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

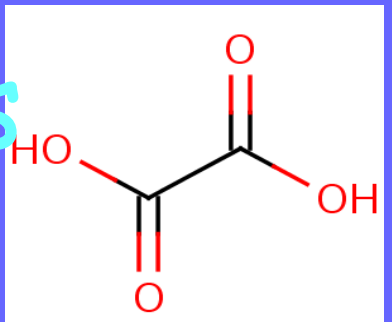
- Tests are <u>not</u> available 24h a day
- Done in a small number of centres and can take time to arrange and send

Specialist tests

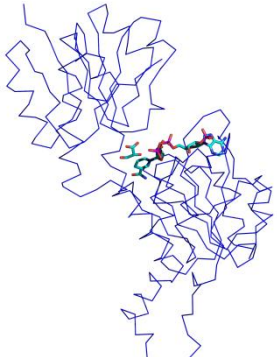
- Tests often MANUAL
  - take time (days)
  - run in batches to keep down costs
- RESULT
  - Will take time (up to one month) to get all the results

Steps involved in diagnosis

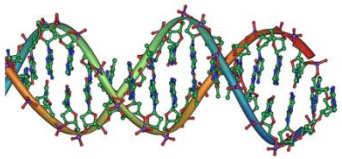
Metabolite



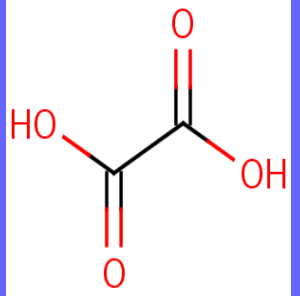
Enzyme (protein)

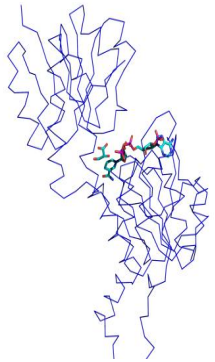


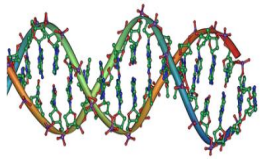
Gene (DNA)



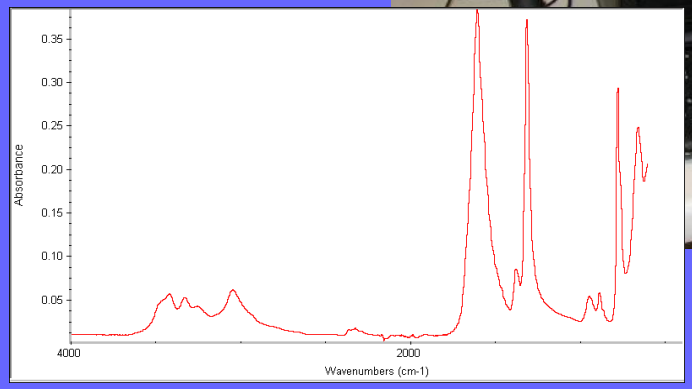
- Symptoms may suggest presence of kidney stones
  - e.g. blood in urine, pain
- Prompts laboratory investigations
  - Urine
    - stone forming substances, e.g. Oxalate, calcium
    - substances that help stop stones forming, e.g. citrate
  - kidney stone content

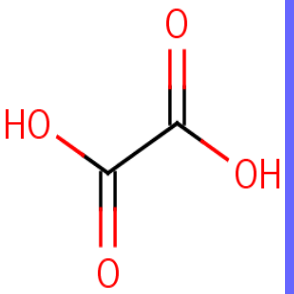


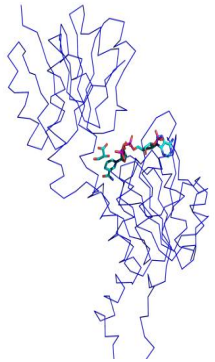


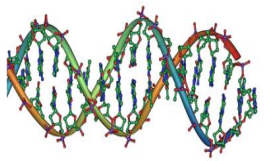


Analysis of kidney stones









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?

