MetBioNet training meeting, 22nd Sept 2023

Purines and Pyrimidines

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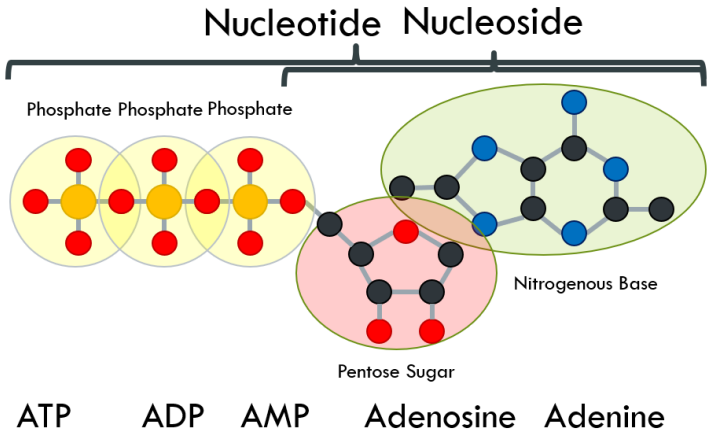
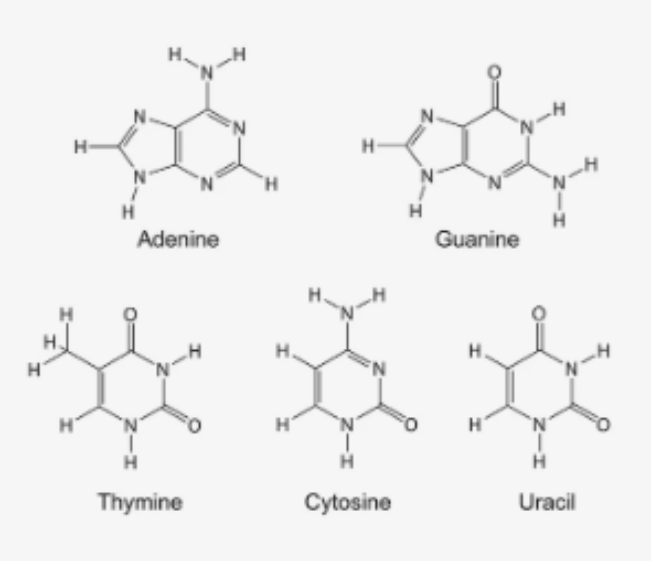
What we'll cover

1 | Introduction
2 | The Purine pathway
3 | The Pyrimidine pathway
4 | Analytical methods
5 | Cases
6 | Summary and questions

Purines and Pyrimidines

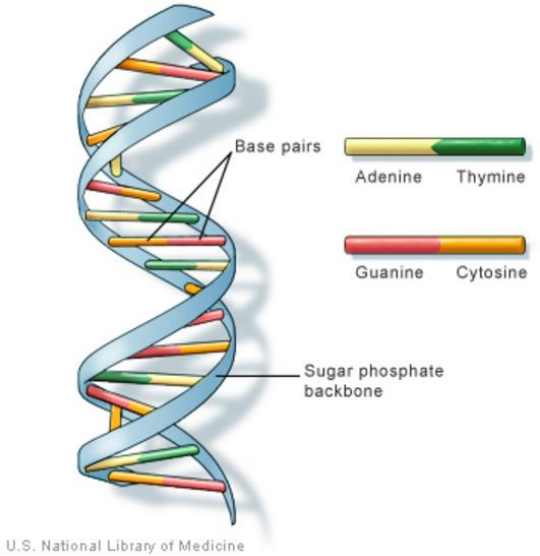
- Bases
- Nucleosides = base + sugar
  - E.g. adenosine, thymidine, deoxyuridine
- Nucleotides = base + sugar + phosphate (mono, di or tri)
  - E.g. UMP, ADP, dATP

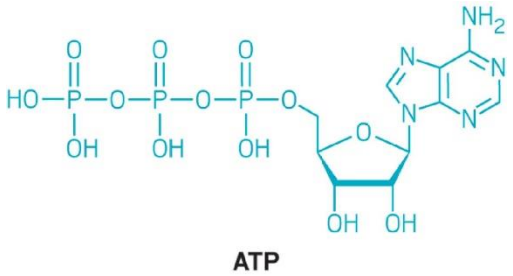




Purines and Pyrimidines

- Biological functions include:
  - Energy storage and transfer, e.g. ATP
  - Components of genetic material (DNA and RNA)
  - Cell signalling, e.g. cAMP
  - Neurotransmitters, e.g. adenosine
  - Enzyme regulation
  - Intermediates of glycosylation reactions, e.g. UDP-glucose
  - Substrates for production of other biological molecules, e.g. thiamine





