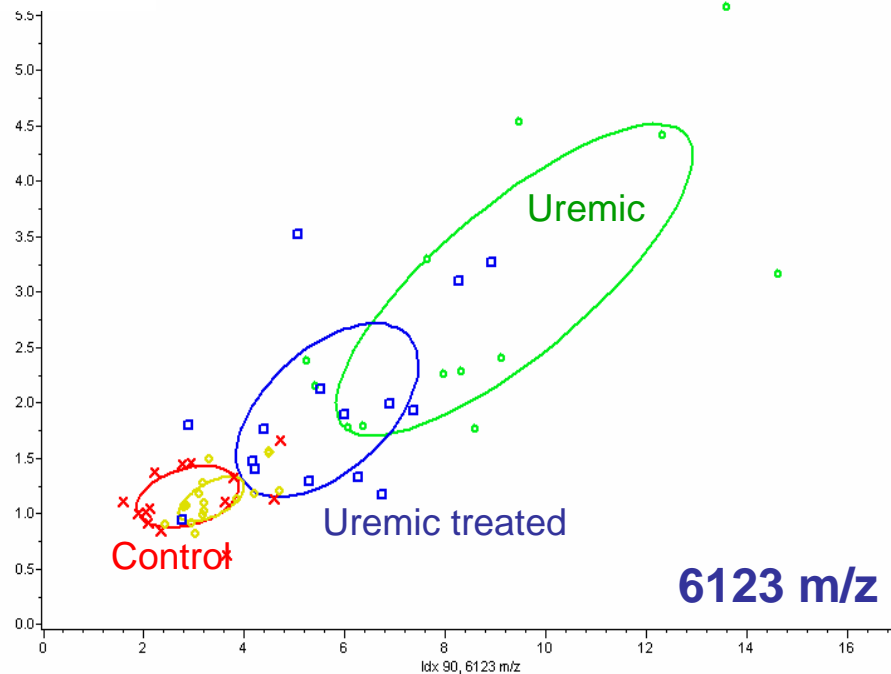
*Massy ZA et al. Uremia accelerates both atherosclerosis and arterial calcification in apolipoprotein E knockout mice. *J Am Soc Nephrol* 2005; 16:109-116

ClinProt Tools® analysis

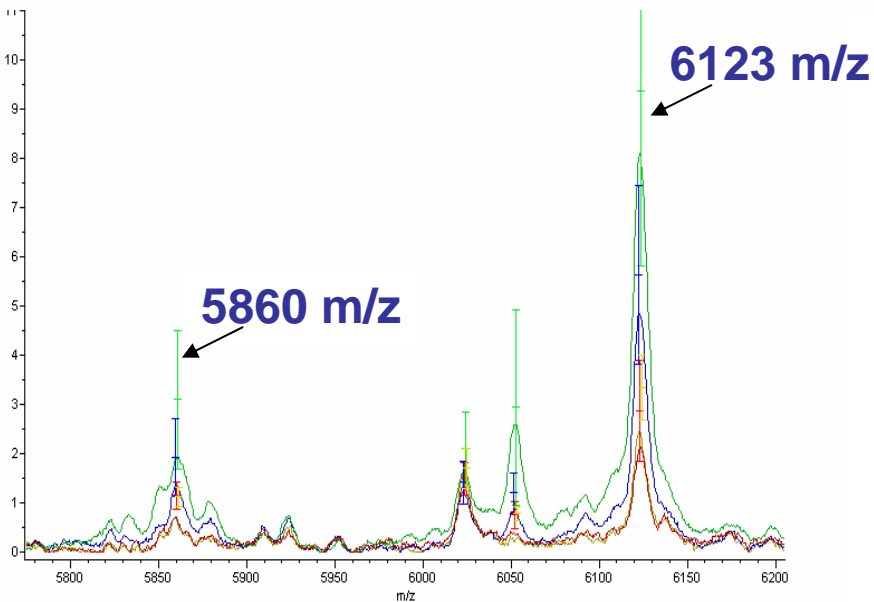
2236 m/z



5860 m/z



6123 m/z



Control-Placebo (n=14)

