University College London Hospitals NHS
NHS Foundation Trust

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Laboratory investigation of metabolic stone disease with emphasis on primary hyperoxaluria

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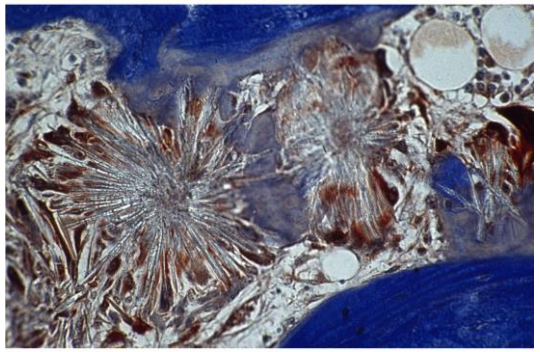


Aim

- Introduction to metabolic stone disease
- Basic laboratory investigations and factors affecting interpretation
- Specialist testing
- Role of genetic testing

Introduction

- Renal stone disease affects up to 10% of the population in the Western world
- Risk factors:
  - Fluid intake
  - Occupation
  - Dietary
  - Drugs
  - Developmental disorders
  - Genes

Metabolic stone disease

- Arising from derangement of a metabolic pathway, inherited or acquired
- Higher index of suspicion if a child, bilateral stones, recurrent stone former, consanguinity in family
- Leading to:
  - too much of an insoluble substrate, e.g. dihydroxyadenine
  - diversion to an insoluble product, e.g. oxalate
  - failure of transport, e.g. cystine
  - loss of stone inhibitors, e.g. effect of topimirate on citrate

Analysis of stone

- Helpful for the following reasons:
  - Can identify rare stone types and thus initiate appropriate investigations
  - Can direct treatment
- Only useful if laboratory analysis is good
  - Wet chemistry tests are prone to problems
  - Infra red analysis preferred but requires expertise to interpret
  - The % of components is a useful indicator

EQA scheme findings

Stone material sent out	
Cystine	2/30 labs did not recognise
Protein (Albumin)	1 cystine 1 xanthine 1 carbonate, CaOx
Silica	2 infrared labs failed to recognise this, one calling it carbonate 4 wet chemistry found CaOx

Investigations for stone formers

24h plain urine

- Volume
- Creatinine
- Sodium
- Urate
- Protein
- Cystine (spot test)

24h acidified urine

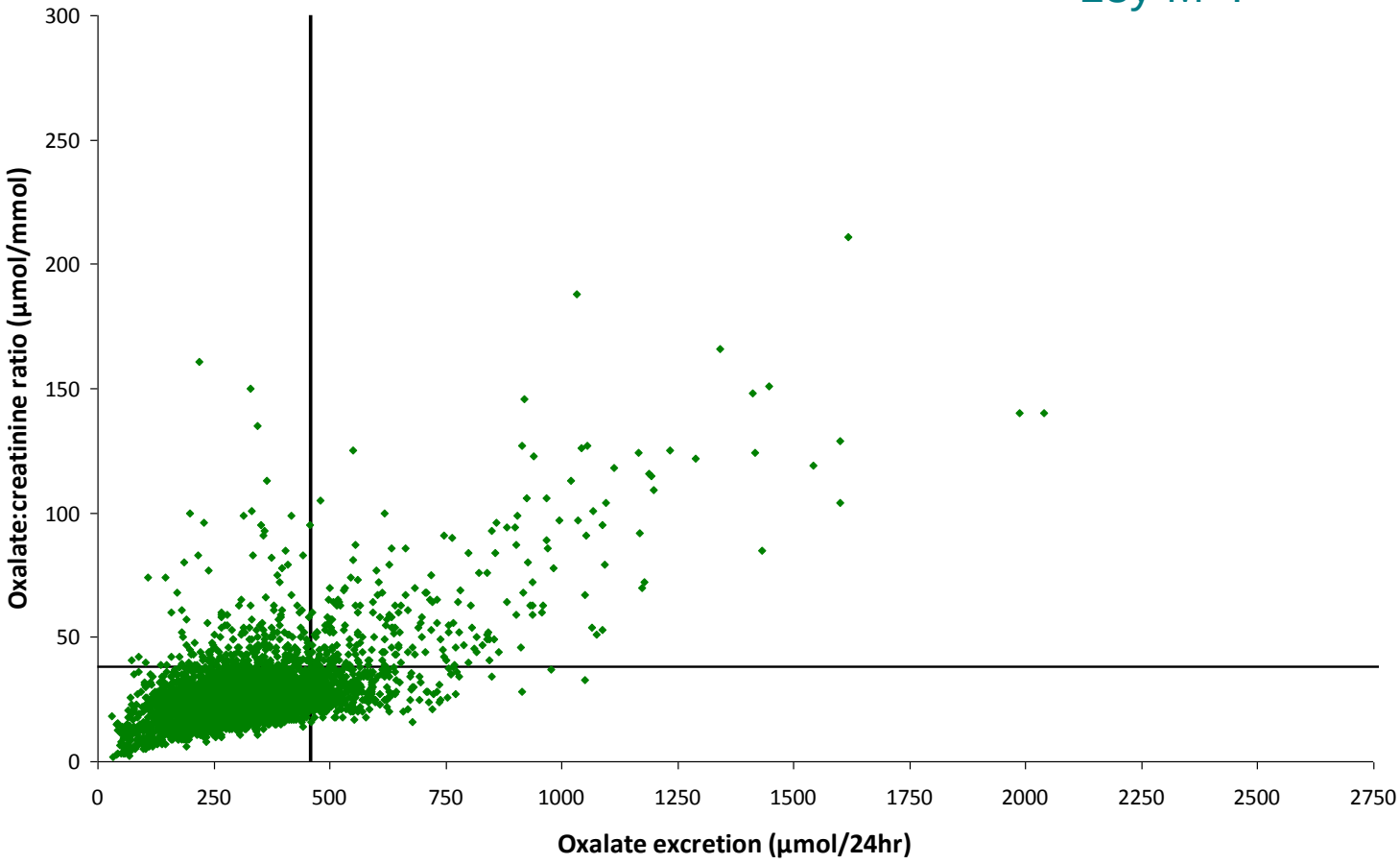
- Volume
- Creatinine
- Calcium
- Oxalate
- Citrate
- Magnesium

Does it have to be a 24h collection?