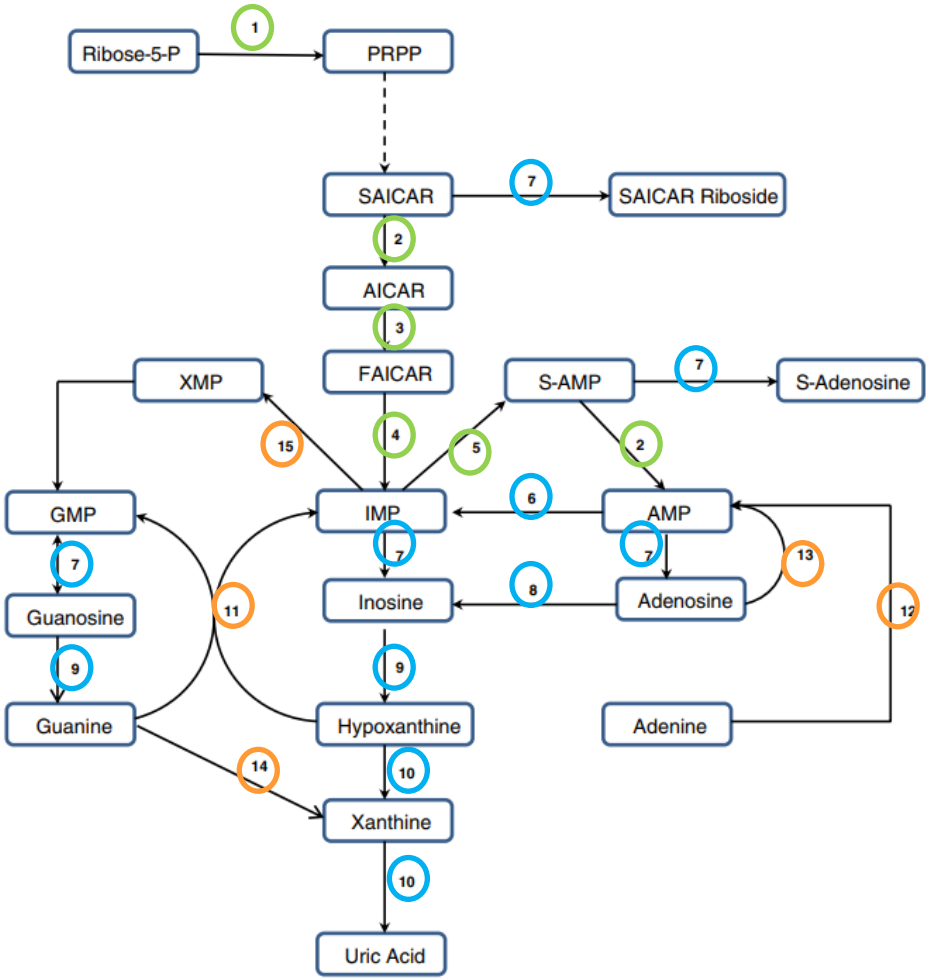
Purine and Pyrimidine metabolism

- >35 defects of purine and pyrimidine metabolism identified
- ~20 associated with serious clinical consequences
- As with most IMDs these are individually rare disorders, but prevalence thought to be underestimated
- Very diverse and often non-specific clinical spectrum, but can be roughly grouped into:
  - Neurological
  - Immunological
  - Haematological
  - Renal (stones)

Purine metabolic pathway

Biosynthesis

1. PRPP synthetase
2. Adenylosuccinate lyase (ADSL)
3. AICAR transformylase
4. IMP cyclohydrolase
5. Adenylosuccinate synthetase



Catabolism

6. AMP deaminase
7. 5'-nucleotidase
8. Adenosine deaminase (ADA)
9. Purine nucleoside phosphorylase (PNP)
10. Xanthine dehydrogenase

Salvage

11. Hypoxanthine-guanine phosphoribosyltransferase (HPRT)
12. Adenine phosphoribosyltransferase (APRT)
13. Adenosine kinase
14. Guanase
15. IMP dehydrogenase

Purines

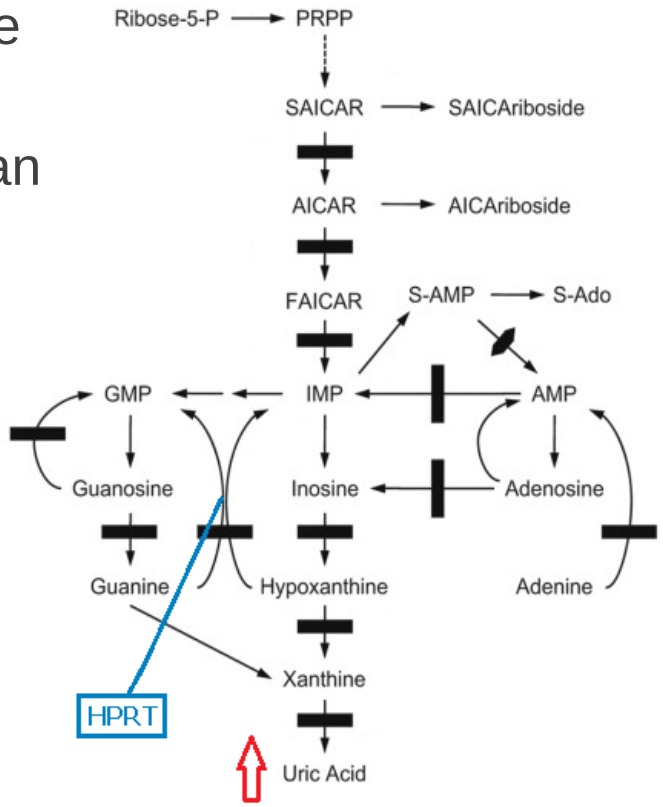
- Non-specific pattern includes urate, xanthine and hypoxanthine

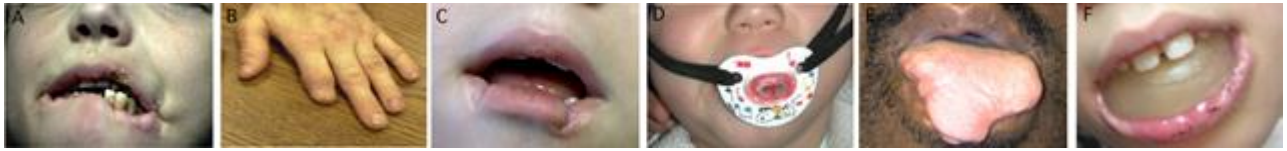
↑ High urate (urine and plasma)	↓ Low urate (urine and plasma)
HPRT deficiency PRPS superactivity	MoCo deficiency Xanthine oxidase deficiency PNP deficiency
High plasma urate with low urine urate	
Autosomal dominant tubulointerstitial kidney disease (ADTKD) – many genetic causes including UMOD, REN and HNF1β mutations	

- Pathognomic compounds include:
  - 2,8-dihydroxyadenine in APRT deficiency
  - SAICAr and SADO in ADSL deficiency (and ATIC)
  - Deoxyadenosine in ADA deficiency
  - (Deoxy)inosine and (deoxy)guanosine in PNP deficiency

