Primary Hyperoxaluria Patient Information Day
Birmingham Children's Hospital, 2013

UCL

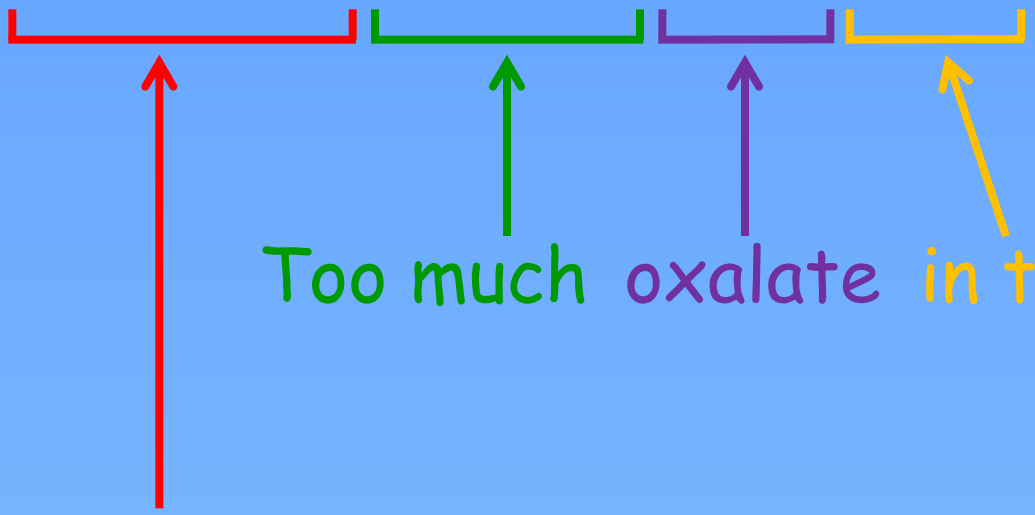
The Biology of Primary Hyperoxaluria

Chris Danpure

Emeritus Professor of Molecular Cell Biology
University College London

Chair, Scientific Advisory Board,
Oxalosis & Hyperoxaluria Foundation

Primary Hyperoxaluria



Directly due to initial defect – often genetic (cf secondary)

Primary Hyperoxalurias

type 1 = PH1

type 2 = PH2

type 3 = PH3

Caused by 3 different gene defects & 3 different enzyme deficiencies

types 4, 5, 6 + ? = uncharacterized/atypical PH

Causes are currently unknown

Rare autosomal recessive disorders

Less than 1 in 100,000 people suffer from PH

Not sex-linked; equally likely to occur in boys and girls

Each parent will carry one copy of the mutant gene & the patient will inherit two copies

Causes illness

Mutations in which genes & deficiencies in which enzymes cause which PH?

PH1 is caused by mutations in the ***AGXT*** gene, which causes a deficiency of the enzyme alanine:glyoxylate aminotransferase (**AGT**)

