

Laboratory diagnosis of Primary hyperoxaluria

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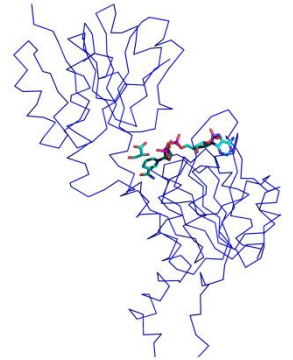
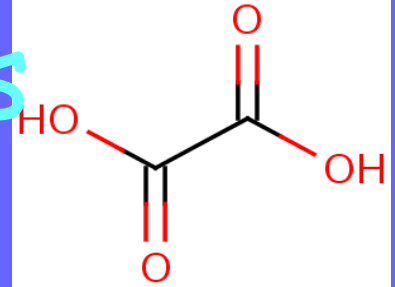
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Steps involved in diagnosis

Metabolite

Enzyme (protein)

Gene (DNA)



Tissue/body fluid
needed for analysis

Metabolite

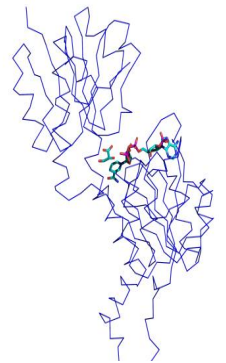
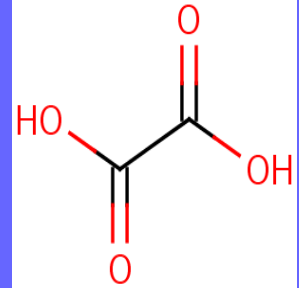
Urine, kidney
stones

Enzyme

Liver biopsy

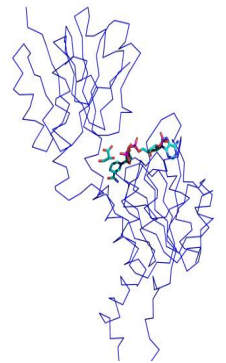
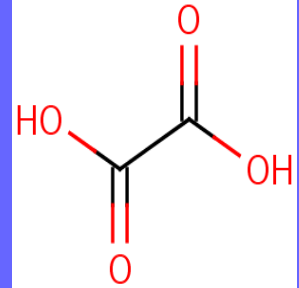
Gene

blood



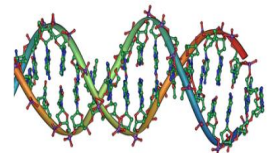
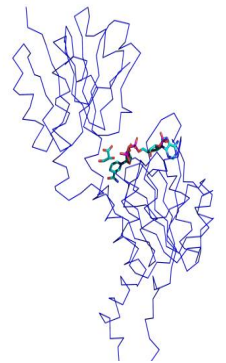
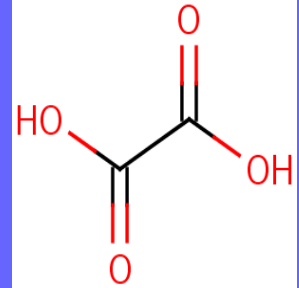
Specialist tests

- 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

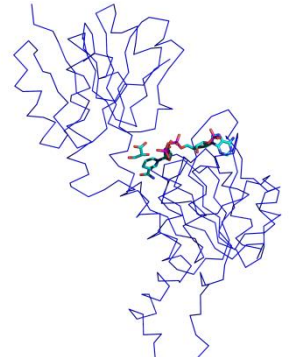
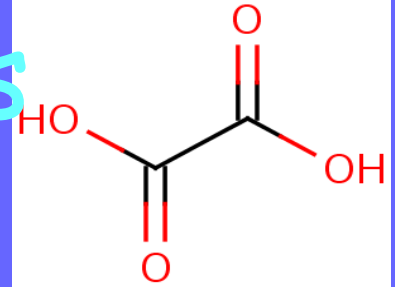


Steps involved in diagnosis

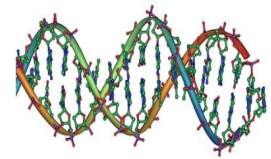
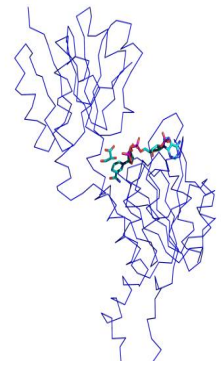
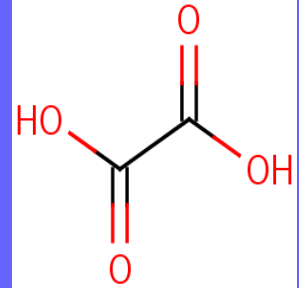
Metabolite

Enzyme (protein)

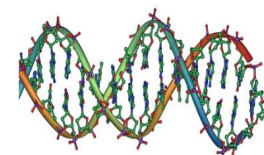
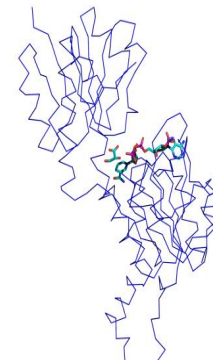
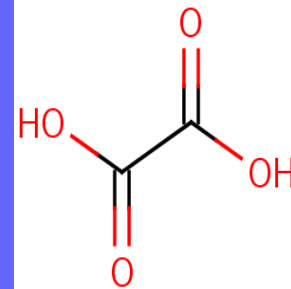
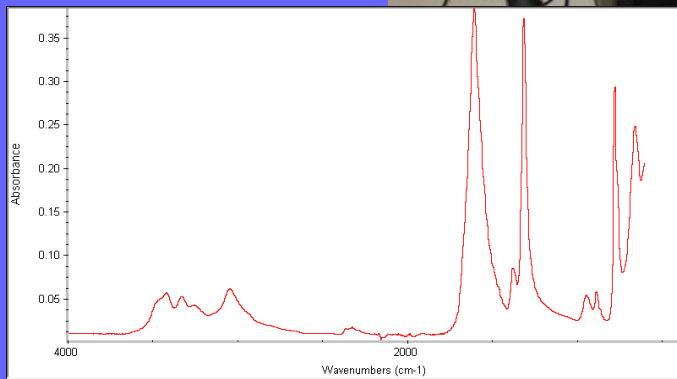
Gene (DNA)