Random	24h
Easy for patient	Inconvenient for patient
Affected by dietary intake prior to collection	Smooths out dietary influences
Affected by concentration (normalise on creatinine)	Affected by under and overcollection

Urine oxalate excretion vs ox:cre ratio

>18y M+F



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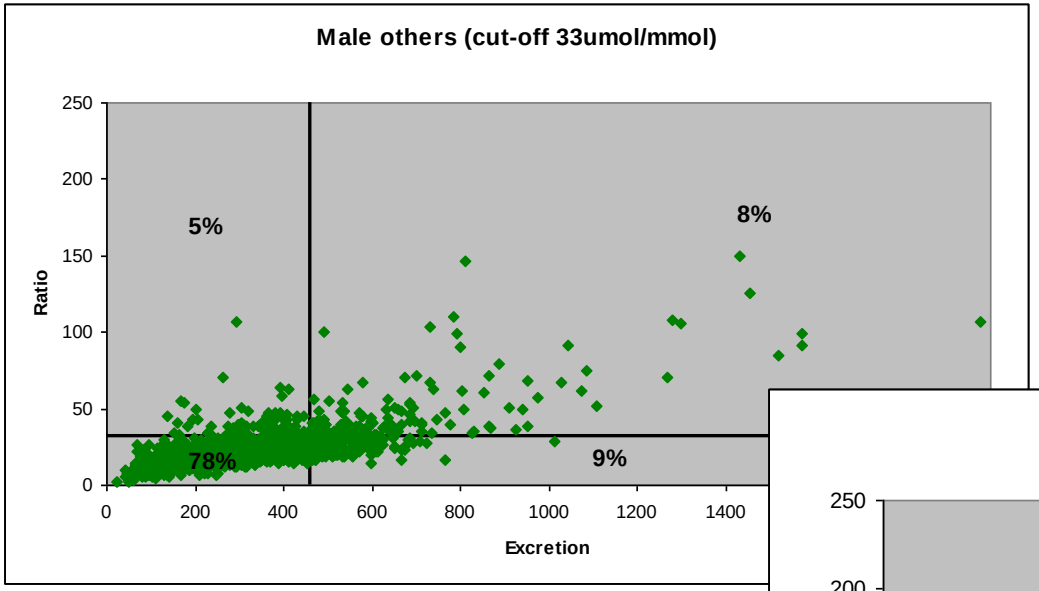
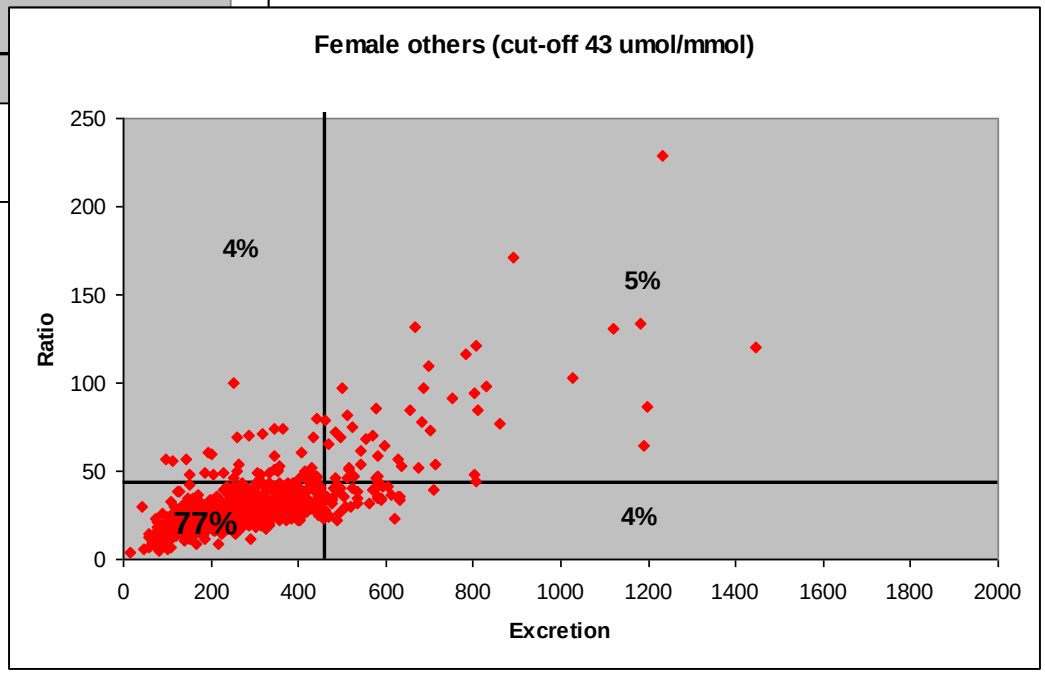
Do sex-related reference ranges fit better for adults?