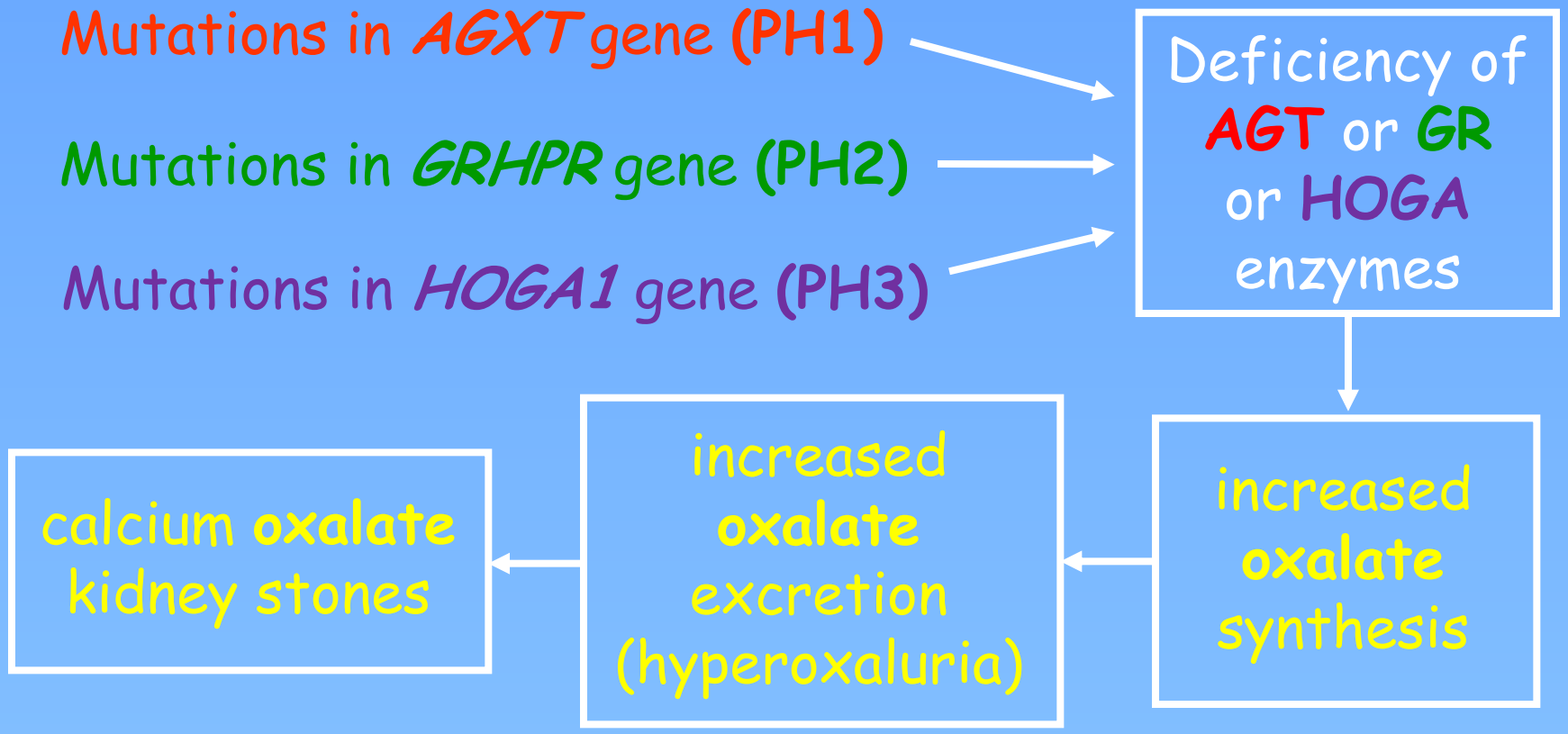
Mutations in which genes & deficiencies in which enzymes cause which PH?

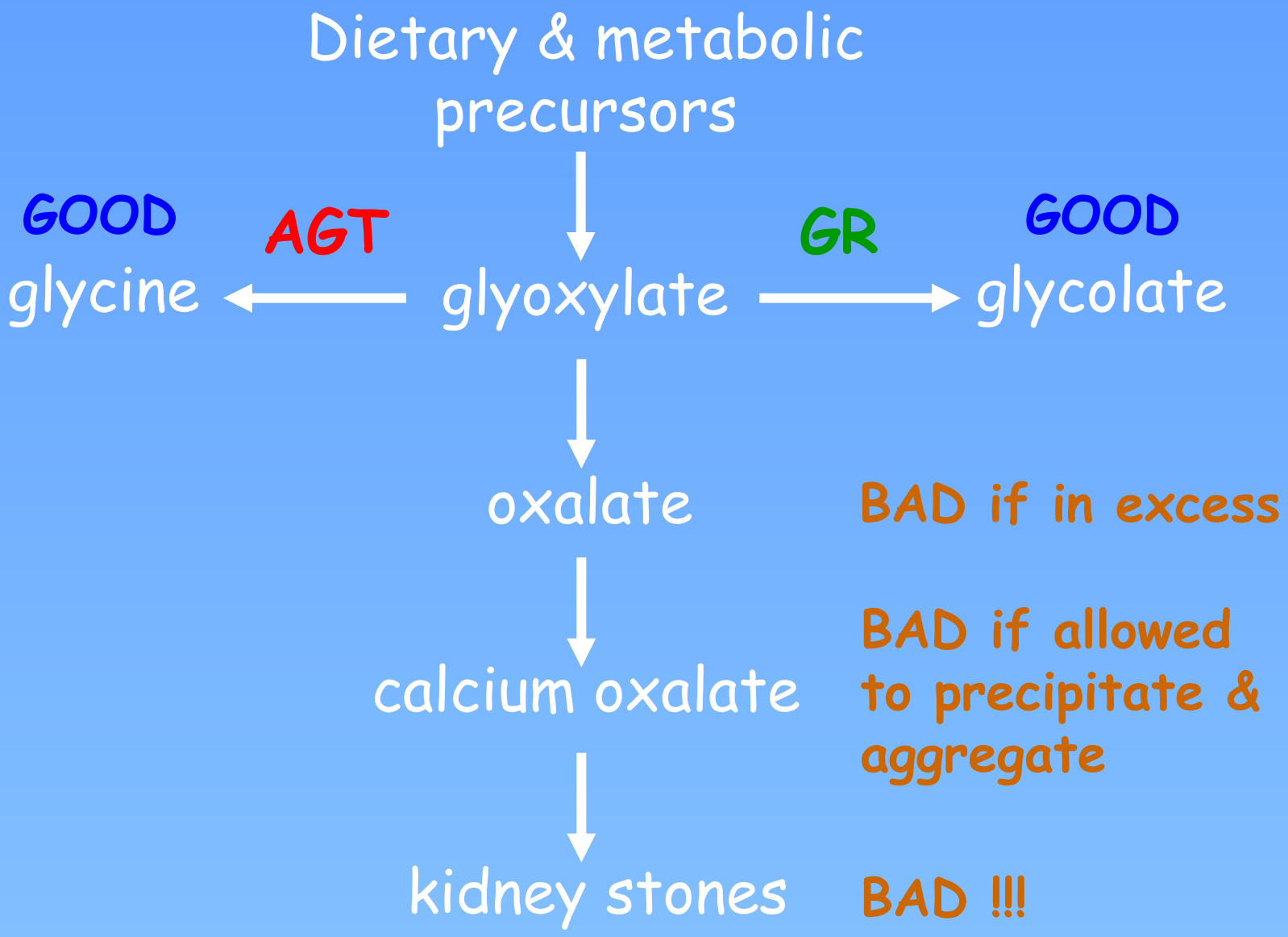
PH2 is caused by mutations in the ***GRHPR*** gene, which causes a deficiency of the enzyme glyoxylate reductase (**GR**)