- 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

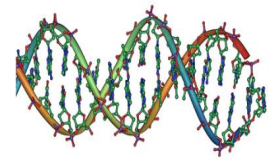
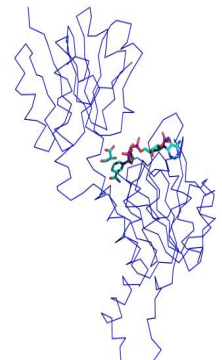
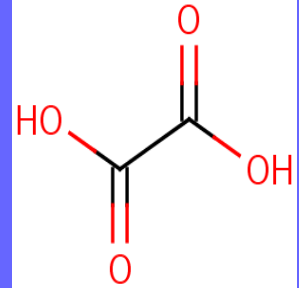


Analysis of kidney stones



Urine oxalate

- Usually one of 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



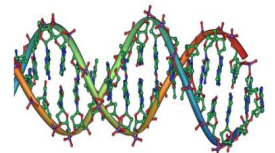
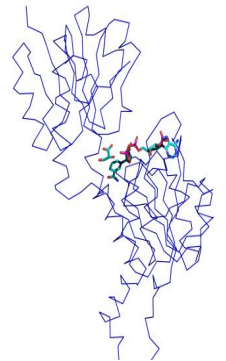
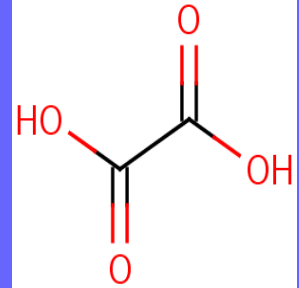
Random or 'spot' urine

or



24h urine collection

Oxalate



24h collection

- Label the bottle with your name, dob and hospital number before you start
- Go to the loo and empty your bladder.
- Write the time¹ and date on the bottle label
- Collect all urine samples after this time and add to the bottle
- When you get back to time ¹ the next day, empty your bladder adding this urine to your collection then STOP collecting.



Urine oxalate

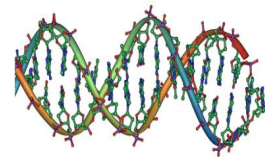
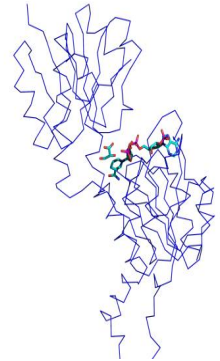
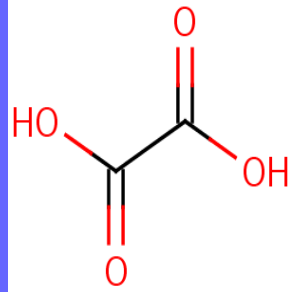
HIGH OXALATE!

Urine oxalate



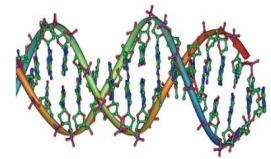
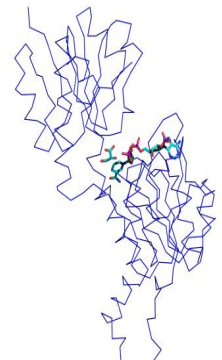
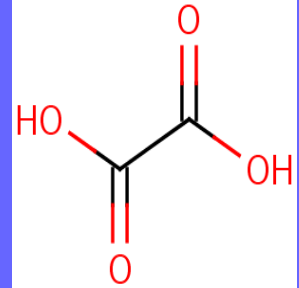
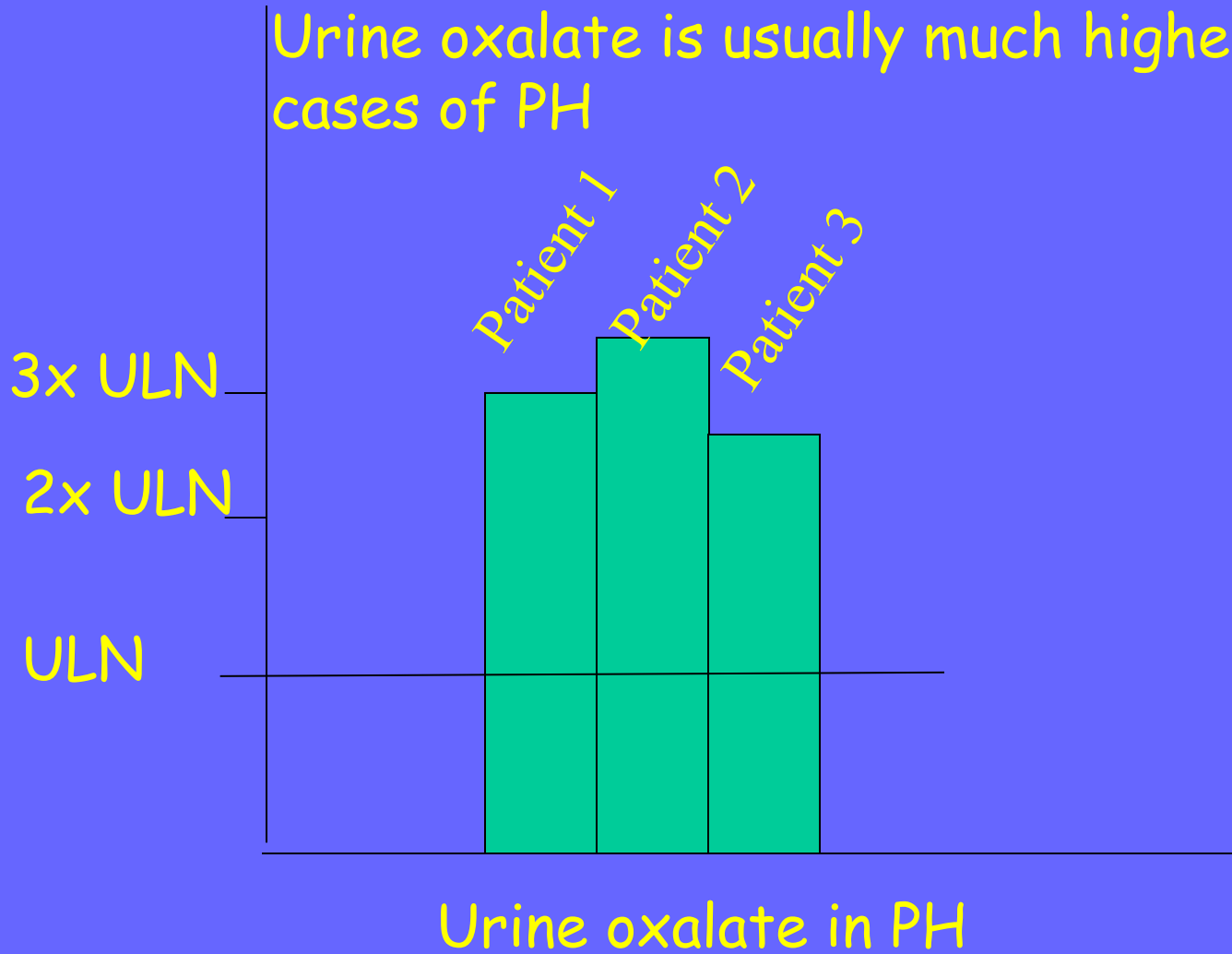
Hours after eating a bar of chocolate