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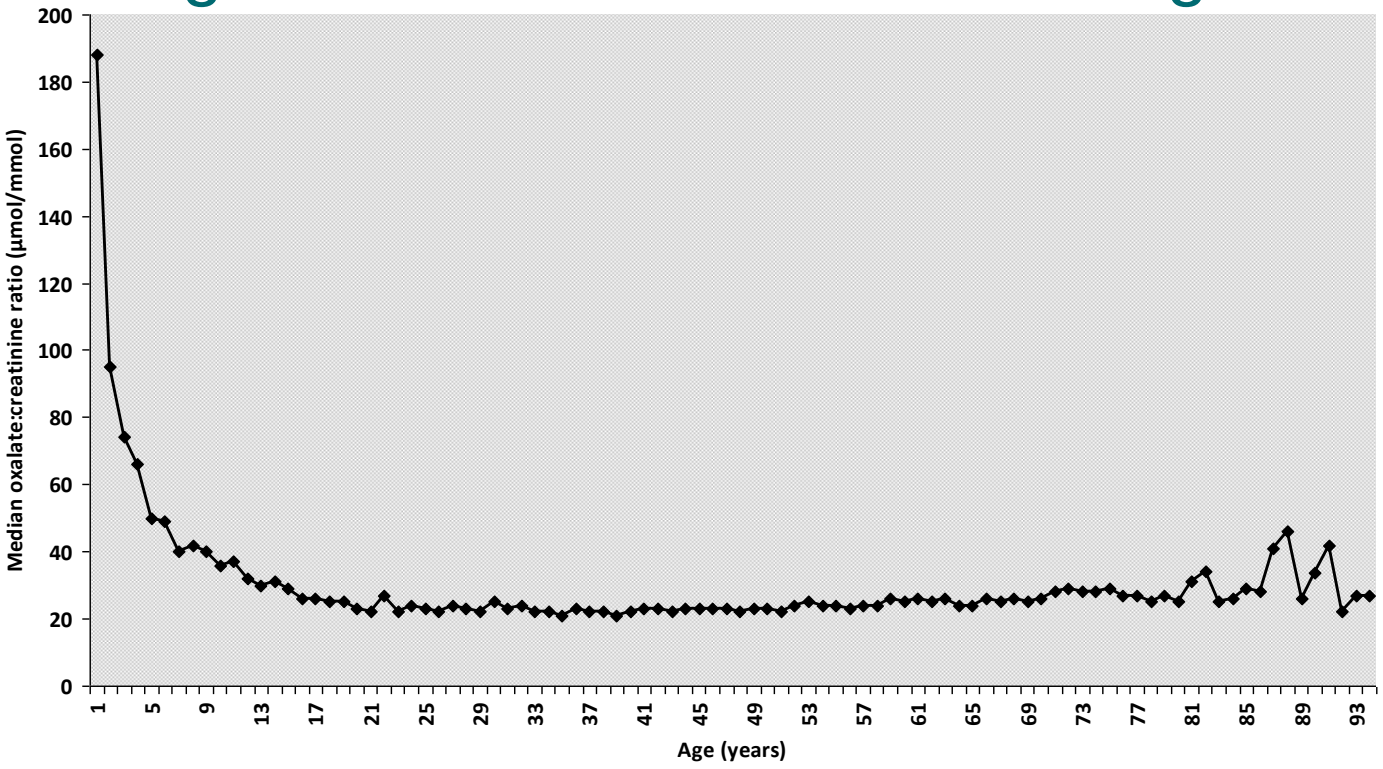
Interpretation of urine oxalate results
Change in oxalate:creatinine with age



n=6569

Clifford-Mobley, Tims & Rumsby, 2015

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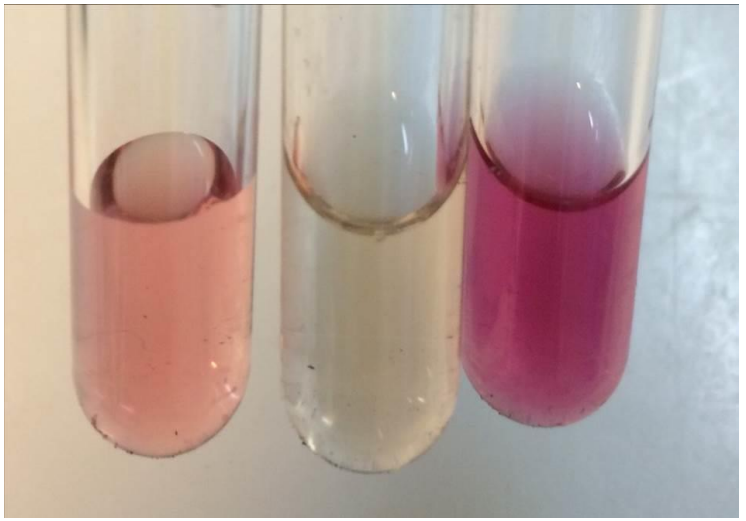
More specialised testing

- Organic acid profile
  - May pick up oxalate, glycerate but less reliable than specific assays
- Purines
  - Urine purines and enzymology if purine disorder suspected,e.g DHA stone (APRT deficiency) or very low serum uric acid as indicator of Xanthine dehydrogenase defy.
- Primary hyperoxaluria metabolites
- Cystine quantitation

Cystinuria

- Two genetic defects recognised, SLC3A1 (type 1) and SLC7A9 (non type 1)
- Heterozygotes for non type 1 may show increased cystine

Screening test with Na nitroprusside and cyanide. Positives are quantitated by amino acid chromatography