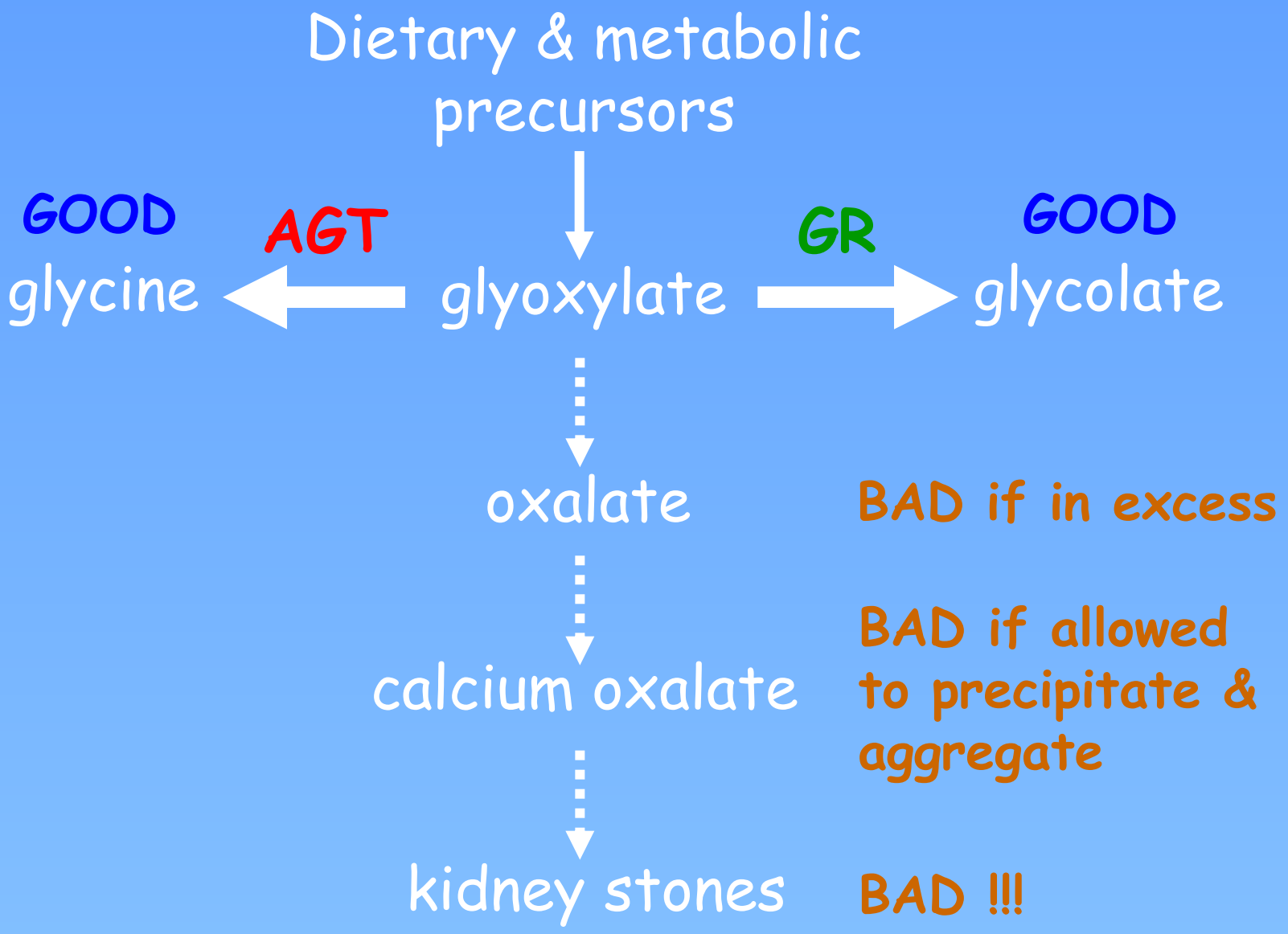
Mutations in which genes & deficiencies in which enzymes cause which PH?