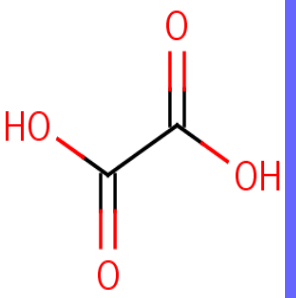
Urine samples

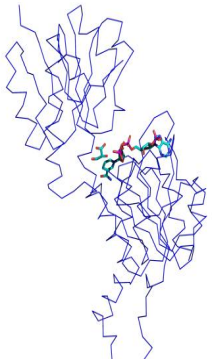
Random or 'spot' urine

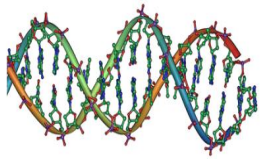
or

24h urine collection

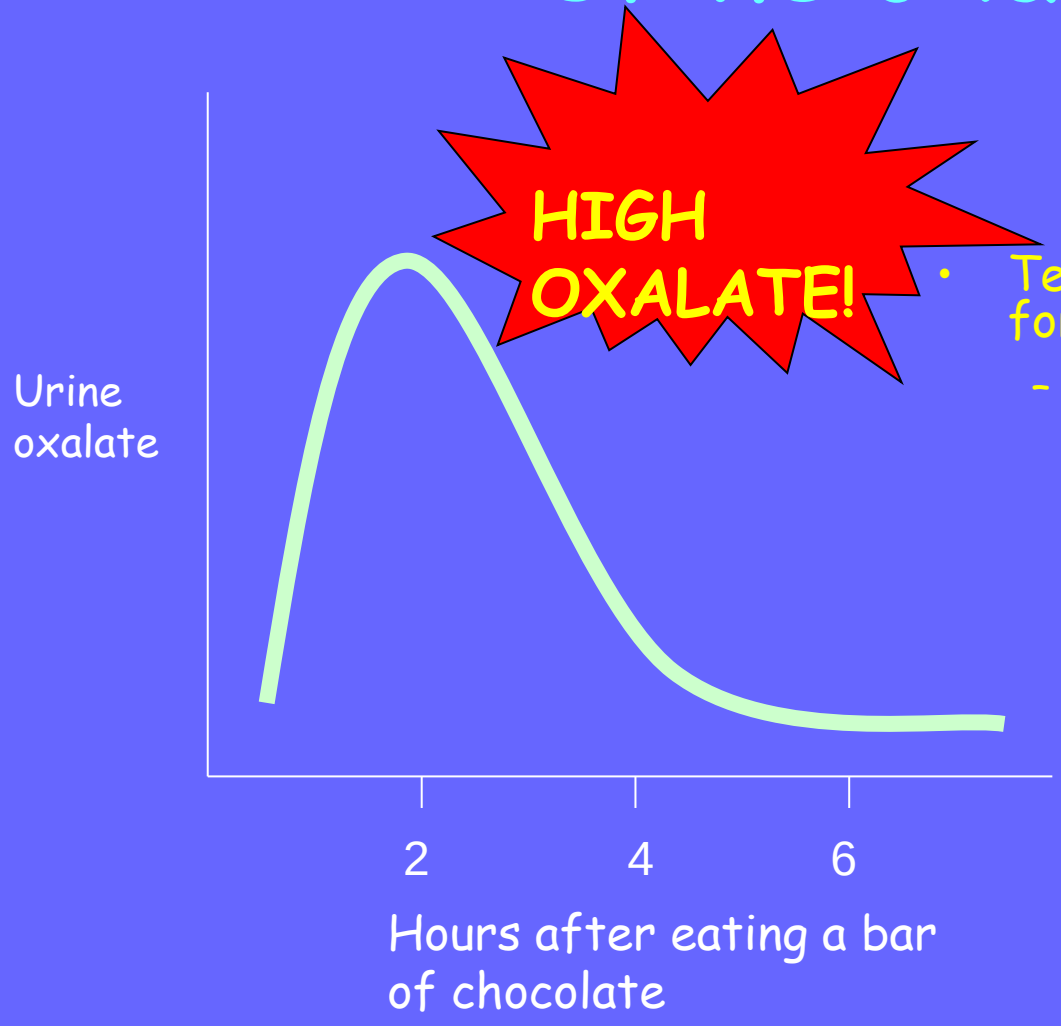
Oxalate



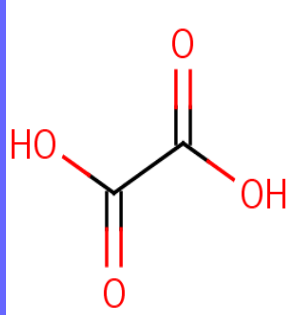


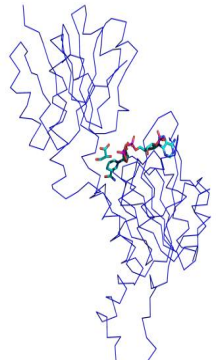


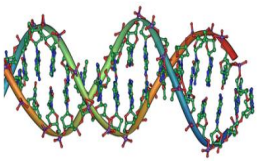
Urine oxalate



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