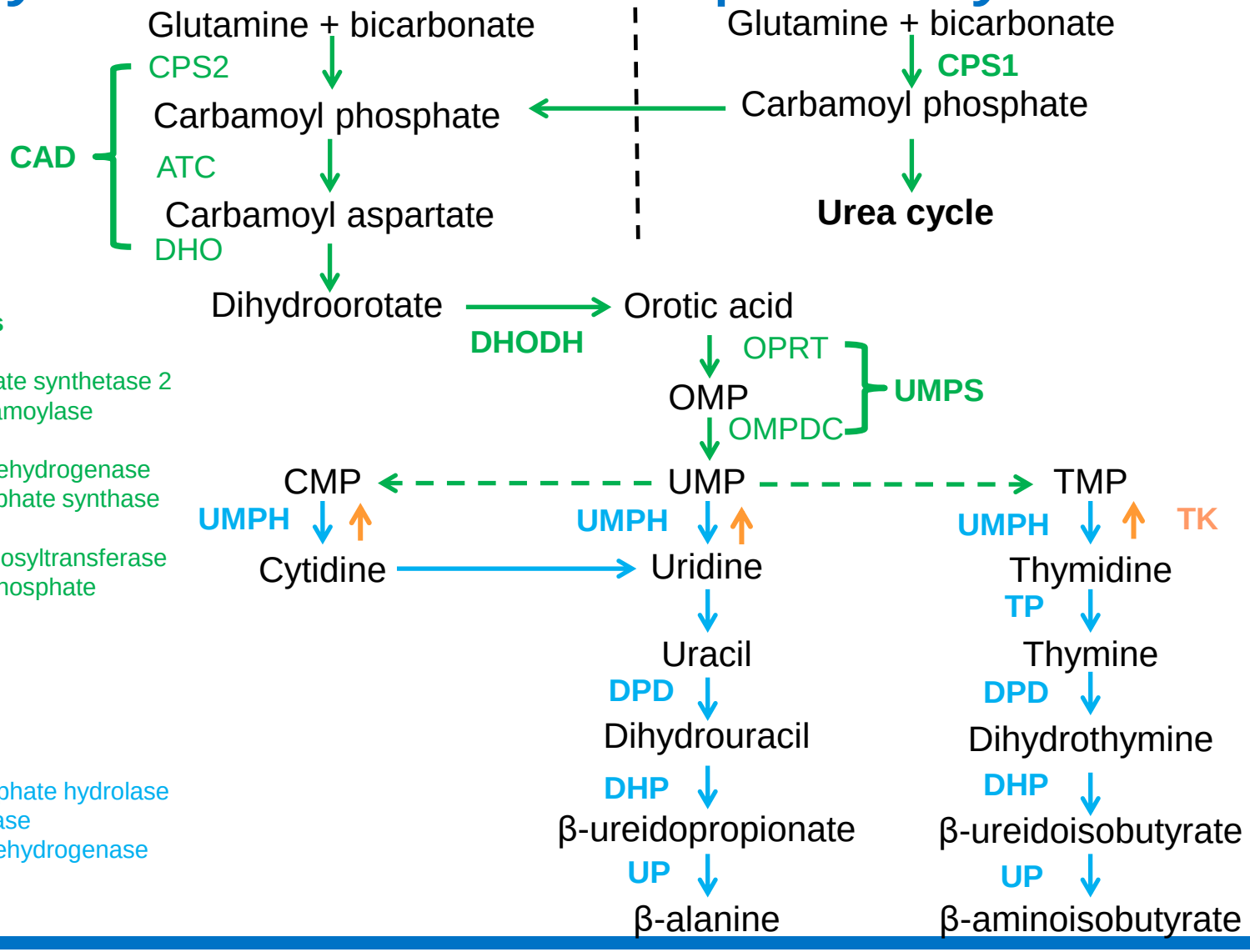
HPRT deficiency

- X-linked mutation in Hypoxanthine-guanine PhosphoRibosyl Transferase
- Complete deficiency results in Lesch-Nyhan disease:
  - Neurological presentation in first six months
  - Self-injurious behaviour
  - Urate stones will eventually form
- Partial deficiency:
  - Neurological disability (but no behavioural disturbance)
  - Hyperuricaemia only, i.e. present with gout or arthritis





Pyrimidine metabolic pathway



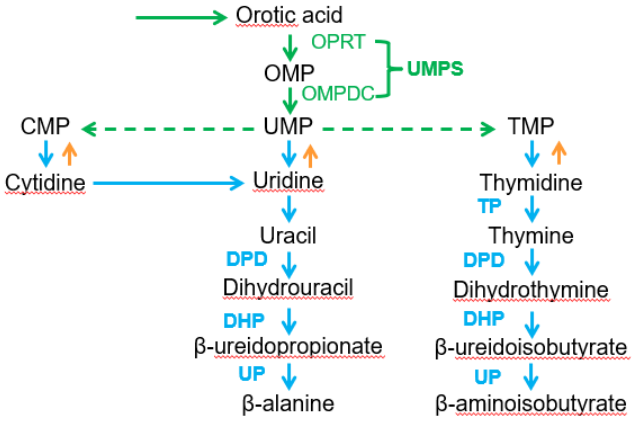
Disorders of Biosynthesis
CAD comprised of:
CPS2 = carbamoyl phosphate synthetase 2
ATC = aspartate transcarbamoylase
DHO = dihydroorotase
DHODH = dihydroorotate dehydrogenase
UMPS = uridine monophosphate synthase
Comprised of:
OPRT = orotate phosphoribosyltransferase
OMPDC = orotidine monophosphate decarboxylase

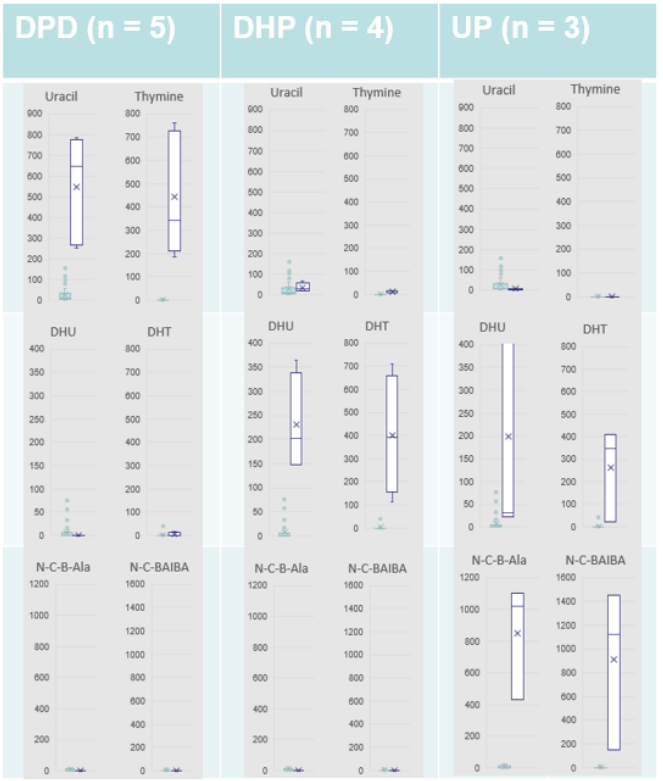
Disorders of Salvage
TK = thymidine kinase

Disorders of Catabolism
UMPH = uridine monophosphate hydrolase
TP = thymidine phosphorylase
DPD = dihydropyrimidine dehydrogenase
DHP = dihydropyrimidinase
UP = β-ureidopropionase

Organic acids as a diagnostic tool for pyrimidine disorders

Condition	Organic acid markers	Further useful tests	Symptoms
Hereditary orotic aciduria (UMPS deficiency)	Orotic acid	Plasma amino acids, ammonia, B12/folate	Macrocytic anaemia
Mitochondrial neurogastrointestinal encephalopathy (MNGIE) aka TP deficiency	Thymine, uracil, lactate, TCA cycle metabolites	Purines and pyrimidines	Mitochondrial depletion,GI dysmotility cachexia, peripheral neuropathy
DPD deficiency	Thymine, uracil	Purines and pyrimidines	Likely benign
DHP deficiency	Thymine, uracil, dihydrouracil, dihydrothymine	Purines and pyrimidines	Likely benign
UP deficiency	Thymine, uracil, dihydrouracil, dihydrothymine, B-alanine, B-AIBA	Purines and pyrimidines	Unclear