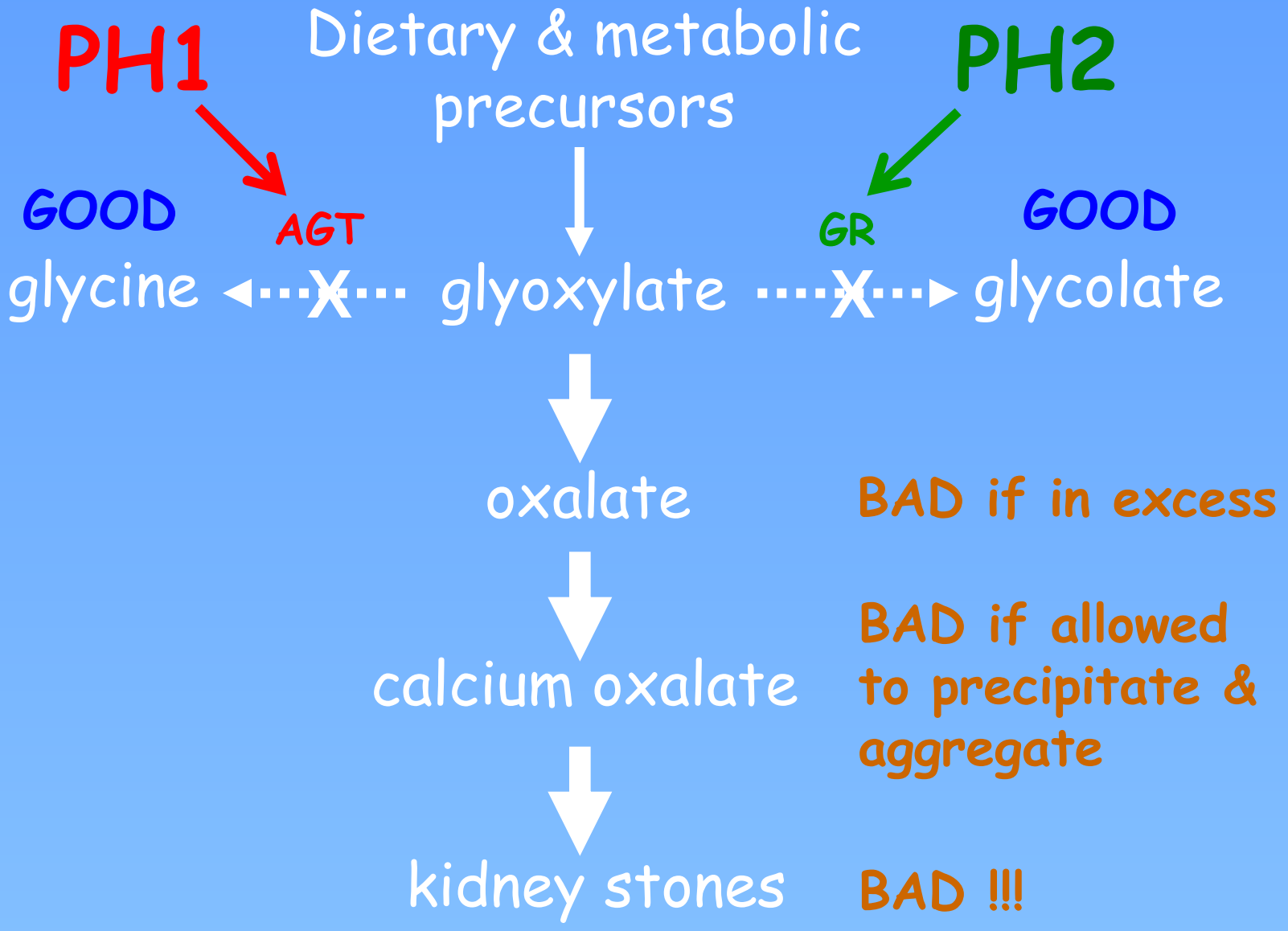
PH3 is caused by mutations in the ***HOGA1*** gene, which causes a deficiency of the enzyme hydroxy-oxoglutarate aldolase (**HOGA**)