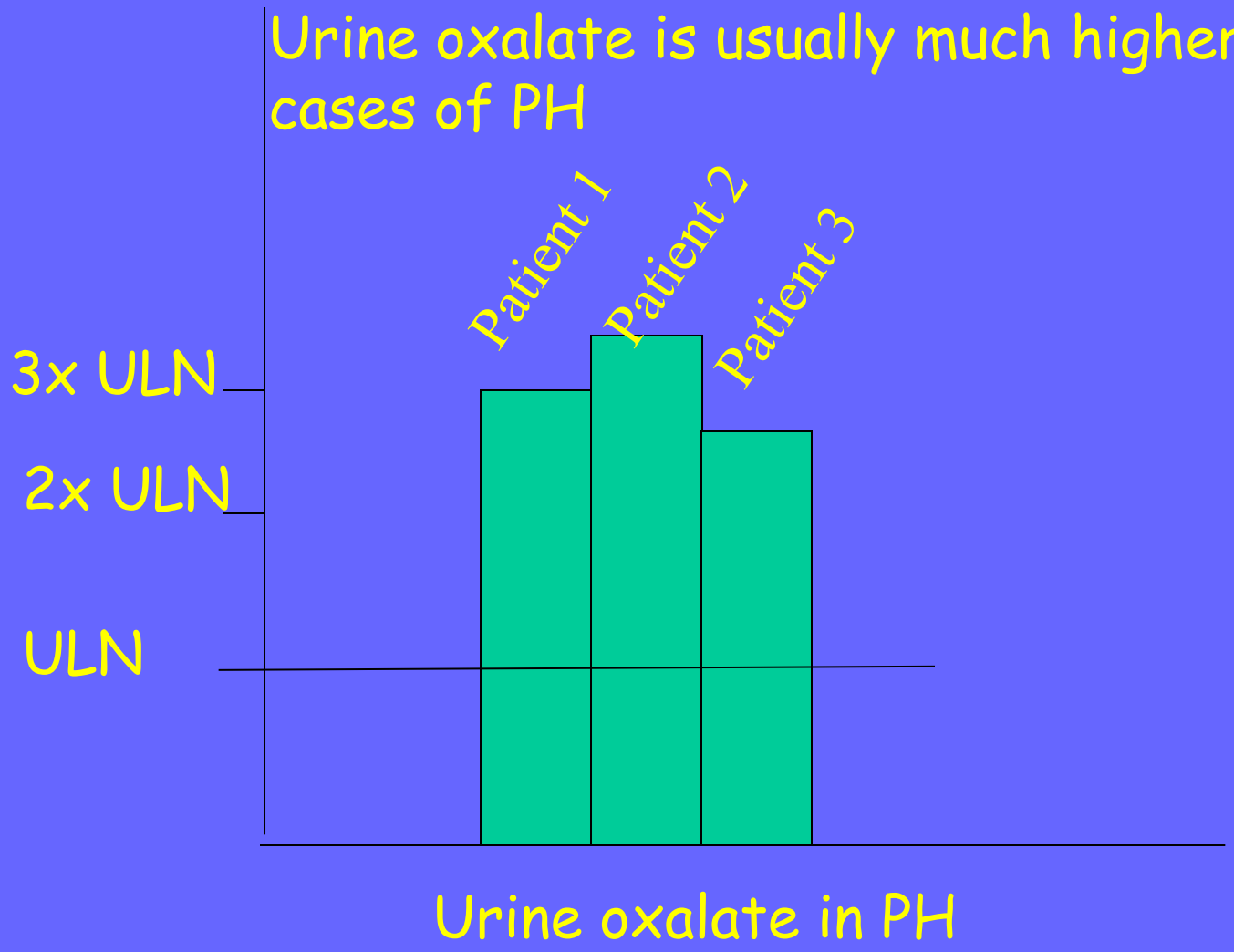




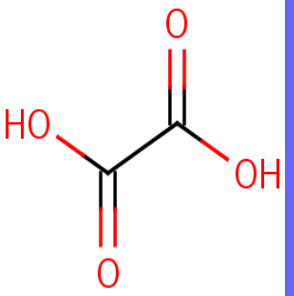


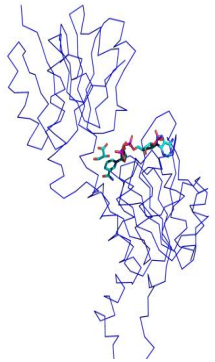
ULN=upper limit found without disease

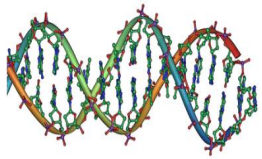
Urine oxalate is usually much higher in cases of PH



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

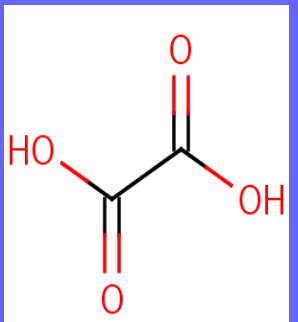
Metabolite

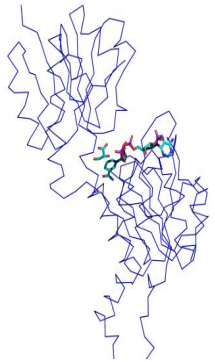
Enzyme (protein)

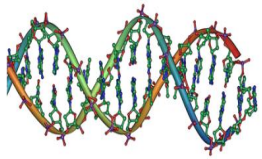
Gene (DNA)