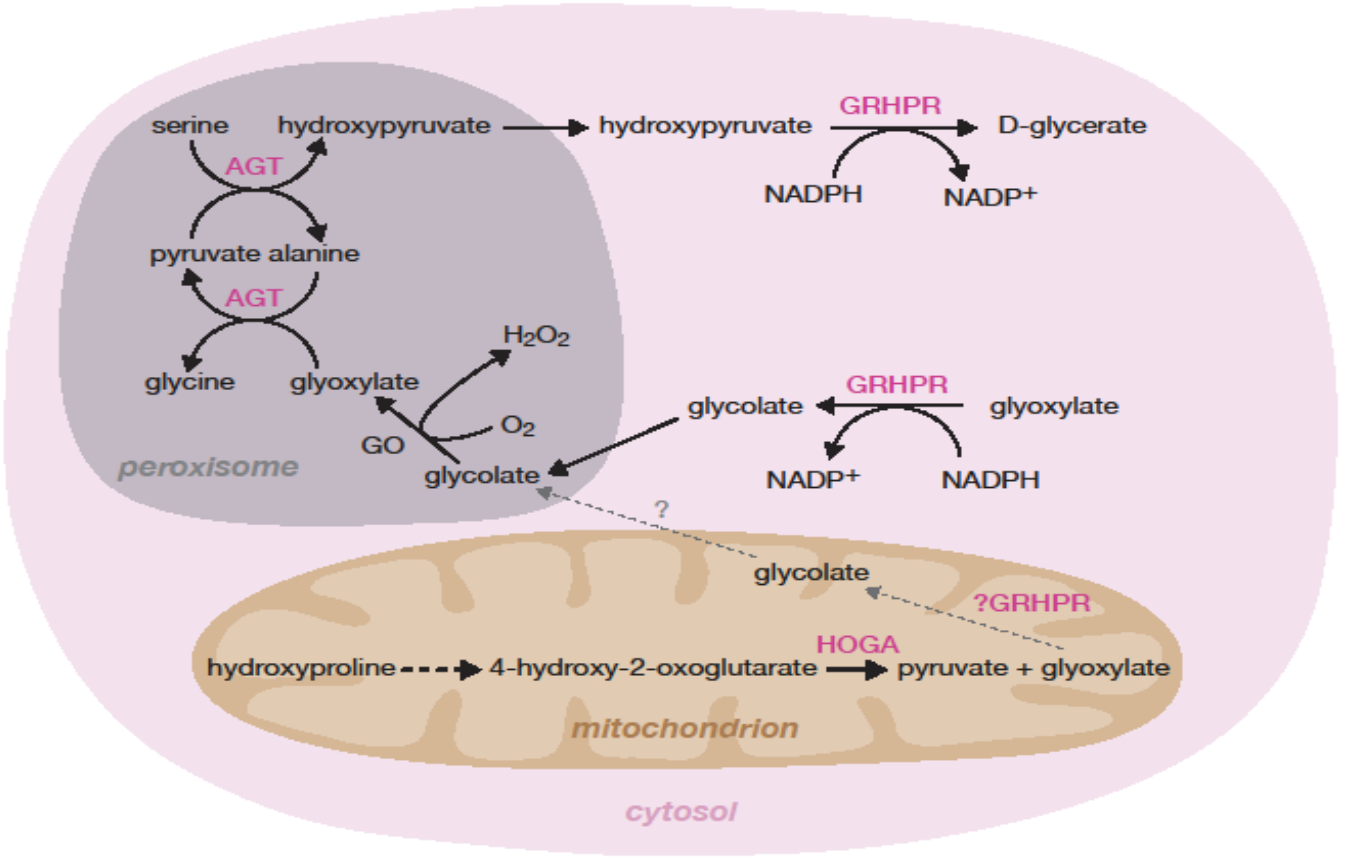


420umol/L

Genes implicated in metabolic stone disease

- Stone type
- Calcium oxalate
  - Primary hyperoxaluria types 1, 2 and 3 (*AGXT,GRHPR, HOGA1*)
  - *SLC26A1* transporter
- Cystine
  - *SLC3A1, SLC7A9*
- Purine stones
  - *APRT, XDH, MOCOS*
- Calcium phosphate
  - Dent's disease *CLCN5*