Different mutations in different genes, but similar symptoms

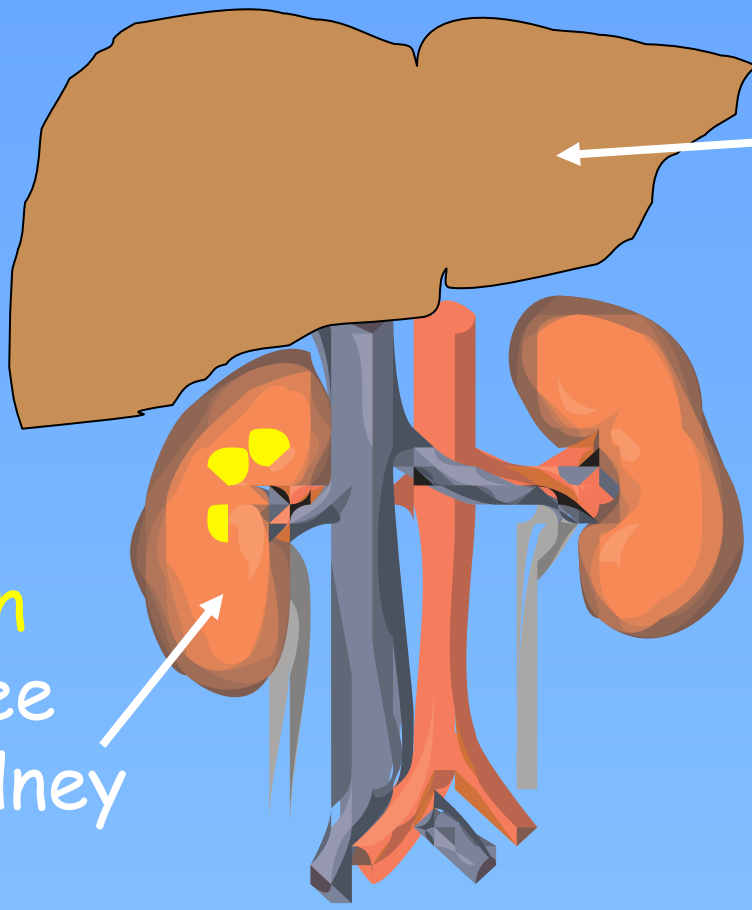



Dietary & metabolic precursors
GOOD
glycine
AGT
glyoxylate
GR
GOOD
glycolate
oxalate
BAD if in excess
calcium oxalate
BAD if allowed to precipitate & aggregate
kidney stones
BAD !!!


Dietary & metabolic precursors
GOOD
glycine
AGT
glyoxylate
GR
GOOD
glycolate
oxalate
BAD if in excess
calcium oxalate
BAD if allowed to precipitate & aggregate
kidney stones
BAD !!!


PH1
Dietary & metabolic precursors
PH2
GOOD
AGT
GR
GOOD
glycine
X
glyoxylate
X
glycolate
oxalate
BAD if in excess
calcium oxalate
BAD if allowed to precipitate & aggregate
kidney stones
BAD !!!