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

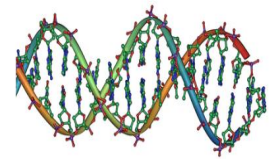
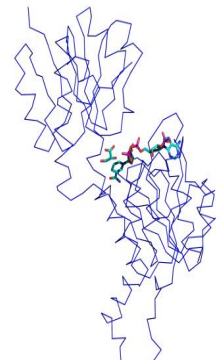
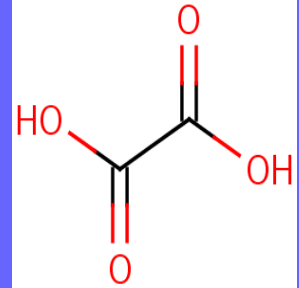


ULN=upper limit found without disease

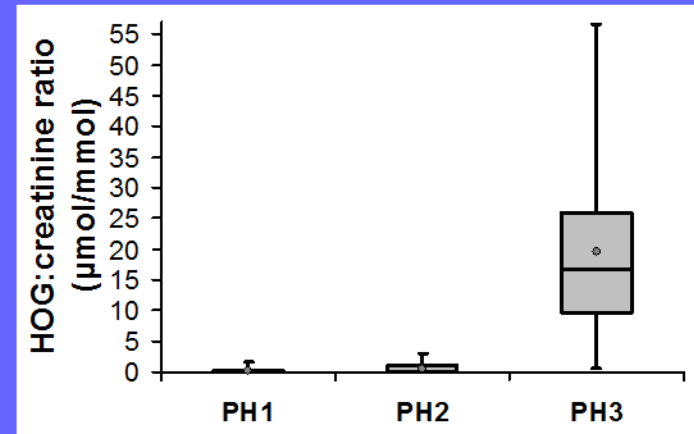
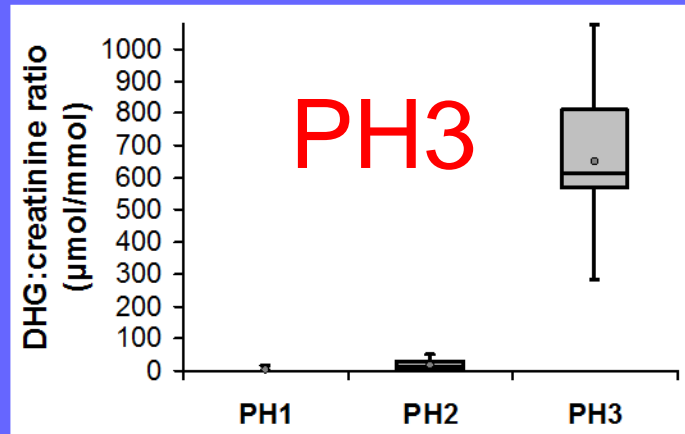
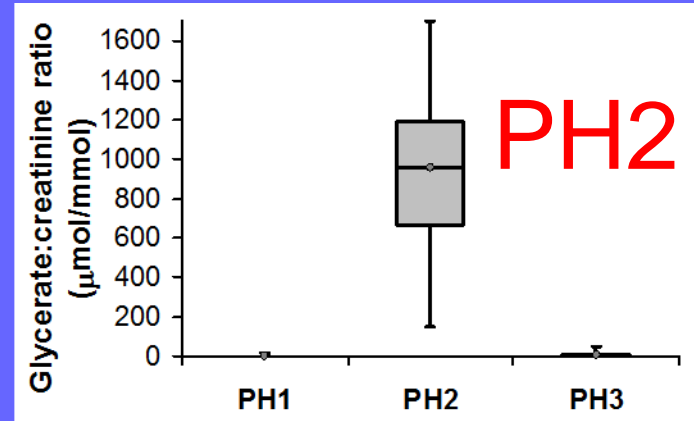
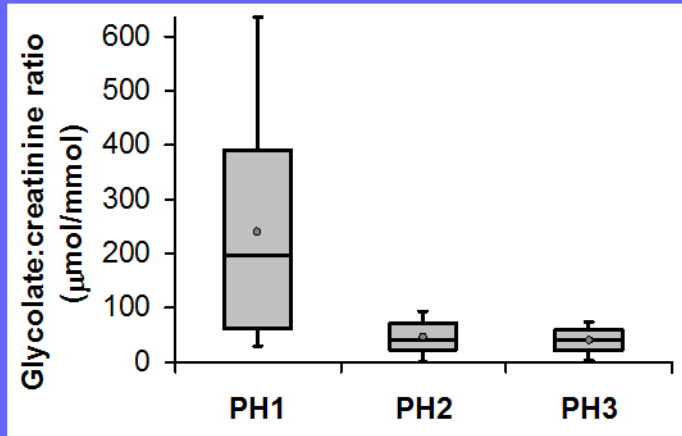
Urine oxalate is usually much higher in cases of PH



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



PH metabolites

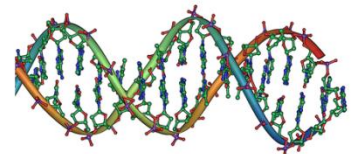
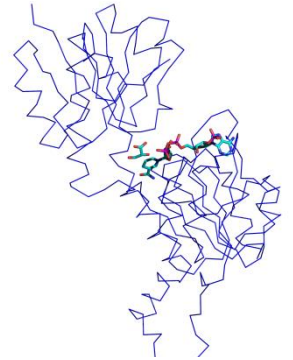
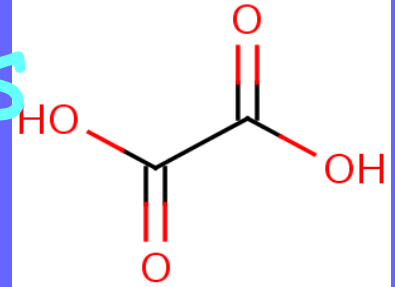