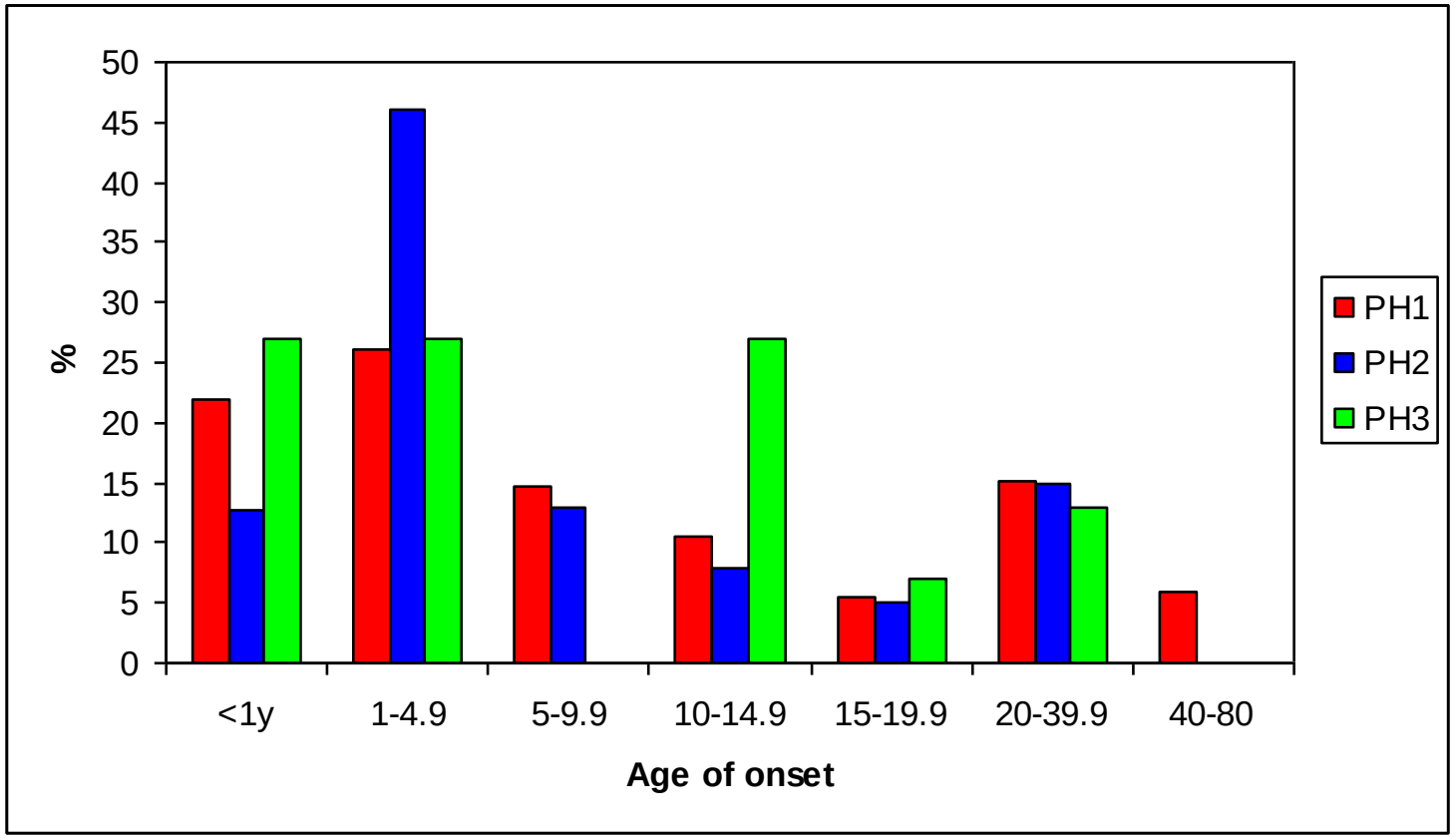
Primary hyperoxaluria



Preliminary diagnosis of Primary hyperoxaluria

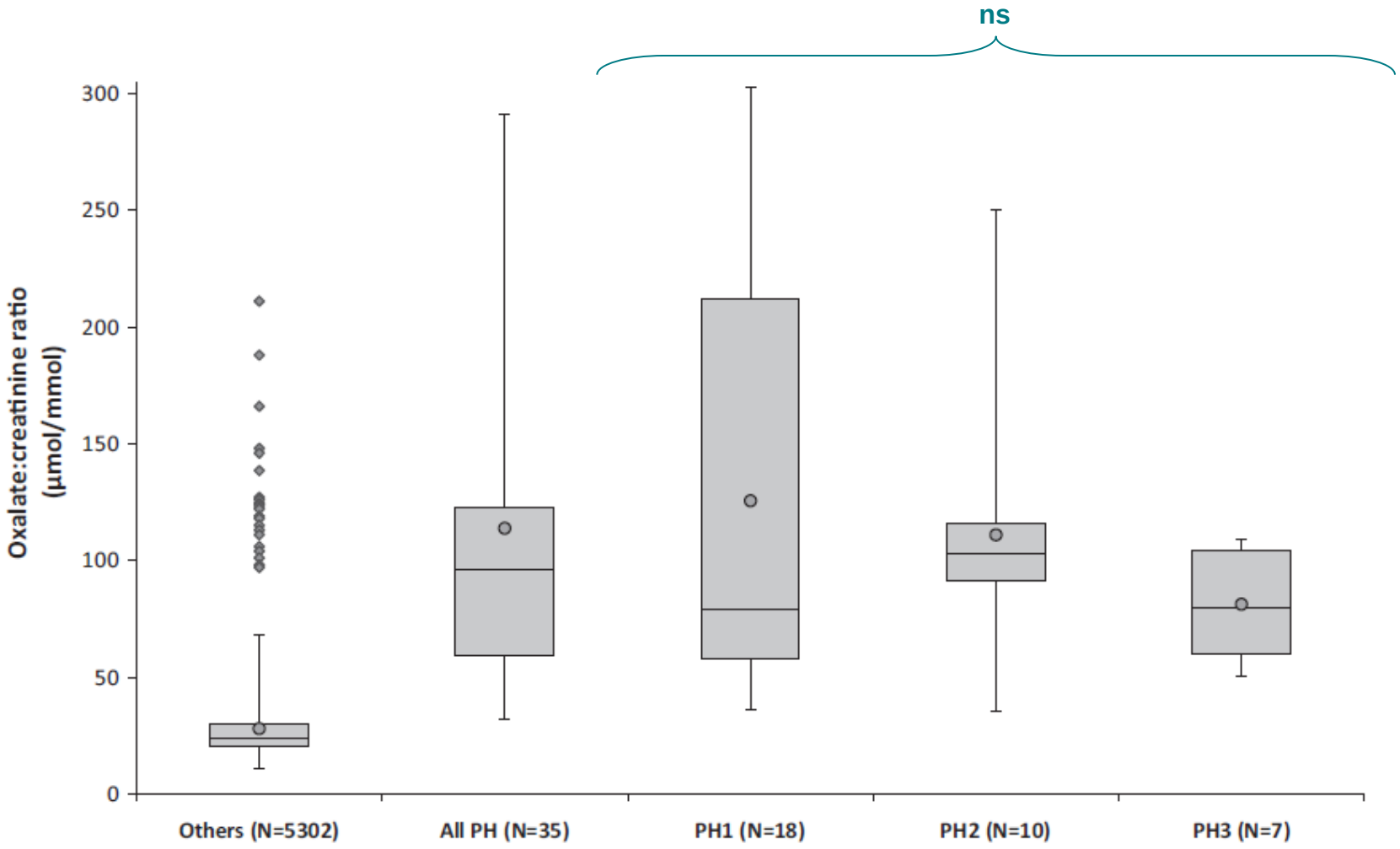
- Raised urine oxalate on more than one occasion
- Exclude diet, GI disease
- Analysis of metabolites
  - Glycolate
  - Glycerate
  - 4-Hydroxy-2-oxoglutarate (HOG) and dihydroxyglutarate (DHG)
- Plasma oxalate
  - Of limited value and only for patients in renal failure

