Laboratory diagnosis of Primary hyperoxaluria

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Steps involved in diagnosis

Metabolite

Enzyme (protein)

Gene (DNA)
Tissue/body fluid needed for analysis

Metabolite  Urine
Enzyme  Liver biopsy
Gene  blood
Specialist tests

- Tests are **not** available 24h a day
- Done in a small number of centres and can take time to arrange and send
Specialist tests

• Tests often MANUAL
  - take time (days)
  - run in batches to keep down costs

• RESULT
  - Will take time (up to one month) to get all the results
Steps involved in diagnosis

Metabolite

Enzyme (protein)

Gene (DNA)
• Symptoms may suggest presence of kidney stones
  - e.g. blood in urine, pain
• Prompts laboratory investigations
  - Urine
    • stone forming substances, e.g. Oxalate, calcium
    • substances that help stop stones forming, e.g. citrate
  - kidney stone content
Analysis of kidney stones
Urine oxalate

- Usually one the first tests done in patients with kidney stones

- Is the amount of oxalate present greater than that found in people without disease?
Urine samples

Oxalate

Random or 'spot' urine

or

24h urine collection
Urine oxalate

- Test is not specific for PH
- can get increased oxalate for other reasons including the diet (BEWARE CHOCOLATE!)

Hours after eating a bar of chocolate

Urine oxalate

HIGH OXALATE!
Urine oxalate is usually much higher in cases of PH

ULN=upper limit found without disease

3x ULN
2x ULN
ULN

Urine oxalate in PH
• added to other clinical symptoms, raised oxalate may suggest further tests need to be done
  
  - e.g. gene (DNA) analysis or liver enzyme studies
Steps involved in diagnosis

Metabolite

Enzyme (protein)

Gene (DNA)
PH is caused by a defective protein (enzyme)

- **PH1**
  - Lack of alanine:glyoxylate aminotransferase = AGT

- **PH2**
  - Lack of glyoxylate reductase = GR

- **PH3**
  - Lack of hydroxyoxoglutarate aldolase = HOGA
Gene
→ Enzyme
→ Metabolite

**PH1**
AGXT
AGT

**PH2**
GRHPR
GR

**PH3**
HOGA1
HOGA

glyoxylate → glyoxylate

**OXALATE**
Enzyme tests for PH

- Need a (tiny) liver biopsy
- Only available for PH1 and PH2
- Rarely required now as genetic testing has taken over.

Data from UCLH lab
Steps involved in diagnosis

Metabolite

Enzyme (protein)

Gene (DNA)
Genetic Testing

- Genes are made of DNA
- DNA is present in the nuclei of all cells, including those in the blood
For genetic tests

• We need blood or saliva samples from the patient and from the parents.
DNA is packaged into chromosomes. One of each pair comes from Mum, the other one from Dad.

PH1, PH2 and PH3 are recessive diseases. This means that the child must inherit two defective genes, one from each parent, to have the disease.
DNA analysis

chromosome
DNA helix
AGXT gene
What we see in the lab
Mutations

• Changes in the DNA sequence which will cause the gene to fail are called MUTATIONS
  - mutations in AGXT will cause PH1
  - mutations in GRHPR will cause PH2
  - mutations in HOGA1 will cause PH3
We find these changes by 'reading' the DNA sequence.

'N' shows us that something is different.
How can DNA testing help?

• We can use it to diagnose PH1, PH2 and PH3 in:
  - A patient with symptoms
  - other family members e.g. brothers and sisters
  - In a fetus, i.e. prenatal diagnosis
T is the normal gene, C is the faulty gene.

Any child inheriting the green marker from Dad and the blue marker from Mum will have PH2.
Chorionic villus biopsy taken under ultrasound guidance at 10-12 weeks gestation.

Isolate DNA

Look for mutation

Result available within 10 days (normal, carrier or affected)
Disease genes are the green and pink markers. The fetus has inherited the green but not the pink marker and is therefore a carrier only and does not have the disease.
Summary

• Diagnosis of PH requires specialised tests that take time to perform
• Urine oxalate is one of the first tests
• DNA testing
  - makes the final diagnosis of PH1, PH2 or PH3
  - can also be used for diagnosis of other family members and prenatal diagnosis
• Liver enzyme testing may be needed if no mutation found
Thank you

- www.uclh.nhs.uk/biochemistry
Some research questions

• Can urine samples taken at a fixed time of day substitute for 24h collections?

• Can we establish methods to measure specific metabolites in urine from PH3 patients?