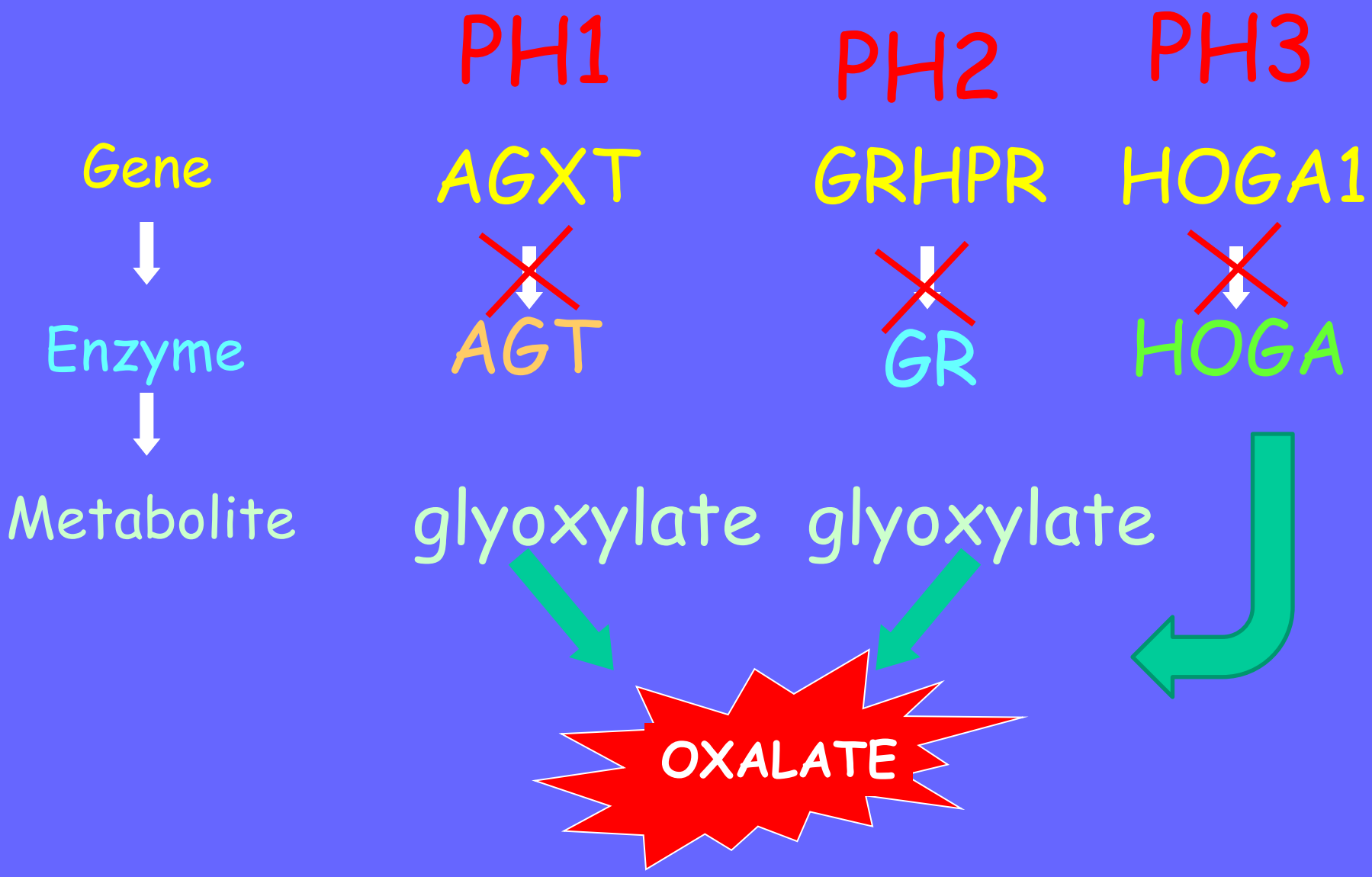
PH is caused by a defective protein (enzyme)

- PH1
  - Lack of alanine:glyoxylate aminotransferase =AGT
- PH2
  - Lack of glyoxylate reductase =GR
- PH3
  - Lack of hydroxyoxoglutarate aldolase =HOGA





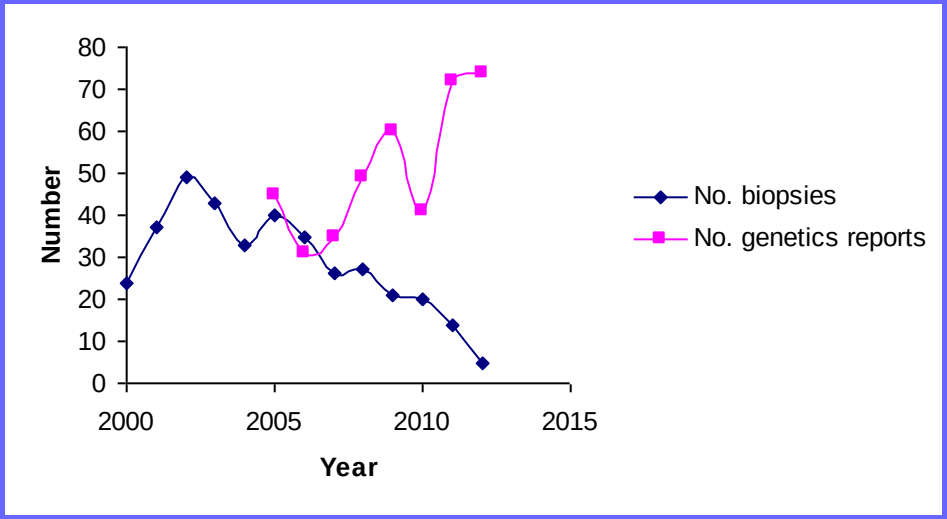



PH1
PH2
PH3
Gene
AGXT
GRHPR
HOGA1
Enzyme
AGT
GR
HOGA
Metabolite
glyoxylate
glyoxylate
OXALATE