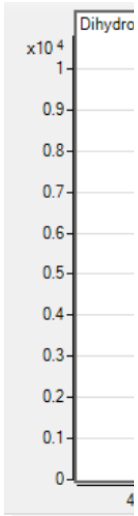


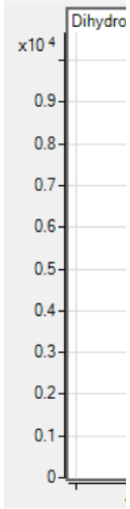


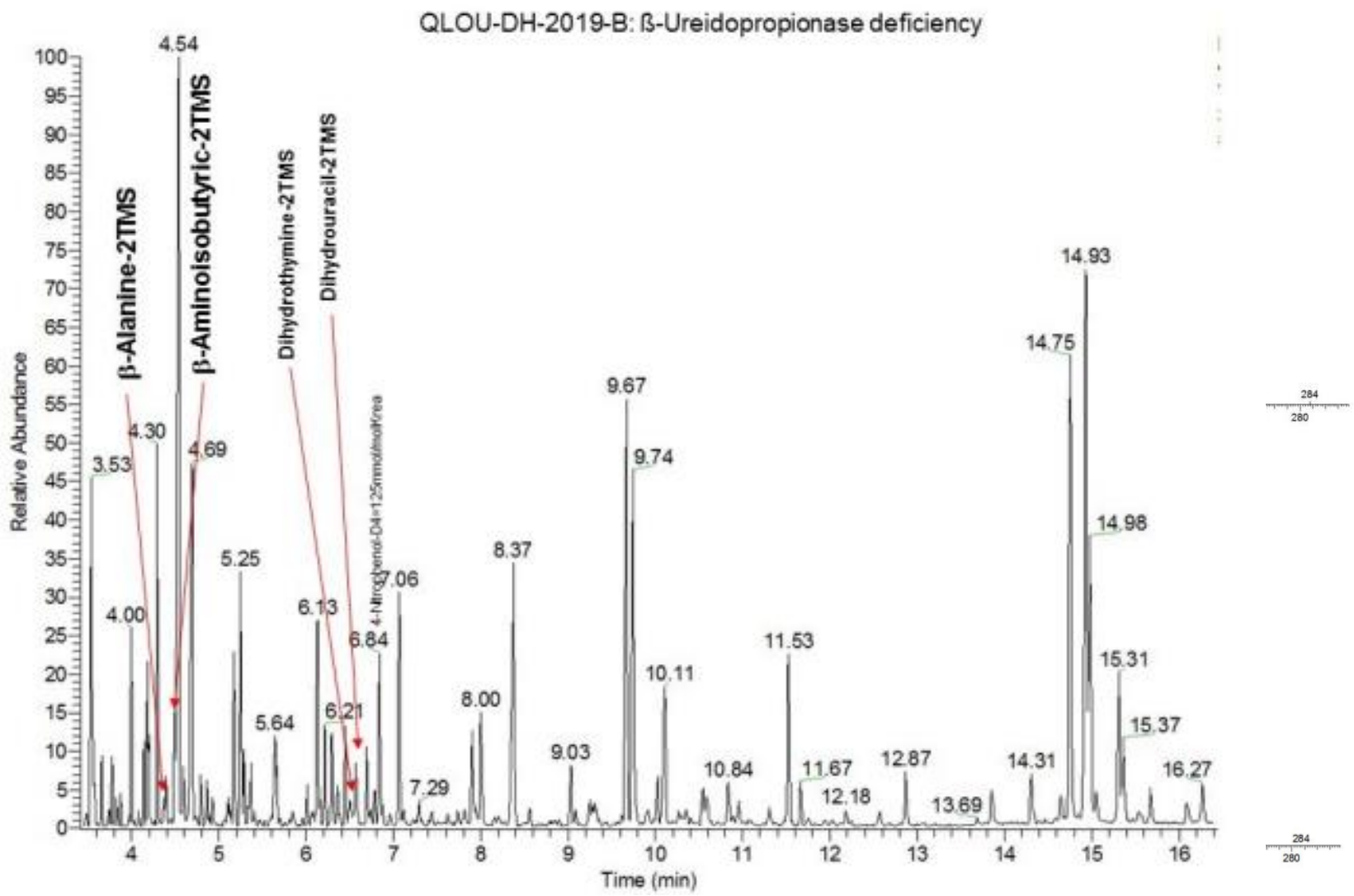
synnovis
A SYNLAB pathology partnership

The Leeds Teaching Hospitals NHS
NHS Trust

Useful GC-MS spectra



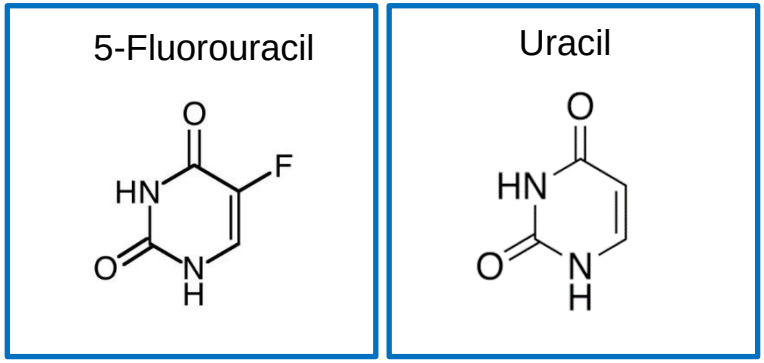


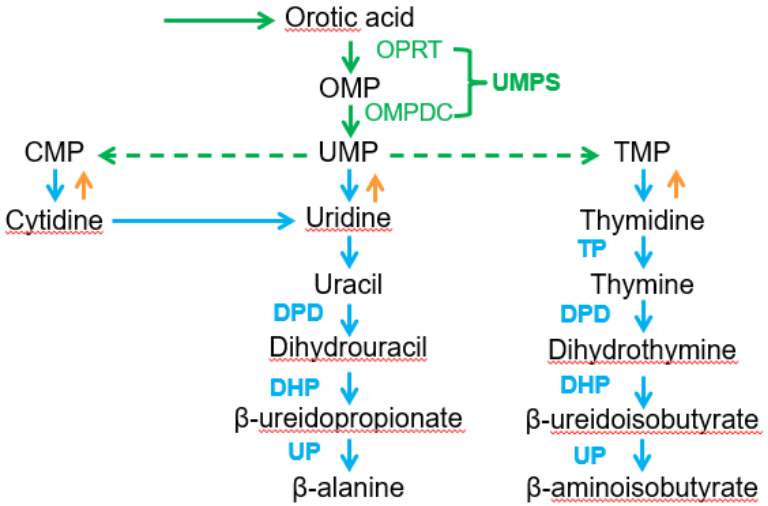


Thanks

5-Fluorouracil toxicity

- DPD and related disorders (DHP and UP) are strongly associated with 5-FU toxicity – even in heterozygotes (?parental risk)
  - Treatment for solid tumours e.g. breast, ovarian and colon cancers.
- Prevalence of low activity variants is very similar to TPMT. Low activity leads to 5-FU accumulation and toxicity.
- Symptoms of toxicity:
  - Neutropaenia
  - Paralysis
  - Death (estimated 1 in 400 patients)
- DPD variants often routinely screened in patients to be given 5-FU, DHP and UP not currently screened for.