Steps involved in diagnosis

Metabolite

Enzyme (protein)

Gene (DNA)



PH is caused by a defective protein (enzyme)

- PH1

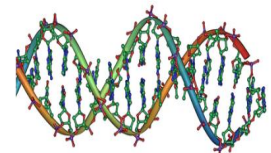
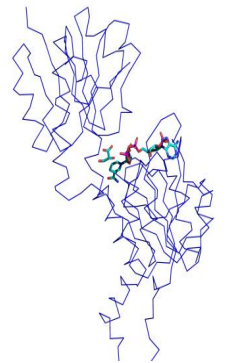
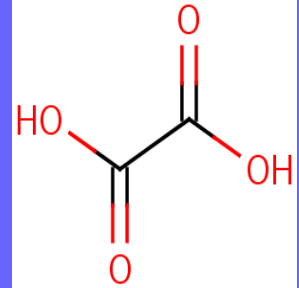
- Lack of alanine:glyoxylate aminotransferase = **AGT**

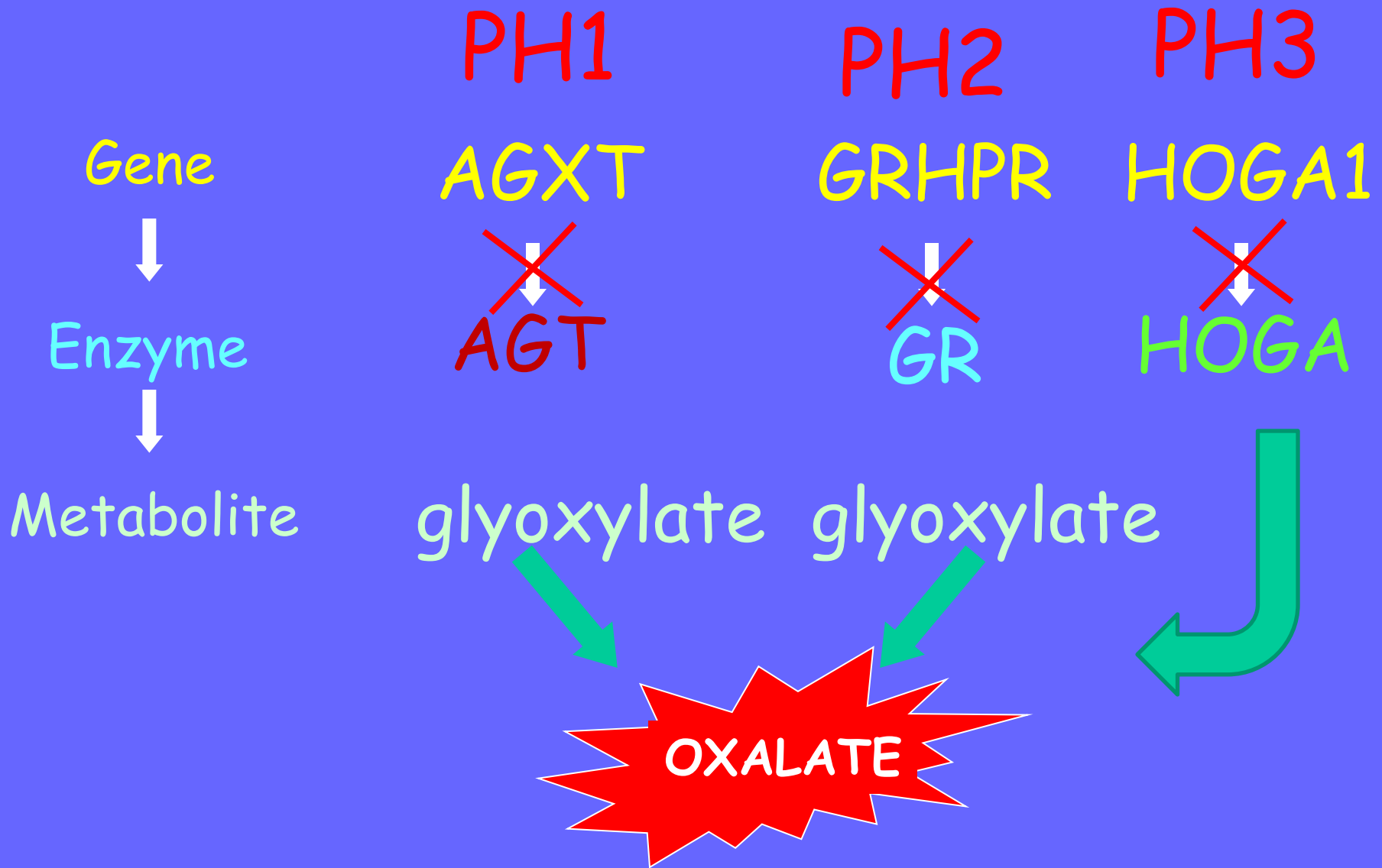
- PH2

- Lack of glyoxylate reductase = **GR**

- PH3

- Lack of hydroxyoxoglutarate aldolase = **HOGA**

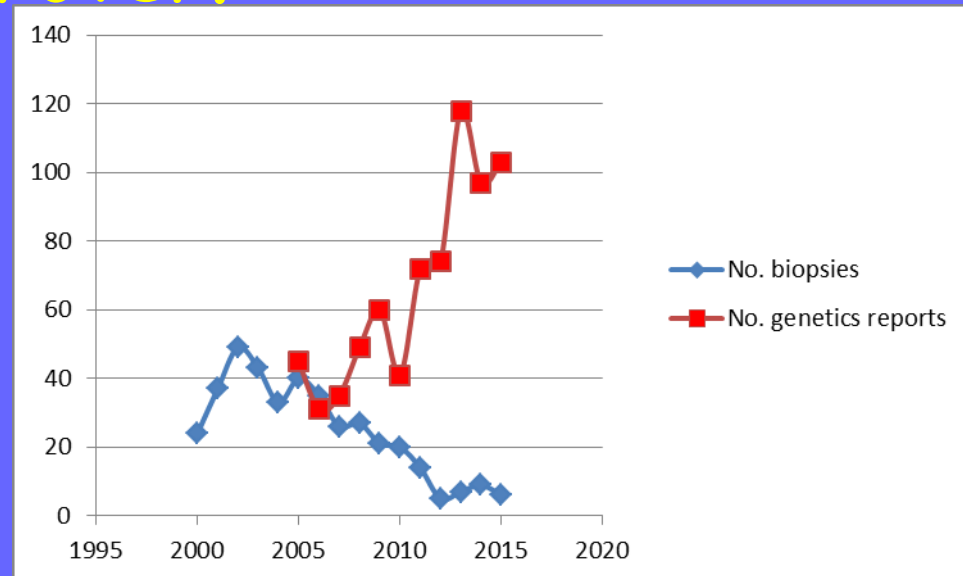




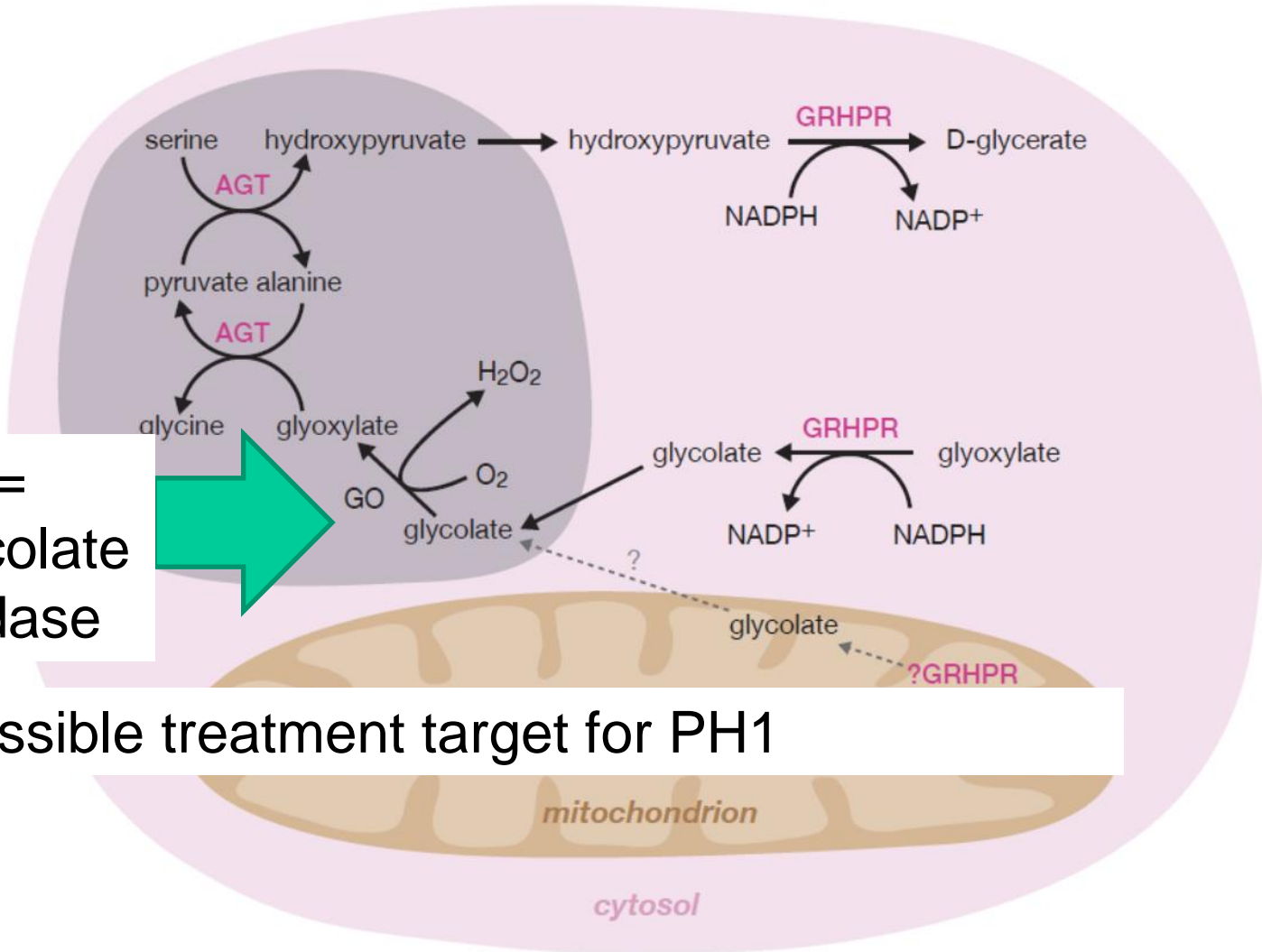