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)

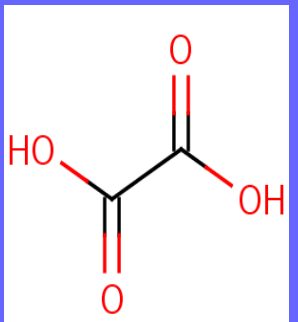
Gene (DNA)

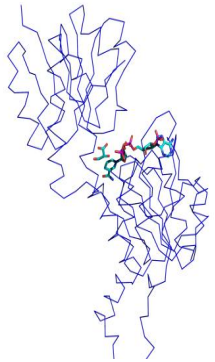
Genetic Testing

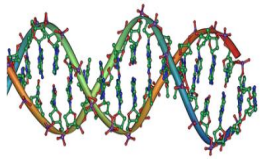
- Genes are made of DNA
- DNA is present in the nuclei of all cells, including those in the blood

For genetic tests

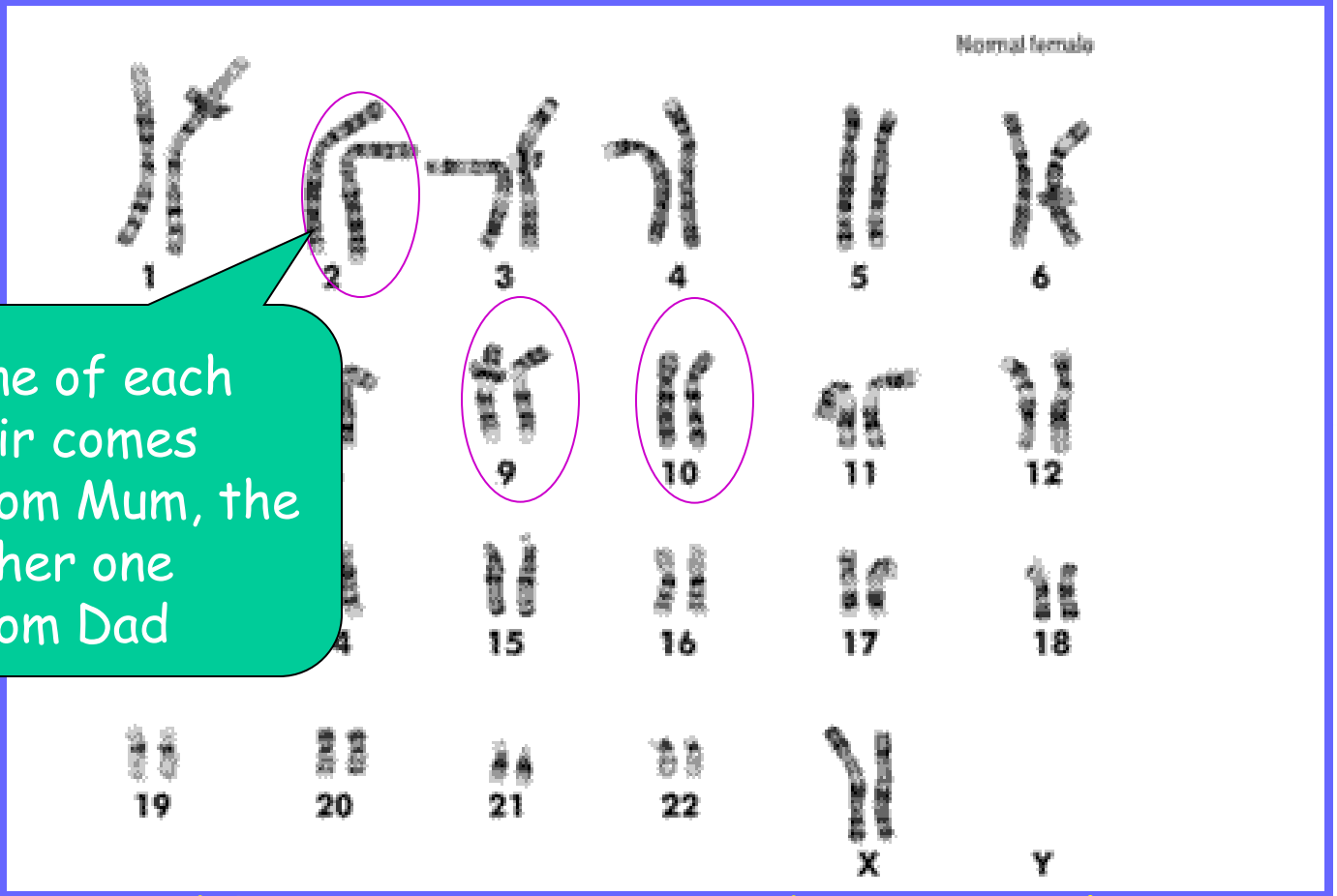
- We need blood or saliva samples from the patient and from the parents.