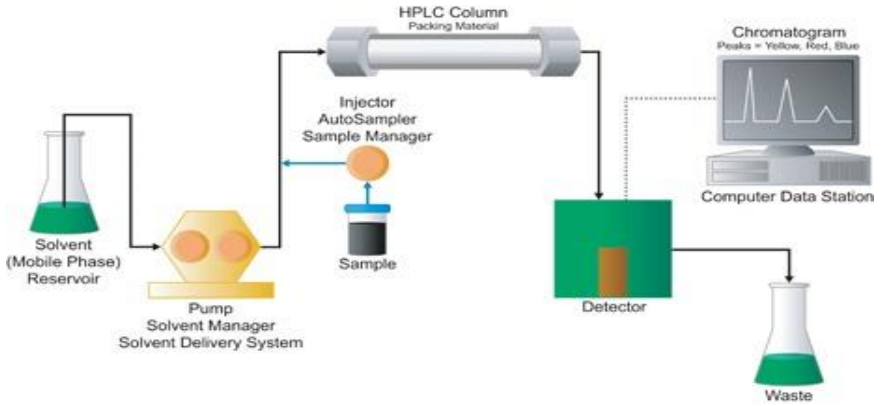




Analytical methods – UPLC

Analysis

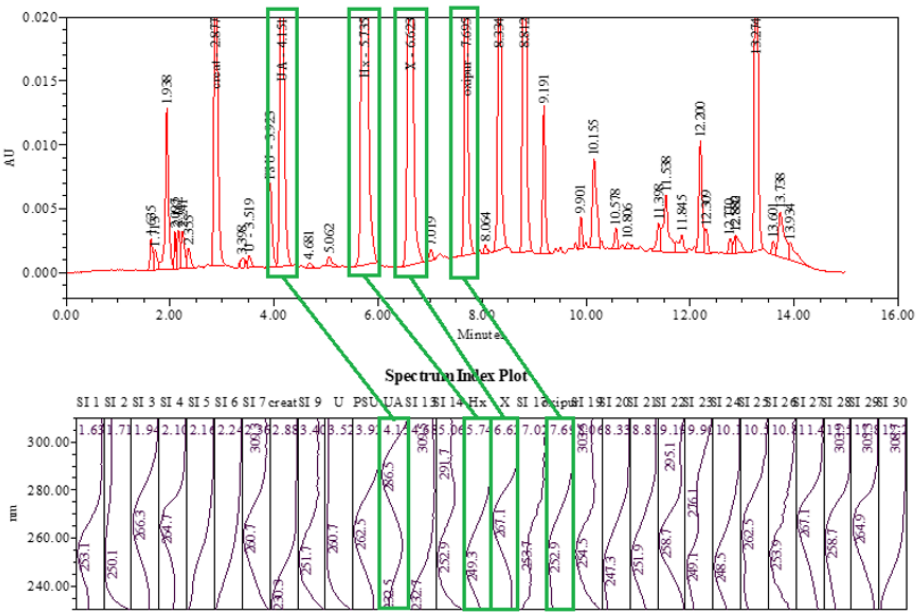
- Urine, plasma, RBC and enzyme extracts run by Ultra Performance Liquid Chromatography (UPLC)
- Reverse phase (non-polar stationary phase, polar mobile phase)
- Column: BEH C18, 1.7 µm, 2.1 x 150 mm
- Mobile phases:
  - A - 40 mM ammonium acetate in water
  - B - 100% methanol
- Detector: photodiode array (based on UV/Vis spec)



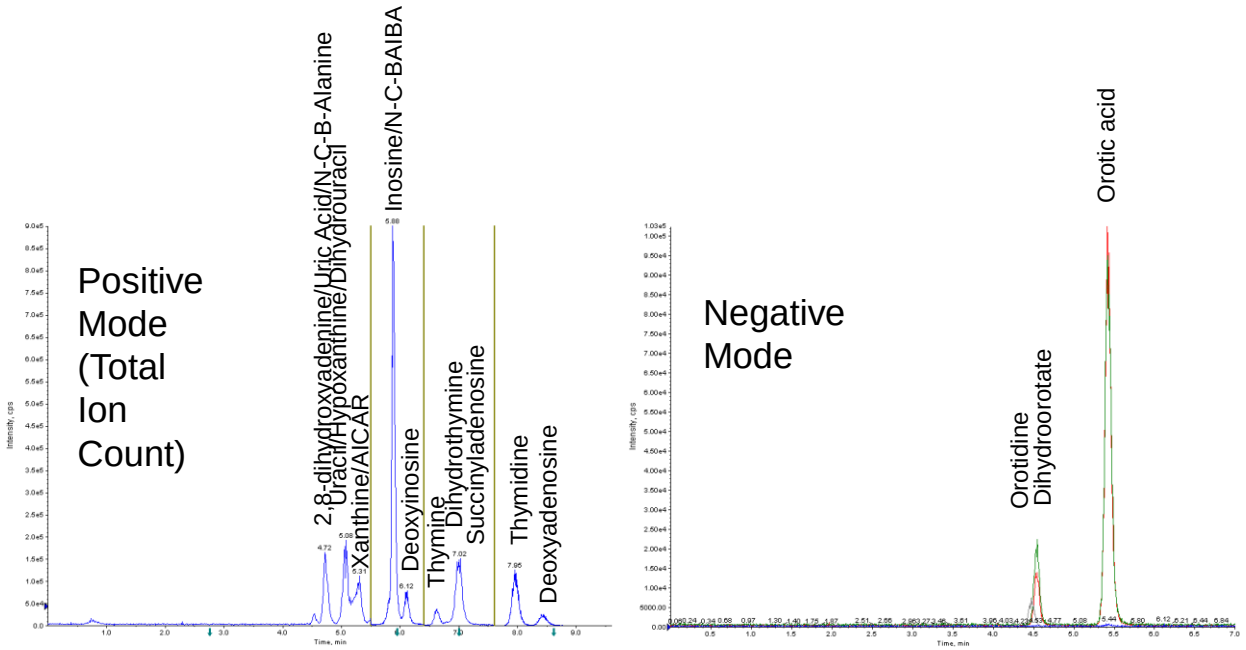
Analytical methods – UPLC

Interpretation

- Analytes identified based on retention time and characteristic absorption profile
- What we report depends on the clinical details
- May phone referring lab/clinician to add enzyme or DNA analysis exclude or confirm a diagnosis



Analytical methods – LC-MS/MS



- Urine samples are incubated and diluted for analysis by reverse phase LC-MS/MS.
- Currently each sample is run twice:
  - Positive mode – 4 analytical periods totalling 38 MRMs
  - Negative mode – 4 MRMs, more polar solvent