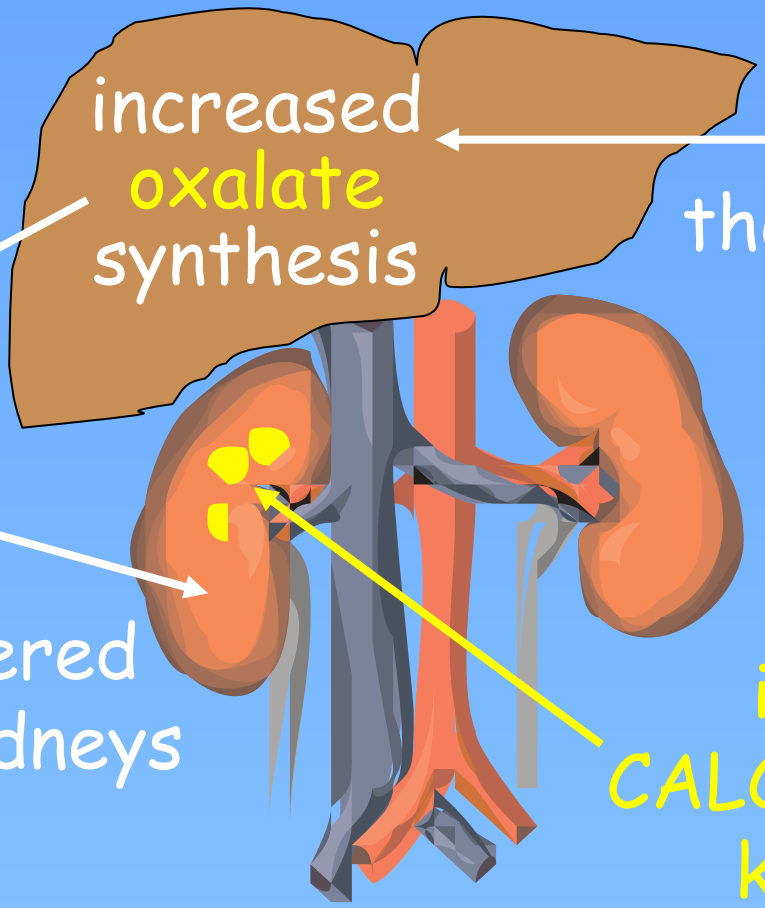
… but the basic laboratory scientist (molecular cell biologist) sees PH1 as a liver disease



The clinician & patient see PH1 as a kidney disease …

The basic defect in PH1 is in the LIVER …

… not the KIDNEY



How do mutations in the *AGXT* gene in PH1 interfere with the function of the AGT enzyme ?