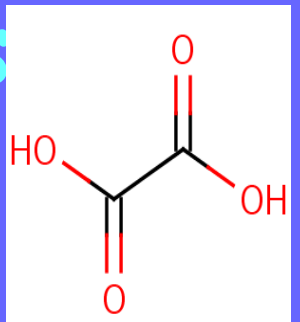


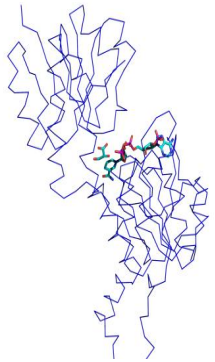


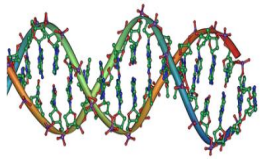
DNA is packaged into chromosomes



One of each pair comes from Mum, the other one from Dad

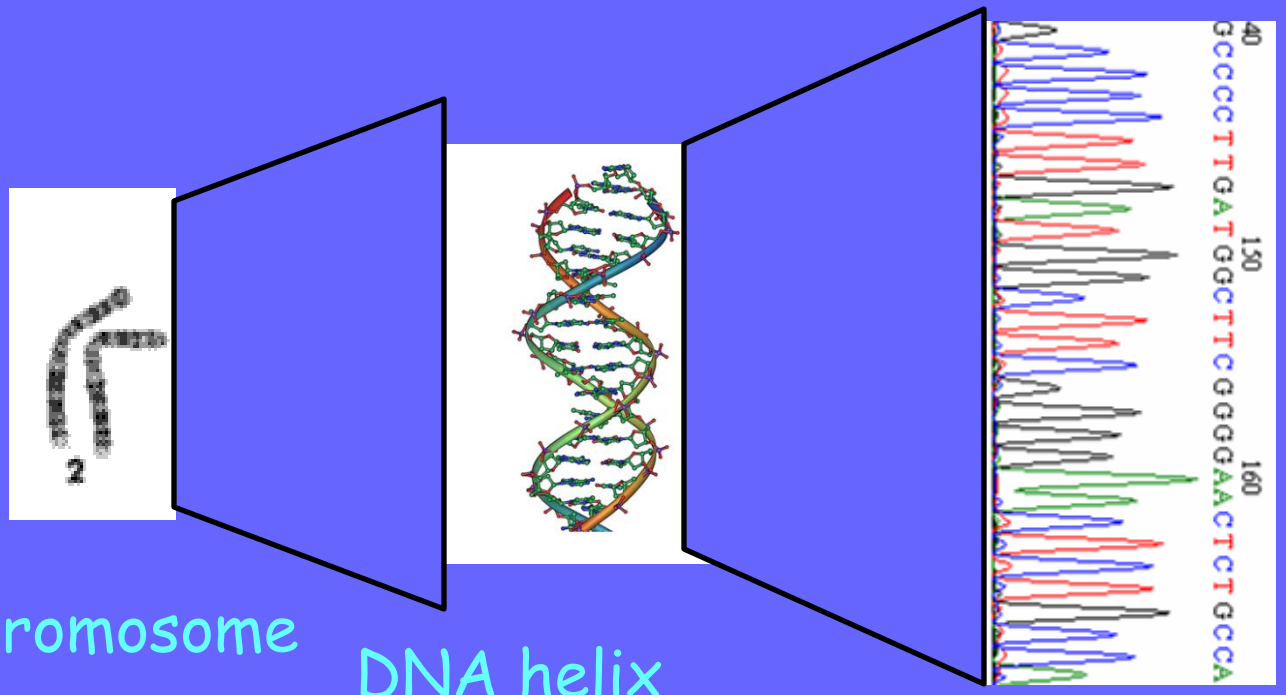






PH1, PH2 and PH3 are recessive diseases. This means that the child must inherit two defective genes, one from each parent, to have the disease