Analytical methods

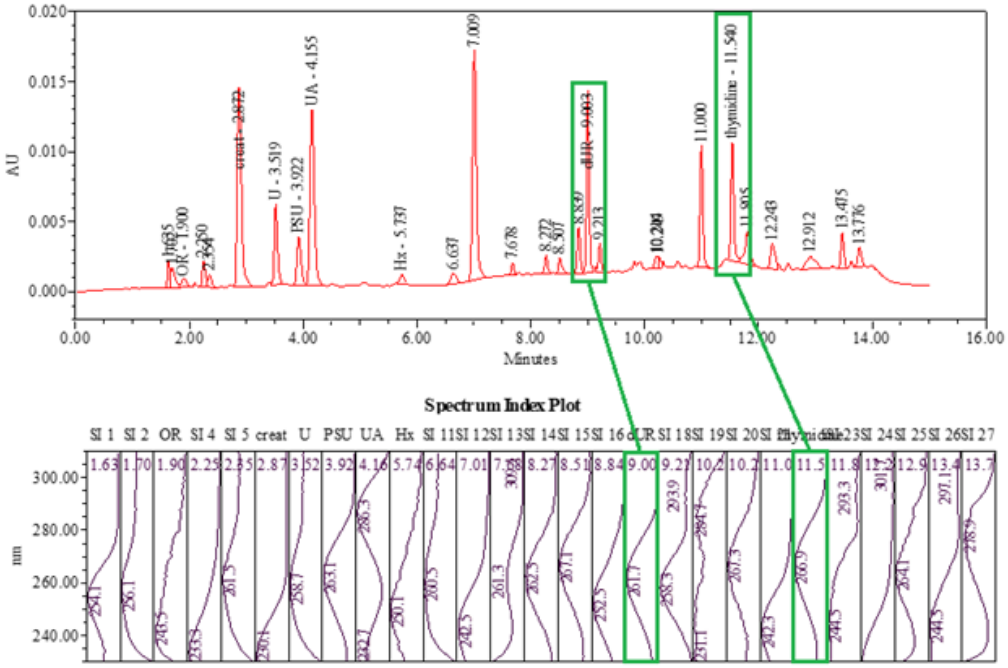
	Advantages	Disadvantages
UPLC	- Simple urine prep – dilute-and-shoot - Plasma and RBC nucleotide analysis possible - Sensitive for most PP compounds	- Identification of analytes based on RT and abs max only (non-specific) - No deuterated ISTD - Infrequent one-point cal - Unable to detect DHT / DHU / β-ureido compounds
LC-MS/MS	- Simple urine prep – dilute-and-shoot - Almost all disorders IDed from urine - Stable-labelled internal standards for most compounds - Sensitive for most PP compounds - Calibrators/QCs run with every batch - Able to detect DHT / DHU / β-ureido compounds - Plasma and RBC nucleotide analysis possible	- Complex development - small and heterogenous molecules with a range of polarities, solubilities, pKas and many interferences - Targeted panel, potential to miss new/unusual compounds - Plasma analysis very contamination prone

Case 1 – History

- 15 year old male
- Admitted to A&E due to severe abdominal pain
- Parents report a history of diarrhoea and periods of abdominal pain
- Low BMI – not concerningly low, although losing weight recently
- Short-sighted since age 9, but recent eye exam abnormal (some signs of ophthalmoplegia) – referral made to Optometry
- Routine Biochemistry, Immunology and Haematology tests revealed no diagnostic abnormalities

Case 1 – Metabolic results

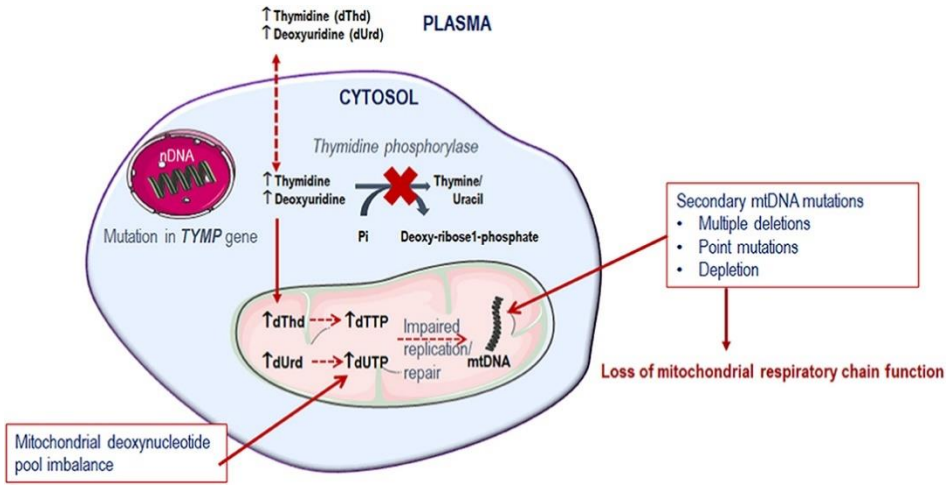
- No diagnostic abnormalities on plasma amino acid and urine organic acid profiles
- Plasma and urine sent for Purine and Pyrimidine analysis:



- Do not usually see deoxyuridine (dUR) or thymidine (Thym)
- Genetic confirmation: homozygous TYMPc.1088delG, p.G363EfsX151

Case 1 – MNGIE

- Mitochondrial Neuro Gastro Intestinal Encephalomyopathy
- Due to thymidine phosphorylase deficiency, *TYMP* gene
- Multi-systemic symptoms:
  - GI: N&V, constipation/diarrhoea, abdominal pain, distension, diverticulosis, perforation, cachexia
  - Neurological: peripheral neuropathy, ptosis, ophthalmoplegia, hearing loss
  - Leukoencephalopathy
  - Metabolic: liver cirrhosis, pancreatitis, DM, high trigs and lactate



- Mean age of death is 37 years (<5% survive after age 50), primarily due to GI and liver complications and cachexia

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): Position paper on diagnosis, prognosis, and treatment by the MNGIE International Network. Michio Hirano et al (2021). *JIMD*, **44(2)**, p376-87

Case 1 – Management

- Management:
  - Was largely supportive
  - Enzyme replacement and haemodialysis only achieve temporary decrease in metabolites
  - Hematopoietic stem cell transplant restores activity and halts disease progression, but high transplant-related morbidity and mortality
  - Liver transplant

- Patient had liver transplant age 17
- Clinical symptoms showing signs of improvement
- Thymidine and deoxyuridine continue to be undetectable
- Regular reassessment of metabolic status, neuropathy and hepatic function; MRI to monitor for development of leukoencephalopathy

Successful Liver Transplantation in Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE). Kimberly A. Kripps *et al* (2020). *Mol Genet Metab,* **30(1)**, p58–64