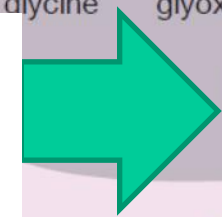
Enzyme tests for PH

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

Data from
UCLH lab



GO=
glycolate
oxidase



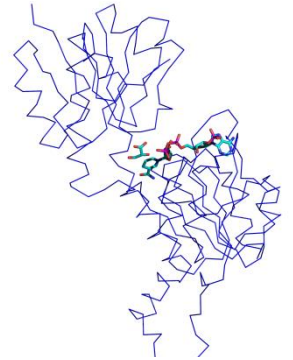
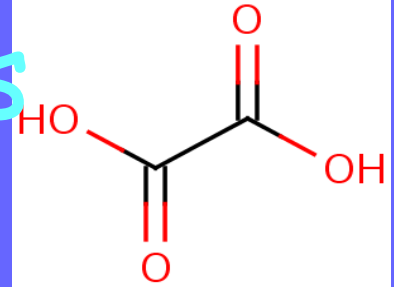
Possible treatment target for PH1

Steps involved in diagnosis

Metabolite

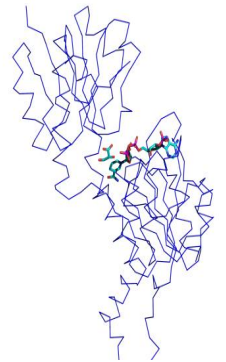
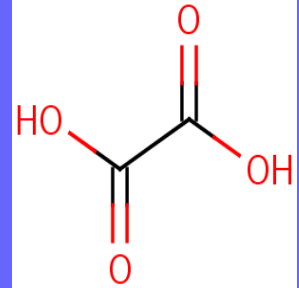
Enzyme (protein)

Gene (DNA)



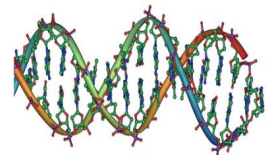
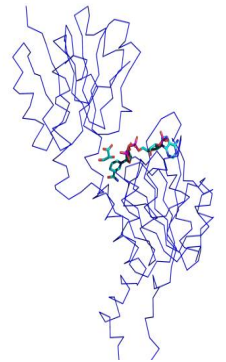
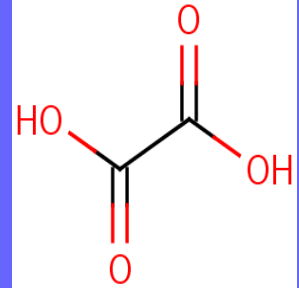
Genetic Testing