1) Stop AGT being synthesised

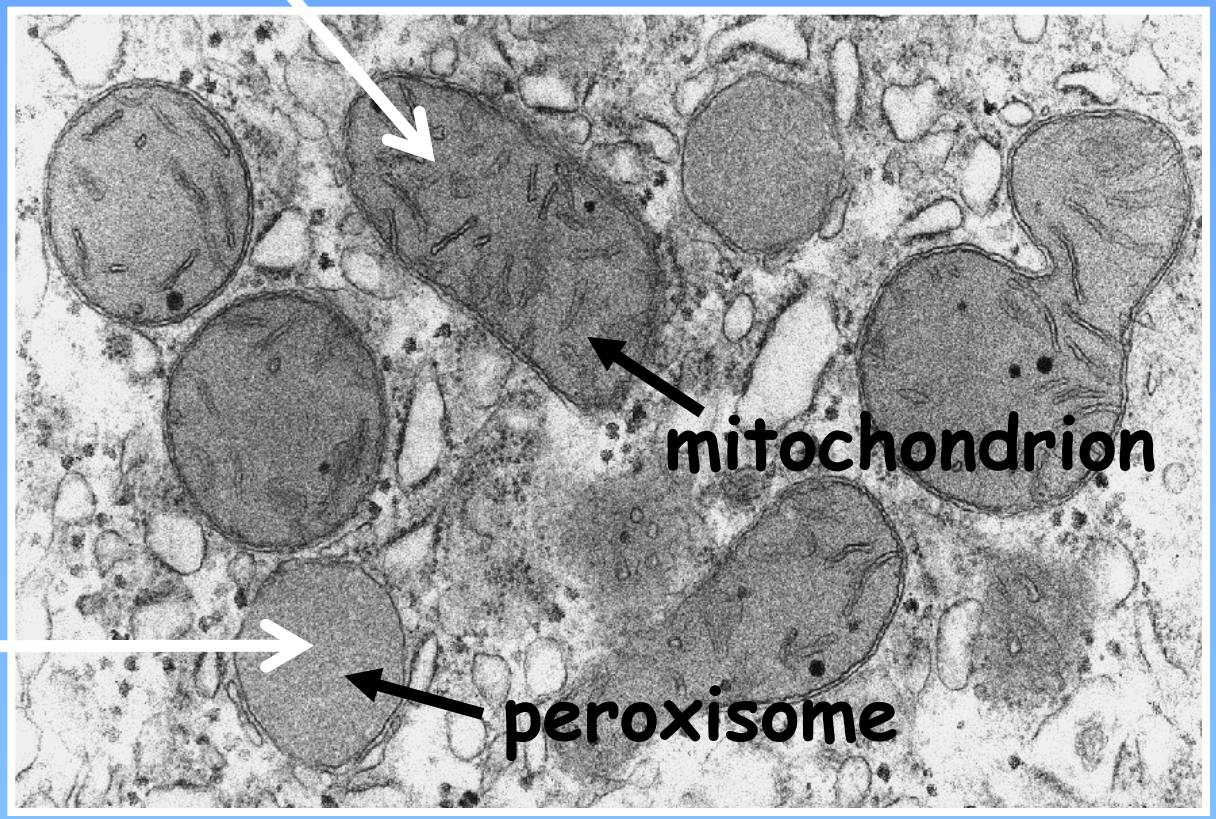
2) Stop AGT working properly

- 2a) Cause AGT to be rapidly degraded
- 2b) Cause AGT to aggregate into lumps
- 2c) Cause AGT to lose enzyme activity
- 2d) Cause AGT to be targeted to the wrong part of the cell

If the *AGXT* gene contains a particular mutation, it will produce abnormal **AGT** which is sent to the mitochondria by mistake.

AGT does not work properly in mitochondria

Normal **AGT** is sent to the peroxisomes where it does its job well



How do we know that mutations in the *AGXT* gene in PH1 cause these problems with the *AGT* enzyme ?

25 years of basic laboratory research using:

- Genetics
- Biochemistry
- Biophysics
- Molecular biology
- Cell biology
- Structural biology …

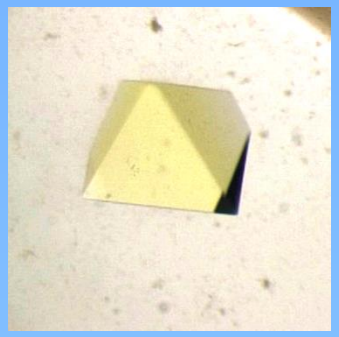
How the structure of *AGT* protein was solved

Isolate human *AGXT* gene

→ Insert into a bacterium (e.g. E. coli)