Case 2 – History

- Male infant presented at 48 hours old to local A&E
  - Focal seizures
  - Limb cycling movements
  - High pitched cry
  - Parents noted he had not been feeding well
- History – no antenatal concerns, born 39 weeks gestation, no issues at delivery apart from hypospadia noted
- Two siblings, no concerns, no parental consanguinity

Case 2 – Management

- Intubated and NG tube
- Prolonged EEG
  - Focal status epilepticus
  - Encephalopathy with burst-suppression pattern
- Levetiracetam, phenytoin, pyridoxal phosphate, antibiotics, antivirals
- Transferred to PICU at the Evelina London on day 3

Case 2 – Investigations

- Head MRI –
  - Imaging differential included a severe hypoxic ischaemic insult, sepsis, or an underlying neurometabolic condition
- No immediately apparent cause on review of routine bloods and cultures
- Further samples included serum urate:
  - Urate = 0.06 mmol/L (RR 0.20 – 0.42)

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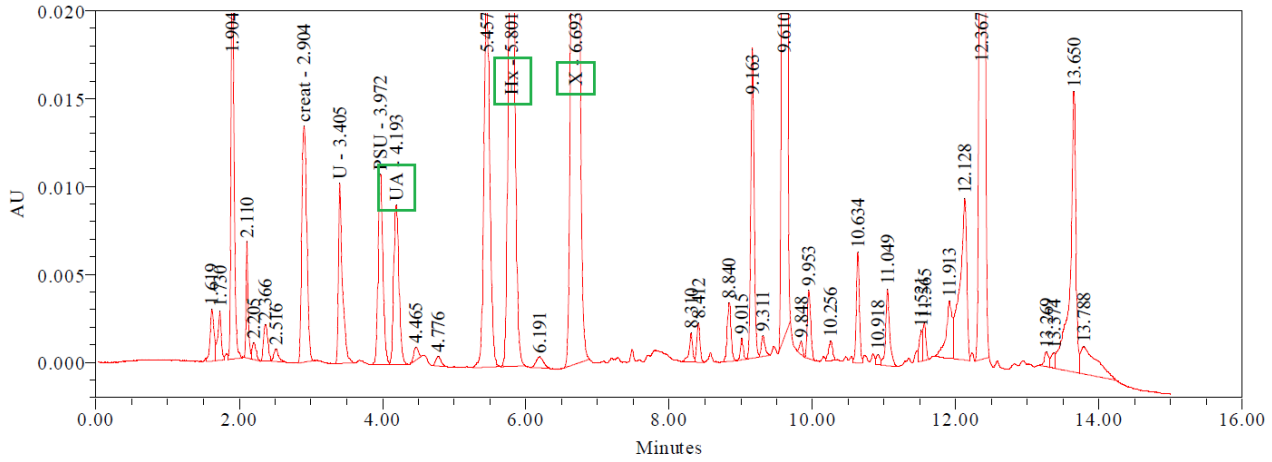
- Clinical history + hypouricaemia → suspicion of MoCoD

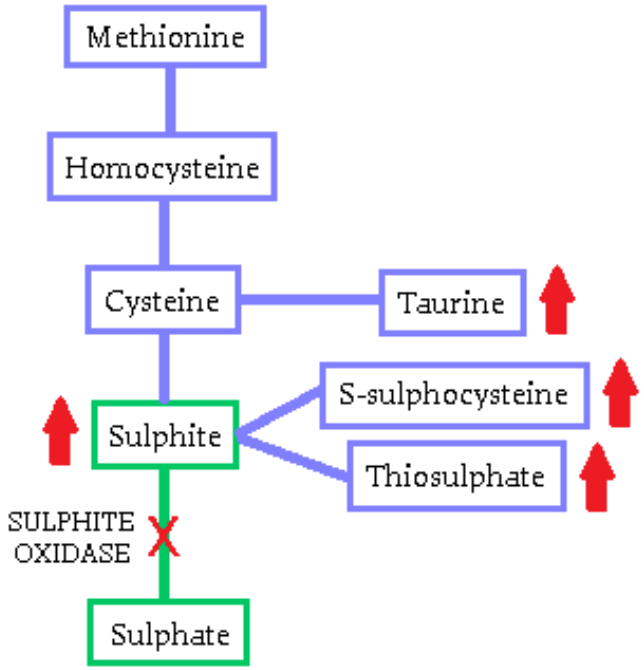
Case 2 – Metabolic results

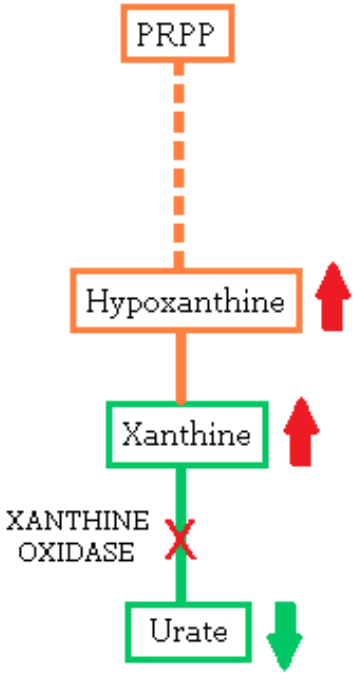
Test	Analyte	Result	Ref. range
Urine P&P screen	UA/Cr	0.23	0.30 – 1.50
	Xanthine	1.390 mmol/L	
	Hypoxanthine	0.385 mmol/L	
Sulphite dipstick		+++	
Urine Scys		73.0 uM/mM Cr	0.0 – 10.0

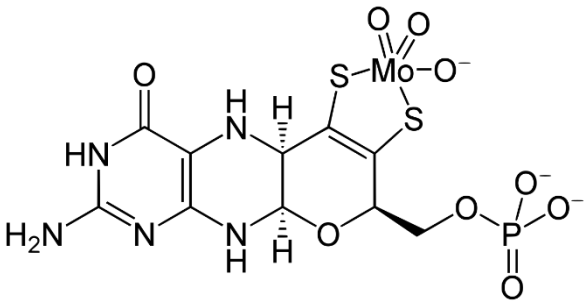
Test	Analyte	Result	Ref. range
Urine AA (LC-MS/MS)	Scys	75 uM/mM Cr	
	Taurine	101	<54
Plasma AA (LC-MS/MS)	Scys	20 umol/L	*(<20)*
	Taurine	163 umol/L	19 - 173
CSF AA (IEC)	Scys	Haemolysed Small peak	