DNA analysis



chromosome

DNA helix

AGXT gene

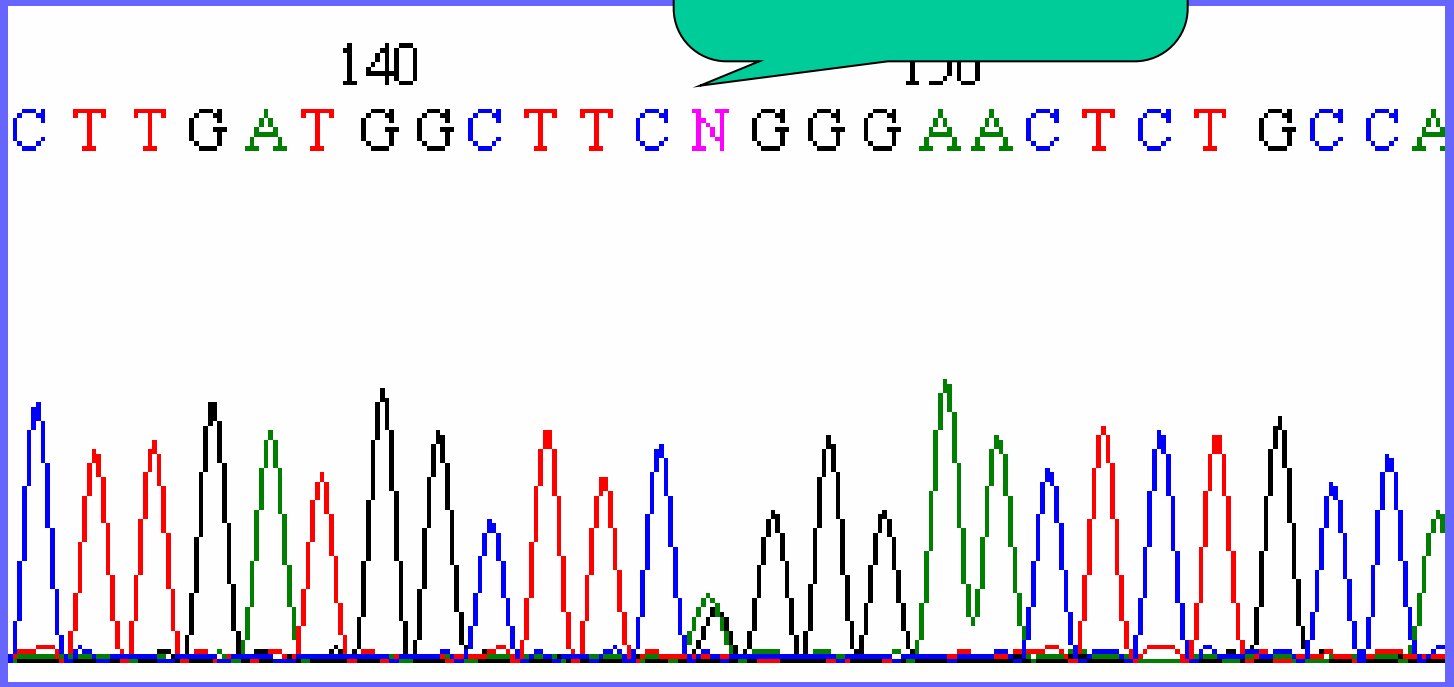
What we see in the lab

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

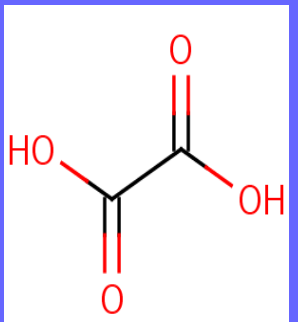
We find these changes by 'reading' the DNA sequence

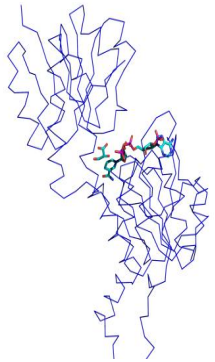
'N' shows us that something is different

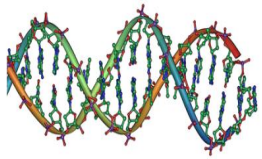


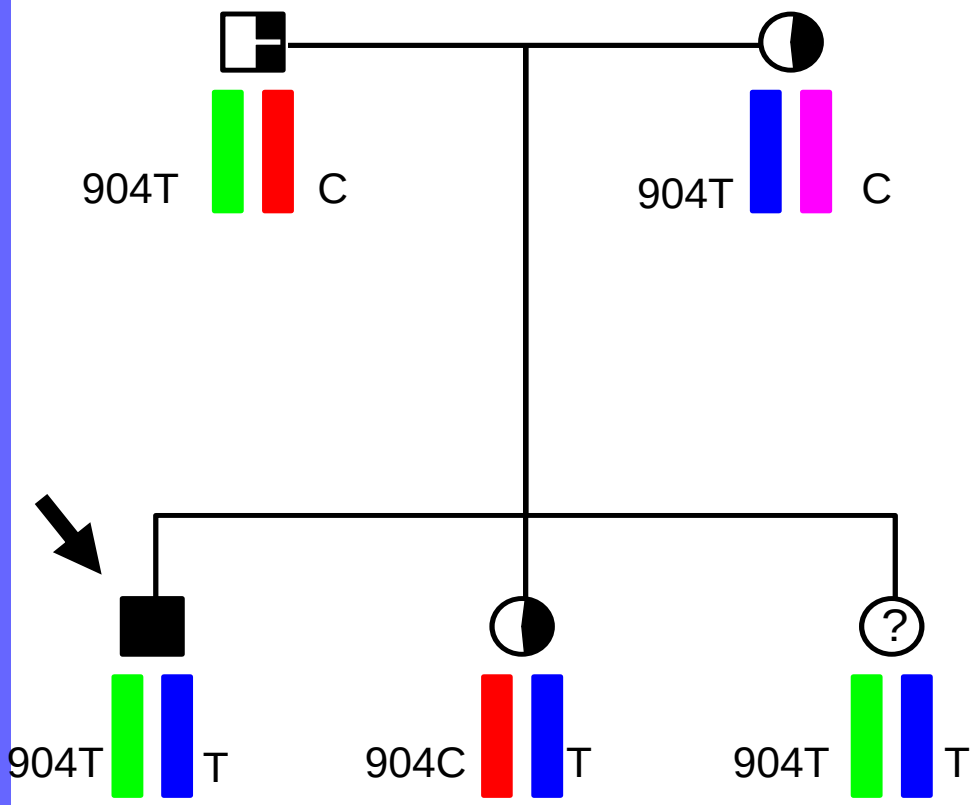
How can DNA testing help?