↓ Get bacteria to make large amounts of human *AGT* protein

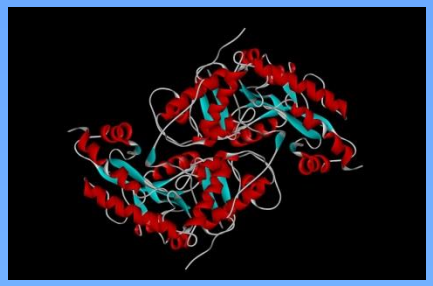
↓ Purify *AGT* protein



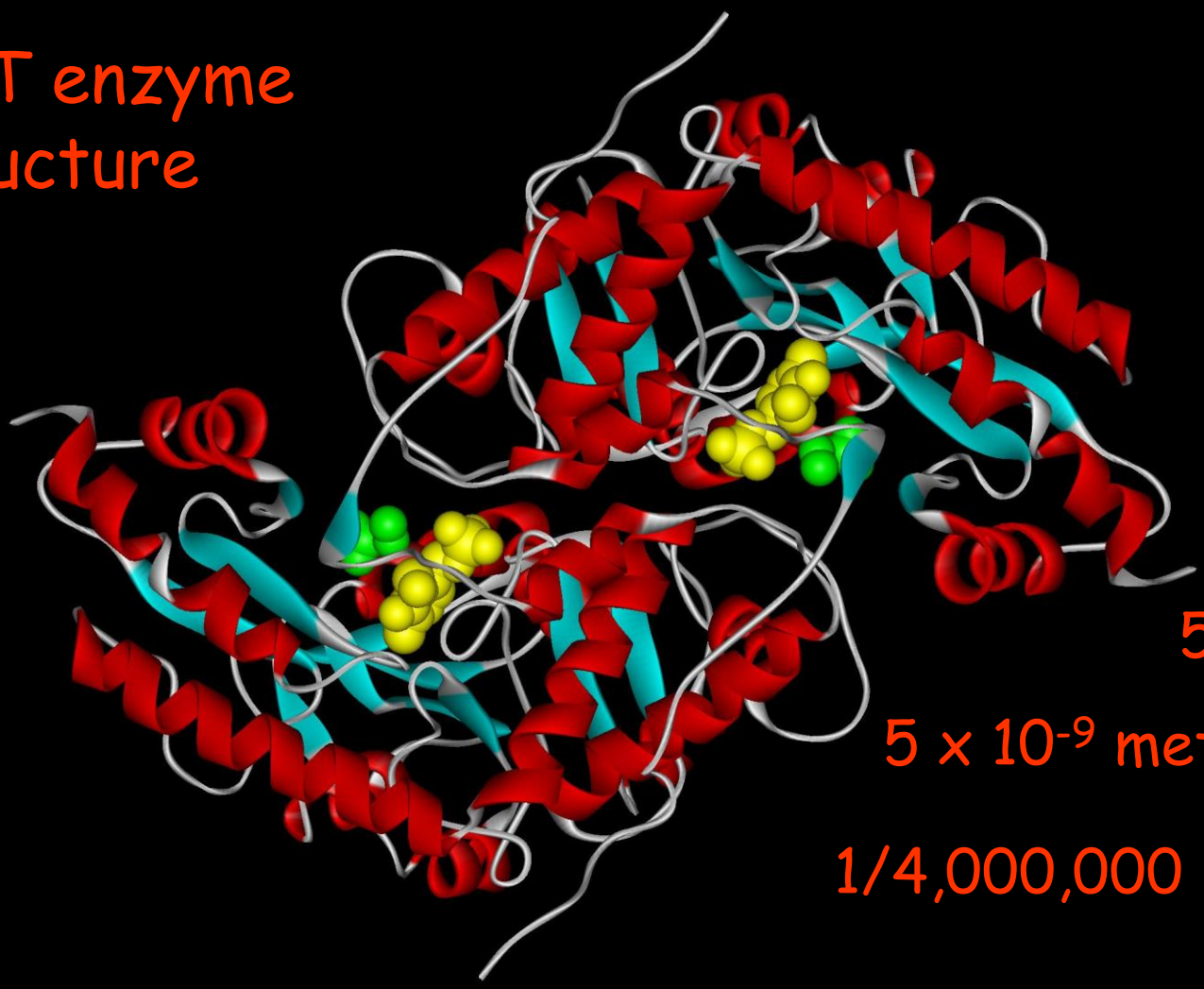
↓ Grow *AGT* crystals

← Bombard with X-rays

↑ Solve structure



AGT enzyme structure



50 Å

5 x 10^{-9} metres

1/4,000,000 inch

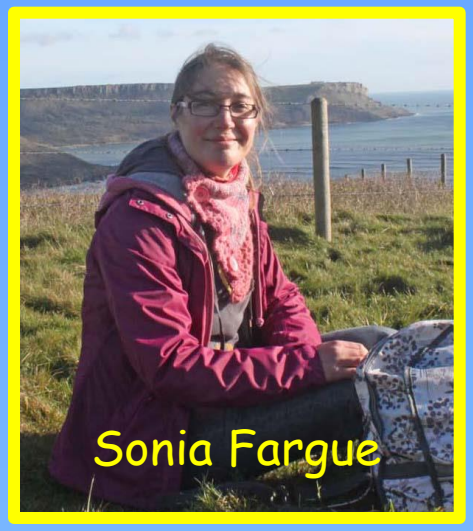
Problems with mutant AGT in PH1 patients

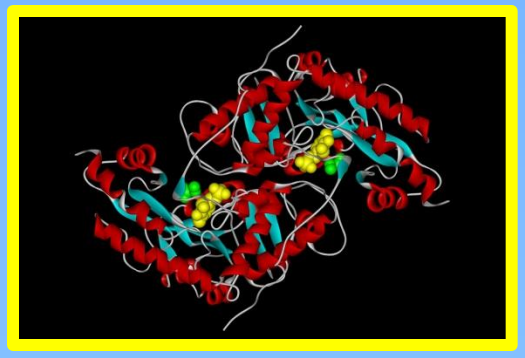
AGT is rapidly degraded

AGT aggregates into lumps

AGT loses enzyme activity

AGT targets to the wrong part of the cell





Pyridoxine (vitamin B6) stops all these things happening in responsive patients

How is our knowledge of the basic molecular & cellular defects in AGT being directed at new potential treatments for PH1 ?

Potential Treatments in the Future:-

	Current research
Gene therapy	Virus-mediated correction of disease in animal models (mouse knock-outs)
Hepatocyte transplantation	Patient-specific virus-mediated correction of stem-cell-derived liver cells
Chemical chaperones	High-throughput screening of chemicals which stabilise mutant enzyme

UCL

Oxalosis & Hyperoxaluria Foundation
STEPPING STONES TO A CURE

MRC Medical Research Council

The Leverhulme Trust

The Wellcome Trust

The Jules Thorn Charitable Trust