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

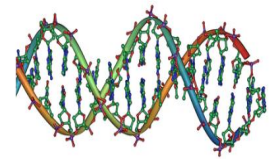
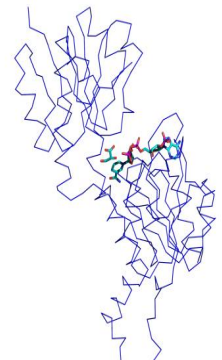
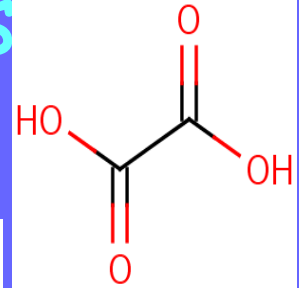
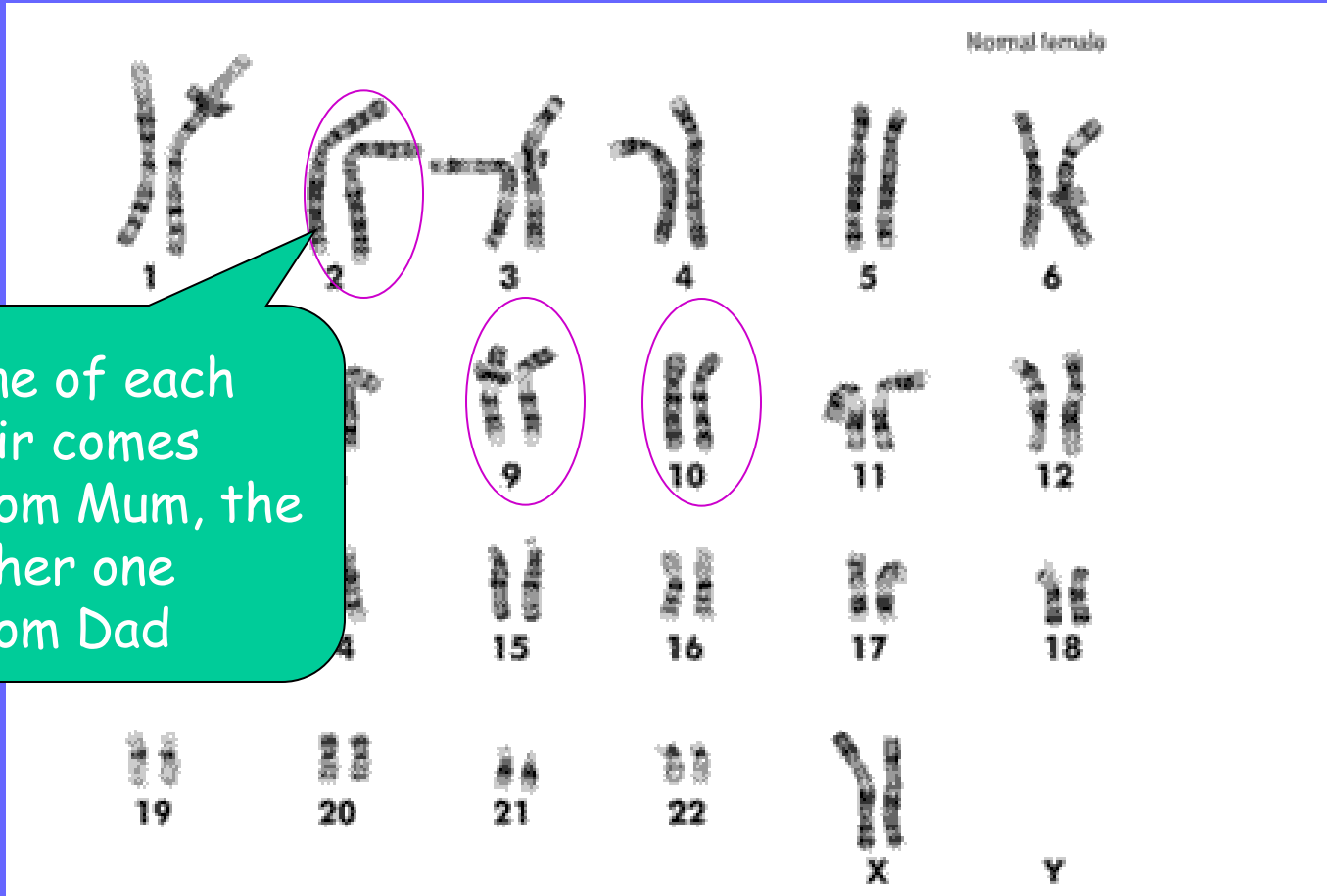


For genetic tests

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



DNA is packaged into chromosomes

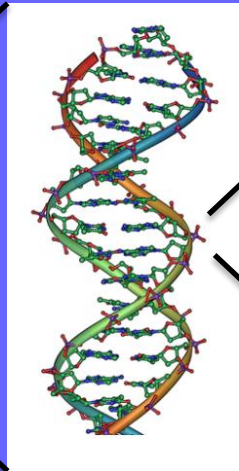


PH1, PH2 and PH3 are recessive diseases. This means that the child must inherit two defective genes, one from each parent, to have the disease

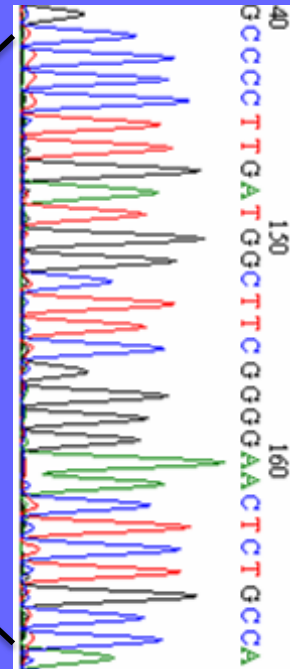
DNA analysis



chromosome

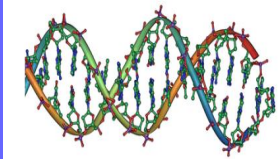
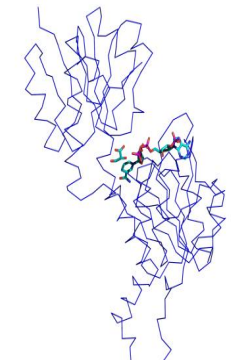
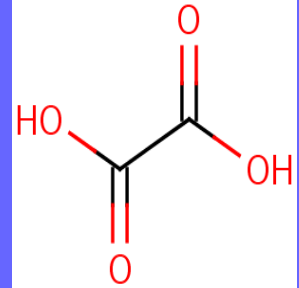