Age of presentation of PH



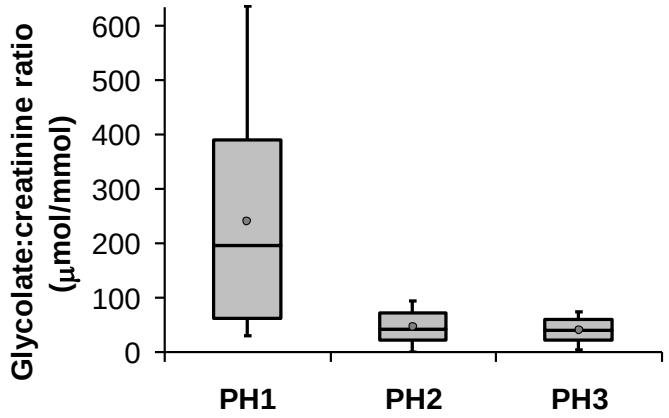
Williams et al. 2011

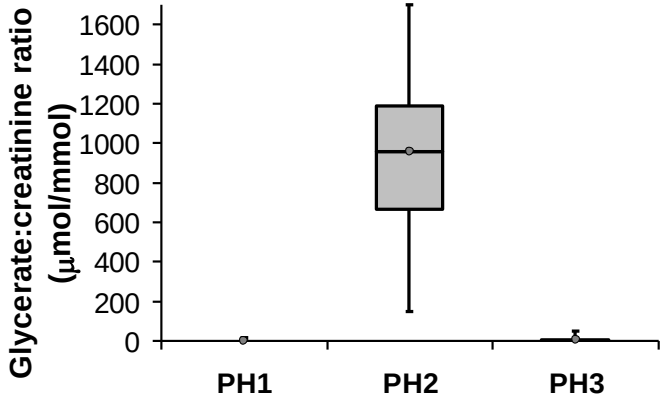
Ox/Cre ratio in all patients according to dx

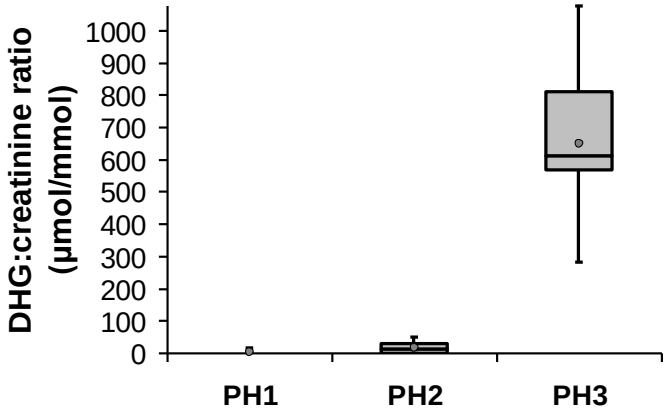


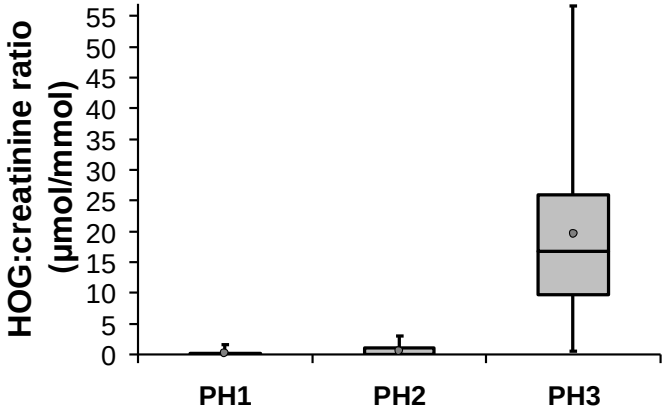
Clifford-Mobley, Tims and Rumsby, 2015

Use of PH metabolites to make a preliminary diagnosis









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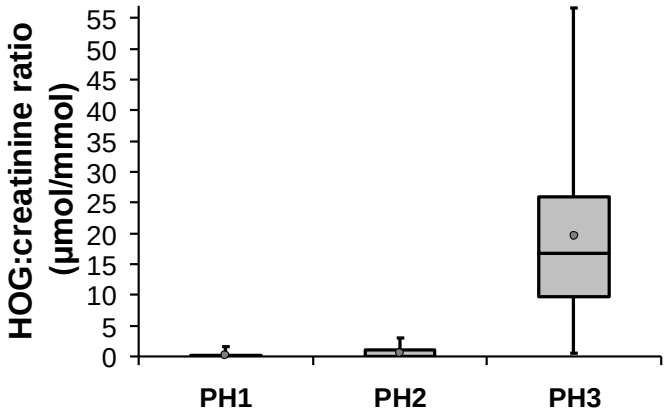