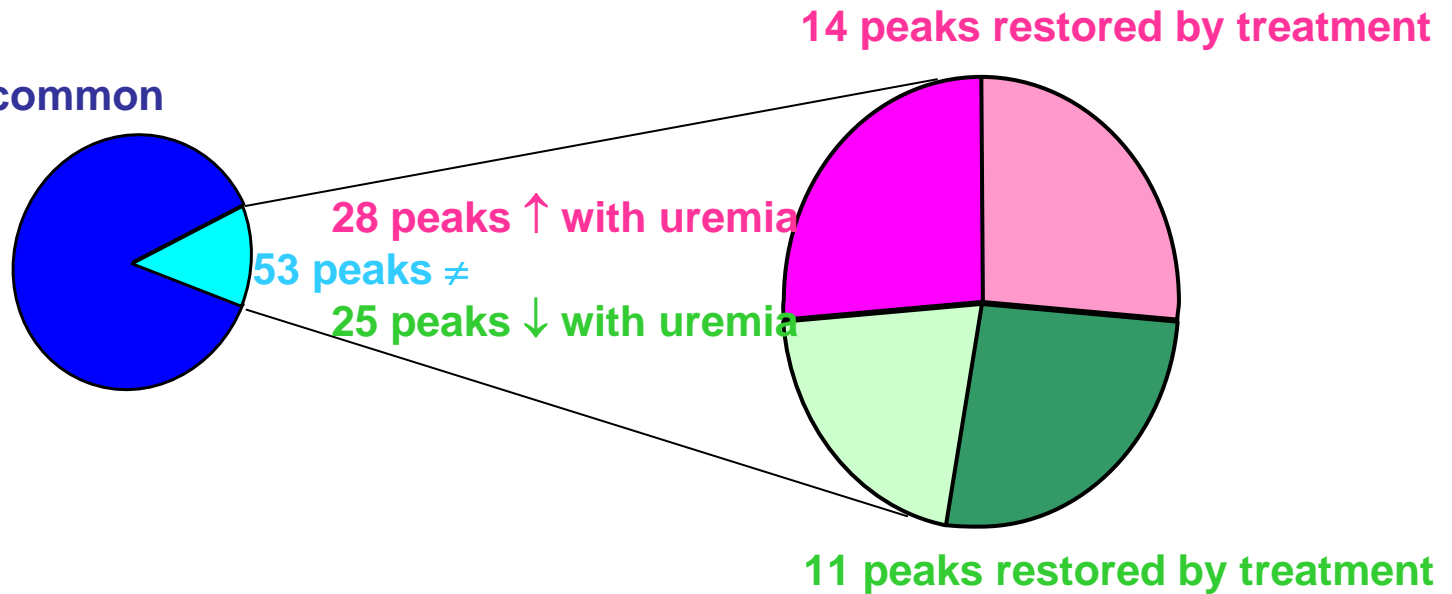
Uremic-Placebo (n=14)

Uremic-Treatment (n=14)

Control-Treatment (n=15)

ClinProt Tools® analysis

365 peaks in common



Proteomic profiling of urine

Cadieux PA *et al.* Surface-enhanced laser desorption/ionization-time of flight-mass spectrometry (SELDI-TOF MS): a new proteomic urinary test for patients with urolithiasis. *J Clin Lab Anal* 2004; 18: 170-5.

ratio in protein peak intensity at 67 / 24 kDa > 1 in patients with urolithiasis and < 1 in controls

Simpson RJ *et al.* Proteomic profiling of exosomes: exosomes as a source of urinary biomarkers. *Nephrology* 2005;10(3):283-90

Thongboonkerd V. Proteomics and kidney stone disease. *Contrib Nephrol* 2008;160:142-58