DNA helix



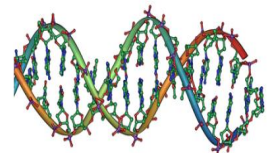
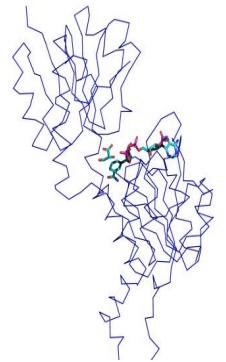
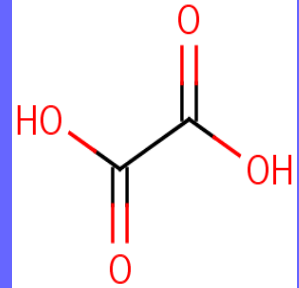
AGXT gene

What we see
in the lab



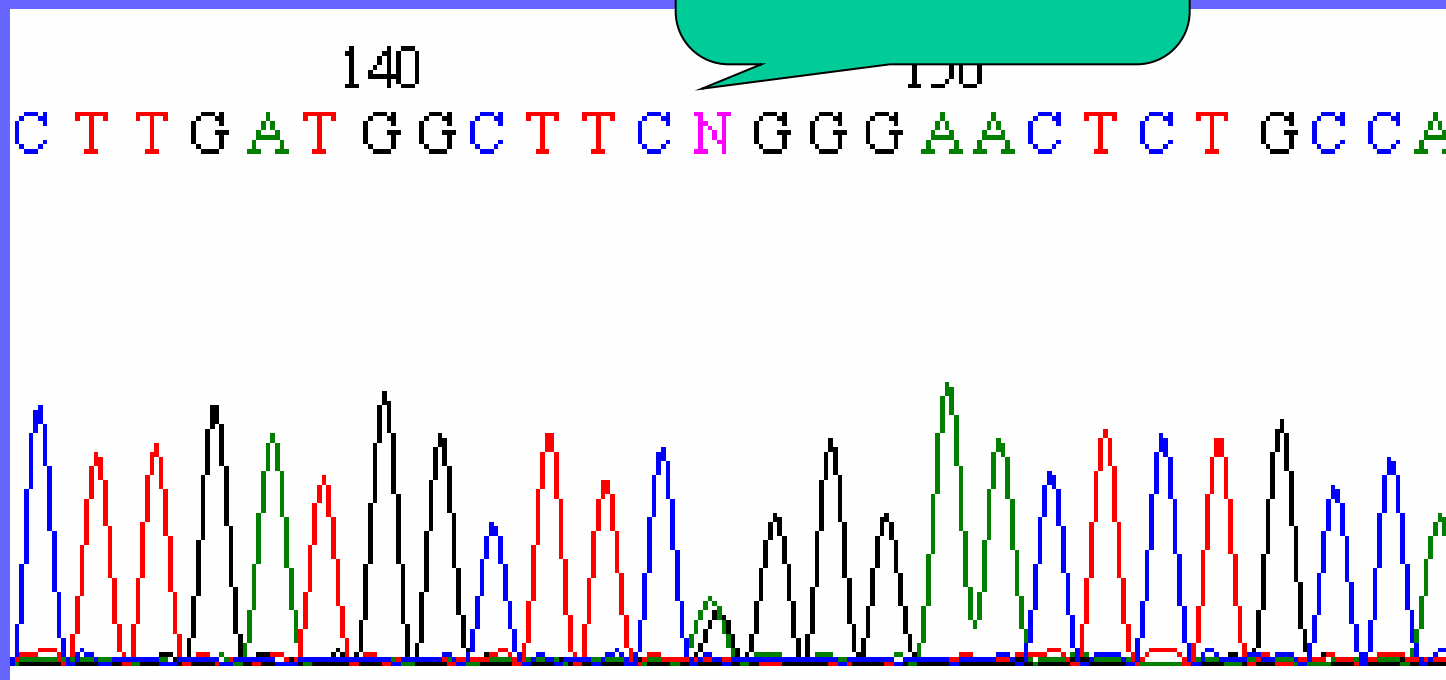
Mutations

- 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



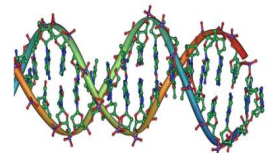
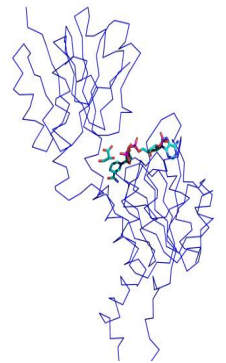
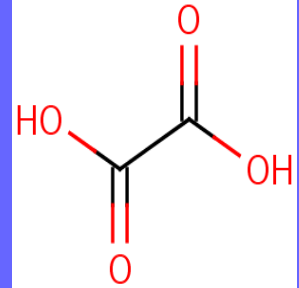
We find these changes by 'reading' the DNA sequence