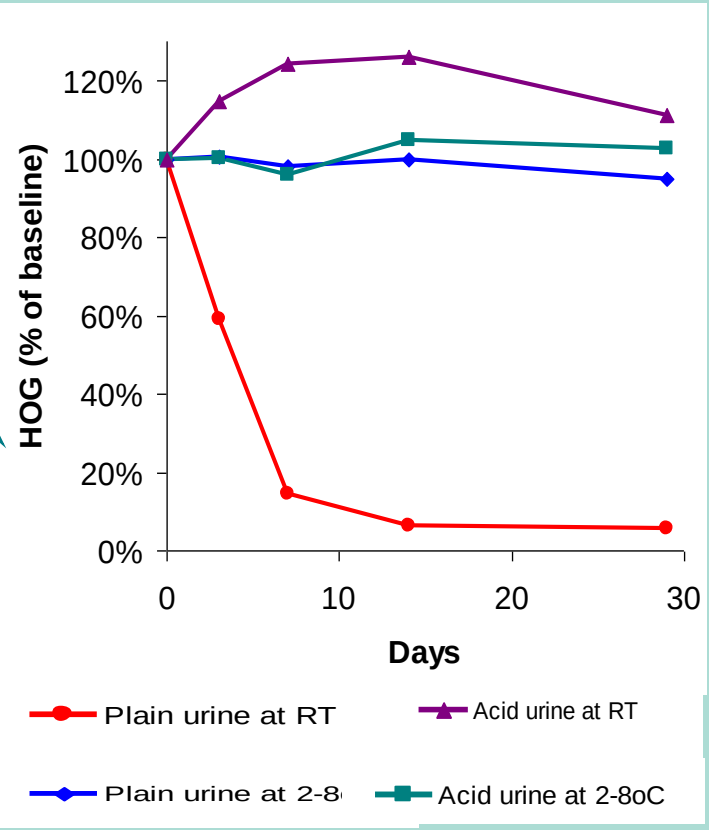

HOG is an unreliable marker

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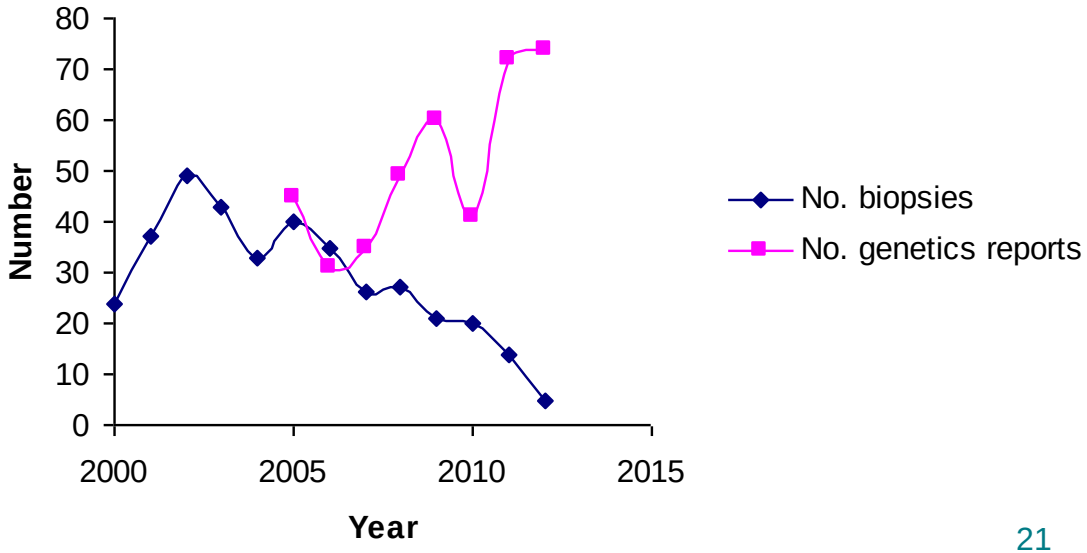
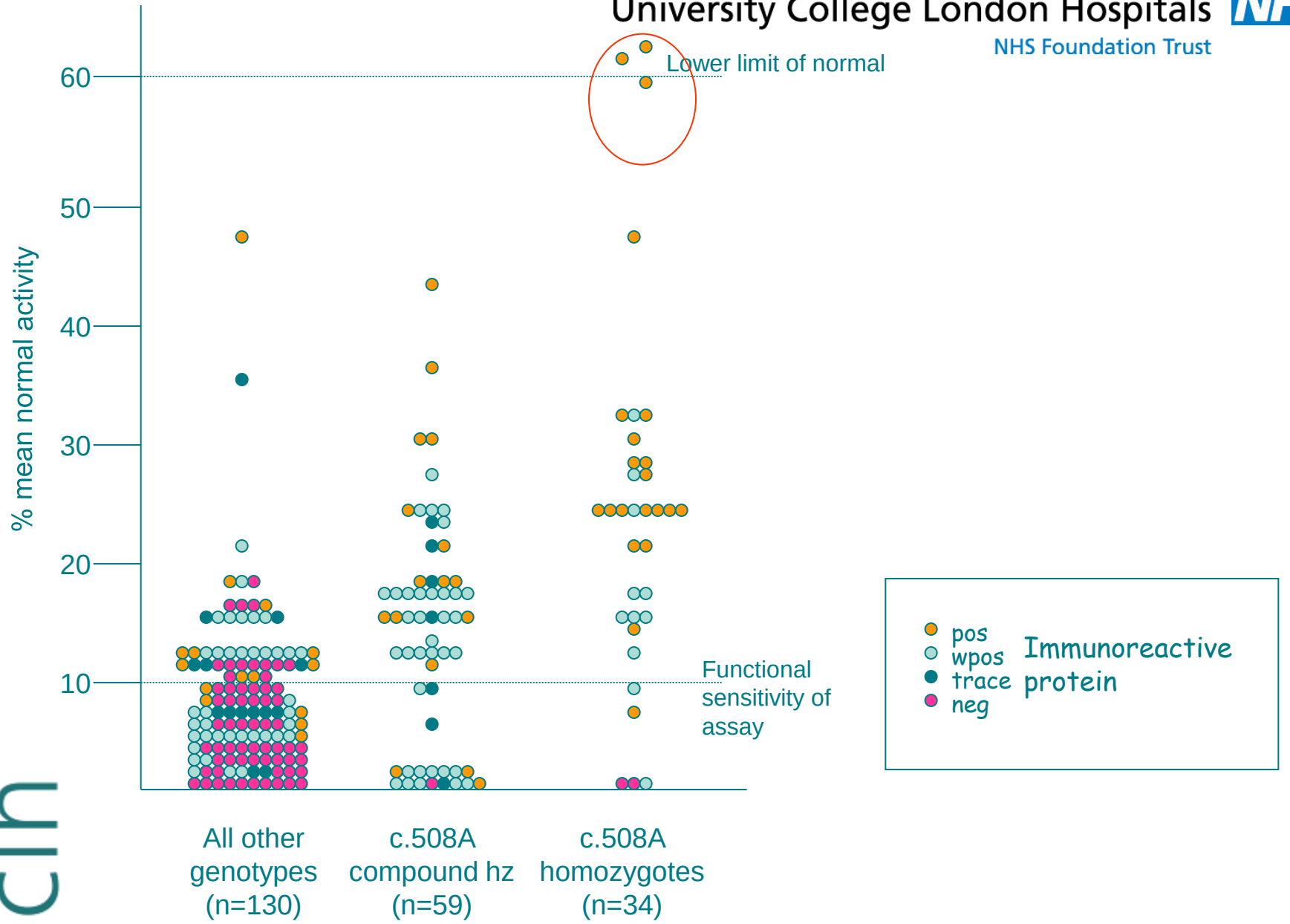
Enzyme activity