- 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

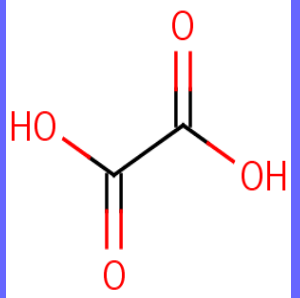


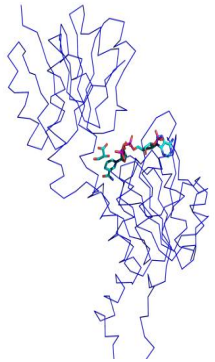






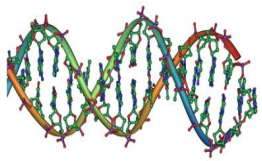
c.904C>T





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.



Prenatal Diagnosis

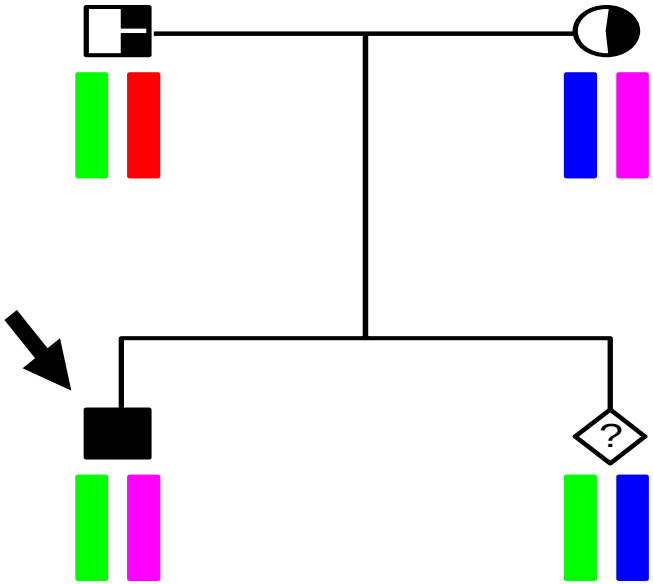
Chorionic villus biopsy taken under ultrasound guidance at 10-12 weeks gestation.

Isolate DNA

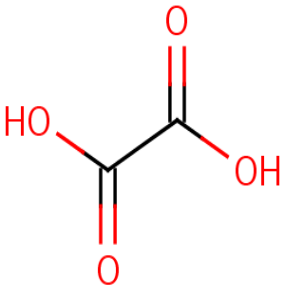
Look for mutation

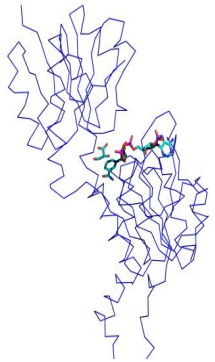
Result available within 10 days (normal,carrier or affected)

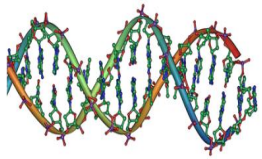
Prenatal diagnosis for PH1



Disease genes are the green and pink markers. The fetus has inherited the green but <u>not</u> the pink marker and is therefore a carrier only and does not have the disease.

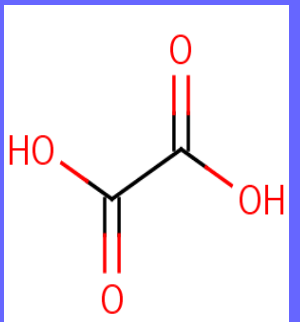


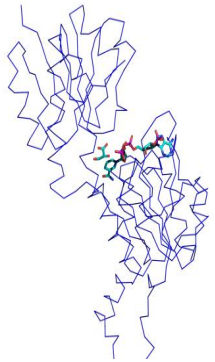


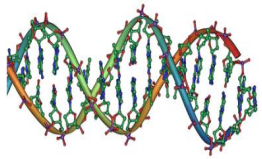


Summary

- 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

University College London Hospitals NHS

NHS Trust

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?