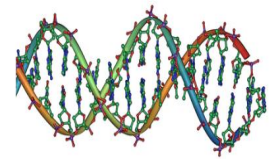
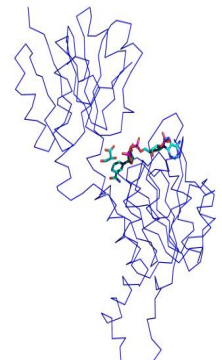
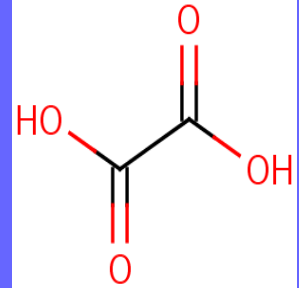
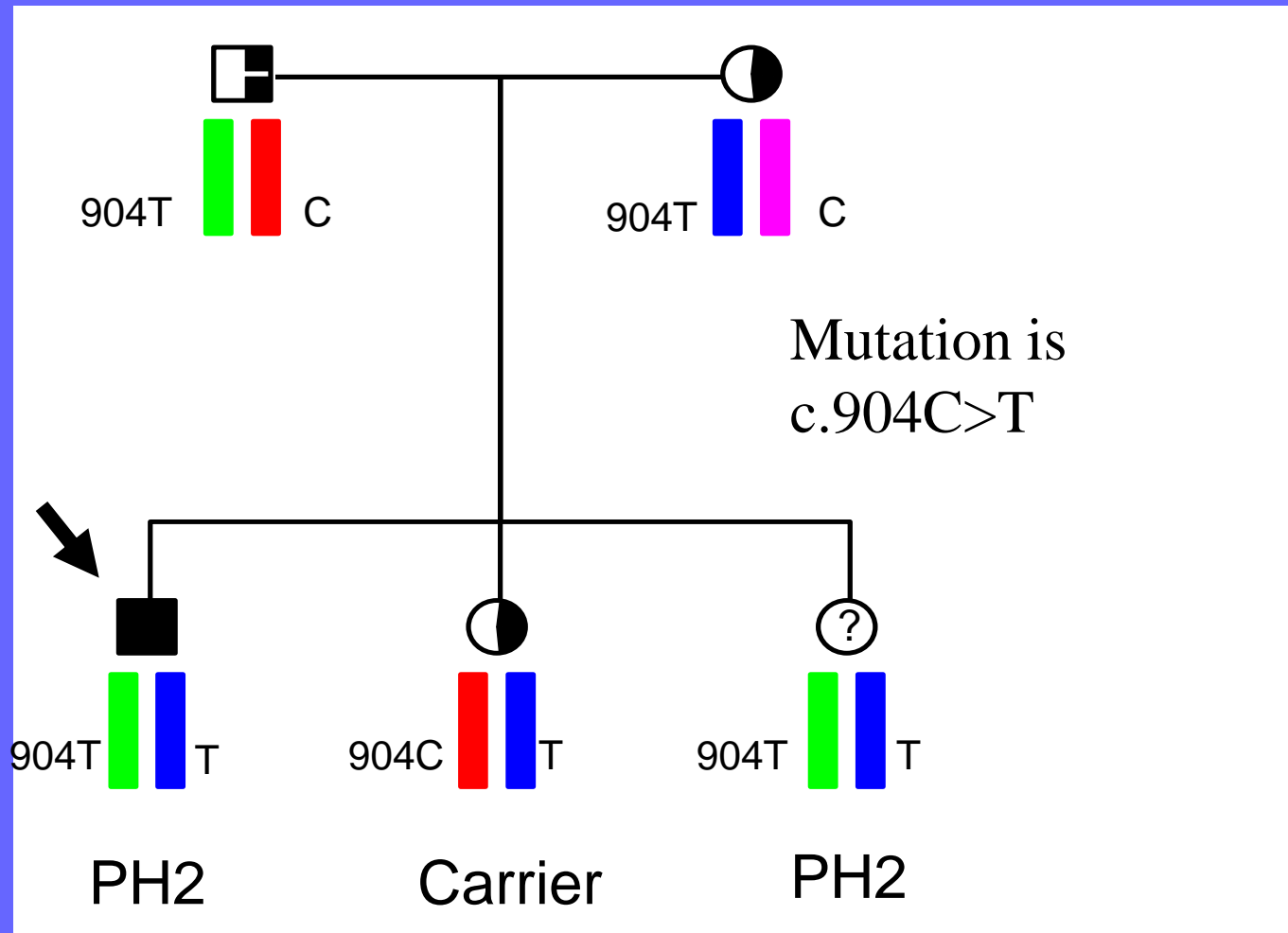
'N' shows us that something is different



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 is the normal gene, T 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.



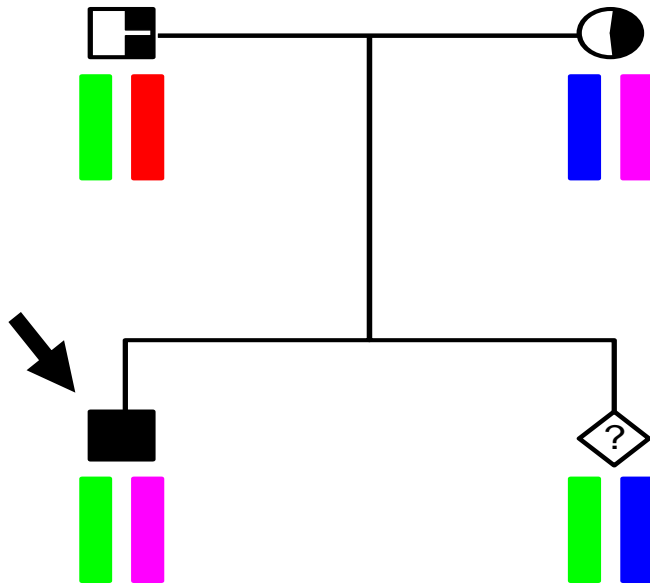
Isolate DNA



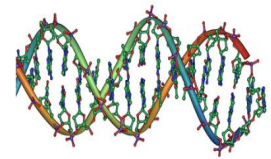
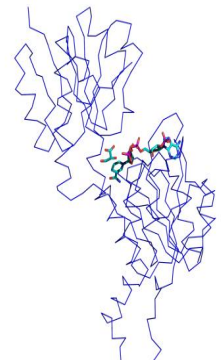
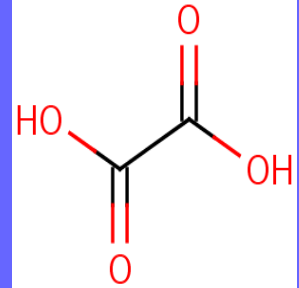
Look for mutation

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

Prenatal diagnosis for PH1

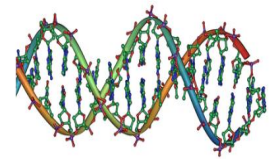
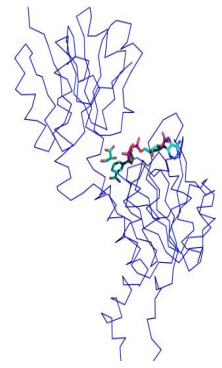
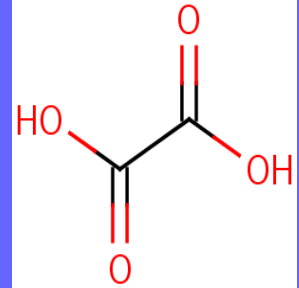


Disease genes are the green and pink markers. The fetus has inherited the green but not the pink marker and is therefore a carrier only and does not have the disease.



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

University College London Hospitals

NHS Trust