- Measurement of enzyme activity and immunoreactivity
- Only available for PH1 and PH2
- Liver biopsy required
  - Sample integrity a problem
  - interpretation
- Fallen out of favour



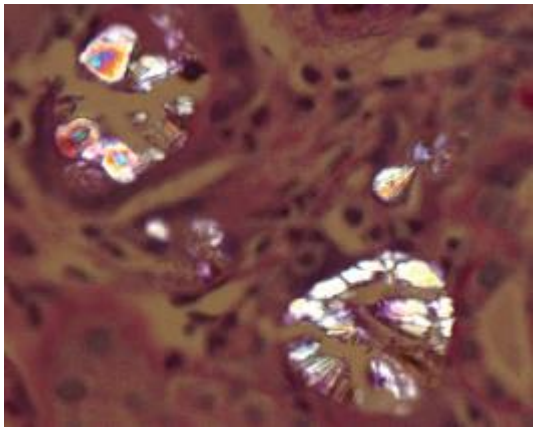
Genetic testing

- Confirmation of disease
  - Implications for treatment, e.g. Primary hyperoxaluria liver-kidney tx vs renal transplant only
  - Responsiveness to pyridoxine treatment (PH1 only)
- Allows testing of other family members, including prenatal diagnosis in severely affected cases
- Removes ambiguity that can sometimes occur with biochemical testing.


Lower limit of normal
% mean normal activity
60
50
40
30
20
10
Functional sensitivity of assay
All other genotypes (n=130)
c.508A compound hz (n=59)
c.508A homozygotes (n=34)
pos
wpos
trace
neg
Immunoreactive protein

Infantile onset

- Full term delivery
- 2 month, hypocalcaemic tonic-clonic seizures, renal failure, acidosis
- Bilateral echogenic kidneys, on biopsy showing oxalate crystals



- Age 4 months
- Urine ox/cre 685 umol/mmol (<98)
- Plasma oxalate 307 umol/L (creatinine 246 umol/L)

Further investigations

- Genetic testing carried out for PH1
- c.[508G>A][466G>A] (p.Gly170Arg, Gly156Arg)

- Dx: consistent with primary hyperoxaluria type 1

  - Living-related liver transplant
  - Kidney transplant age 7y

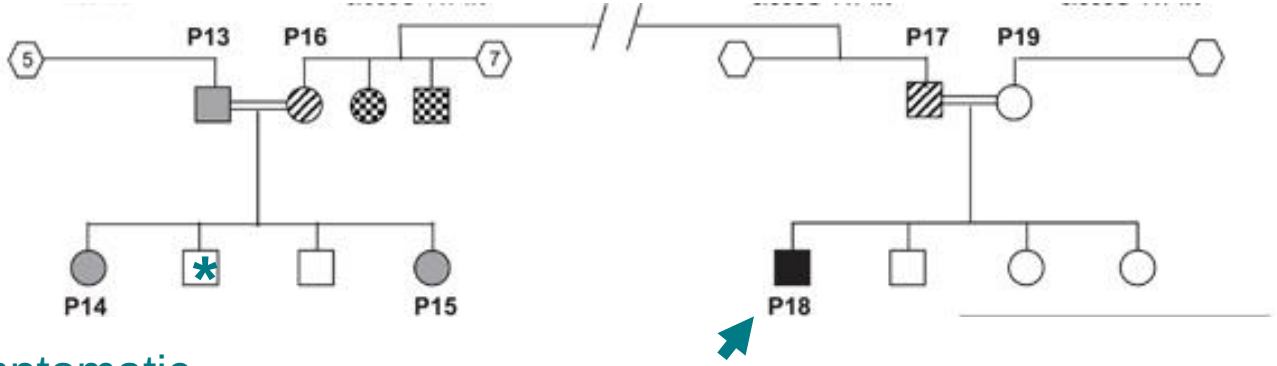
Downside of genetic testing

- Significance of changes found
  - Gene specific database http://www.uclh.nhs.uk/phmd also submitted to ClinVar
  - Includes reported variants plus expression data where available

- What if only a single pathological mutation found?
  - Affected but other mutation not identified?
  - Some other disease and incidental finding of carrier status?

Single mutation only

- Carrier or affected, with undiscovered other mutation?



*Symptomatic

Liver bx: AGT activity just below normal, not as low as index

AGXT: c.603C>A heterozygous

HOGA1: c.569C>T (p.Pro190Leu)

Final dx: PH3!

Index case

Dx PH1 by liver biopsy (nil AGT activity)

DNA: *AGXT*: c.603C>A homozygous

Confirmed PH1

Role of patient registries

- Rare diseases
- Registries allow information to be accrued about natural history of disease
- Identifies patients for clinical trials
- Laboratories should be encouraged to flag up registries when reporting
- But: requires a lot of effort on behalf of clinicians to input data

Summary

- Metabolic stone is rare and often takes a long time to make a diagnosis
- Should be considered in anyone presenting in childhood or with recurrent bilateral disease
- A combination of biochemical and genetic testing provides the best approach to diagnosis