Case 2 – MoCo deficiency

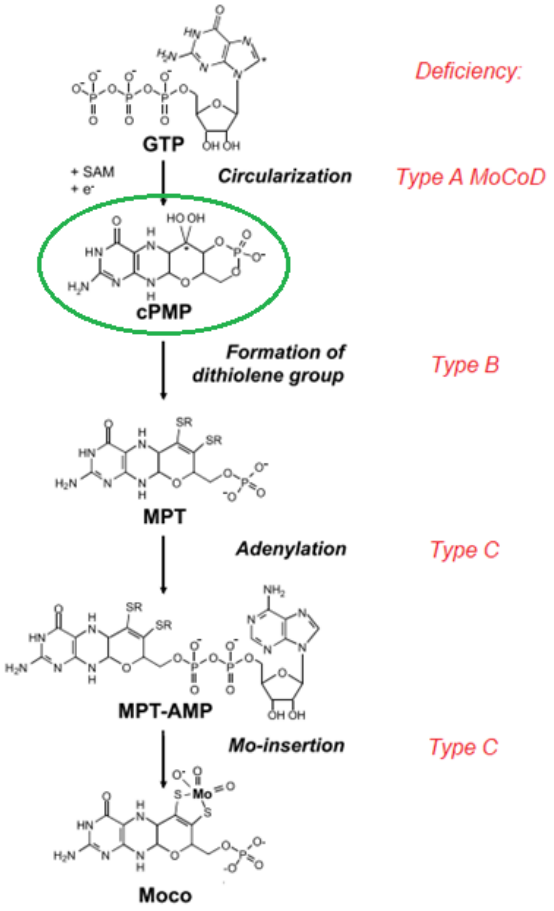






Case 2 – Treatment

- Genetic result available on day 6
- Homozygous for *MOCS2* c.413 G>A, p.G76R mutation in exon 4 (type B)
- Potential treatment for MoCoD type A is to replace cPMP
- Approved by the FDA in the US. Ongoing trials in the UK
- Reduces risk of mortality
- Cannot reverse any neurological damage that has already occurred



Efficacy and safety of cPMP substitution in severe molybdenum cofactor deficiency type A: a prospective cohort study, B Schwahn *et al* (2015). *Lancet*, **386**, 1955-63

Fosdenopterin: a first-in-class synthetic cyclic pyranopterin monophosphate for the treatment of molybdenum cofactor deficiency type A, B Schwahn (2021), *Paed Neur*, **17(2)**, 85-91

Case 3 - Background

- A urine organic acid sample was received in the Leeds lab, with clinical details “dystonia, low molybdenum, anaemia”.
- A moderate peak of orotic acid was seen which was then quantified by purine and pyrimidine analysis.
- Relevant routine blood results:
  - Uric acid undetectable in plasma
  - Lymphocyte count consistently low
- History:
  - Recurrent infections from birth – assumed routine childhood infections
  - Motor delay at 1 year old triggered neurological referral and metabolic investigations.

Case 3 - Results

Compound	Result (µmol/mmol creatinine)	Reference Range
Uric acid	Not detected	419 - 1963
Xanthine	24	4 – 50.9
Hypoxanthine	2230	4 – 61
Inosine	81	0 – 4
Deoxyadenosine	2.9	<1
Deoxyinosine	81	0 – 4
Uracil	34.9	0 – 47.5
Orotic acid	30.9	<6
AICAR	14.3	<3
N-C-B-alanine	18.6	<31.2

Immunoglobulins

Result	Value	Units	Ref. Range	Result Comment
Immunoglobulin IgG	7.6	g/L	3.1-13.8	
Immunoglobulin IgA	**0.14**	g/L	0.30-1.20	
Immunoglobulin IgM	0.54	g/L	0.50-2.20	
Set Comment:				

Specific Haemoph. Ab

Result	Value	Units	Ref. Range	Result Comment
Haemophilus Ab	1.020	ug/mL		
Set Comment:	HAE Ab (ug/mL): Inadequate <0.15, Suboptimal 0.15-1, Adequate >1			

Specific Pneumoc. Ab

Result	Value	Units	Ref. Range	Result Comment
Pneumococcal Ab	27.9	ug/mL		
Set Comment:	PNE Ab (ug/mL): Inadequate <10, Suboptimal 10-30, Adequate >30			

Specific Tetanus Ab

Result	Value	Units	Ref. Range	Result Comment
Tetanus Ab	0.462	IU/mL		
Set Comment:	TET Ab (IU/mL): Inadequate <0.01, Suboptimal 0.01-0.15, Adequate >0.15			

Cell markers DEF

Result	Value	Units	Ref. Range	Result Comment
Lymph(Abs)	136	cells/uL	2600-10400	
CD3 (%)	42	%		
CD19 (%)	33	%		
CD4 (%)	36	%		
CD8 (%)	6	%		
NK (%)	21	%		
CD3 (Abs)	58	cells/uL	1600-6700	
CD19 (Abs)	45	cells/uL	600-2700	
CD4 (Abs)	49	cells/uL	1000-4600	
CD8 (Abs)	8	cells/uL	400-2100	
NK (Abs)	29	cells/uL	200-1200	
ratio	6.13		1.07-1.87	
Set Comment:	Severe lymphopenia affecting all lymphocyte subsets, reduced proportion of naive T-cells and raised proportion of activated T- cells. Note possiblity of PNP deficiency which could explain these findings			

Case 3 - PNP deficiency

Technical Information

Variant details

Gene	Zygosity	Inheritance	HGVS description	Classification
PNP	Homozygous	AR	NM_000270.3:c.172C>T p.Arg58*	Pathogenic

- One of the 2 purine disorders associated with SCID.
  - ADA deficiency is more common (ca. 15% SCID cases), the most common form of SCID in female patients.
  - PNP deficiency is rarer (ca. 1% of SCID cases).
- Deficiency of PNP prevents nucleosides being converted to bases.
- Nucleosides accumulate and uric acid is depleted (urine and plasma).
- Nucleosides are converted to nucleotides (particularly GTP and dGTP).
- Accumulation of a single nucleotide inhibit ribonucleotide reductase leading to reduced DNA synthesis, causing SCID in these patients.
- Patients are also at risk of neurological symptoms and autoimmune conditions.
- Treatment is bone marrow transplantation.

Case 4 - Background

- 6 month old boy presented with renal stones.
- Standard urine stone screens had shown no abnormalities (cystine, urate, oxalate, calcium, magnesium etc).
- FTIR analysis of the stone was inconclusive (?lansoprazole)
- Over the next 6 years:
  - 19 renal/surgical outpatient appointments
  - 3 ESWL, 2 nephrolithotomy and a nephrostomy
  - 34 ultrasounds, 10 X-rays and a CT under general anaesthetic.
  - Recurrent UTIs leading to a total of 24 days in-patient stay.
- At the age of 7 as part of a reference range study at Leeds we ran a sample from the paediatric renal stones clinic through our purine and pyrimidine method.

Case 4 - Results

Compound	Result (µmol/mmol creatinine)	Reference Range
Uric acid	398.7	213.8 - 895.4
Xanthine	11.5	4 – 23
Hypoxanthine	13.3	3 – 33
Uracil	11.4	<20.1
Orotic acid	0.7	0 – 3.1
2,8-Dihydroxyadenine	66.1	<3

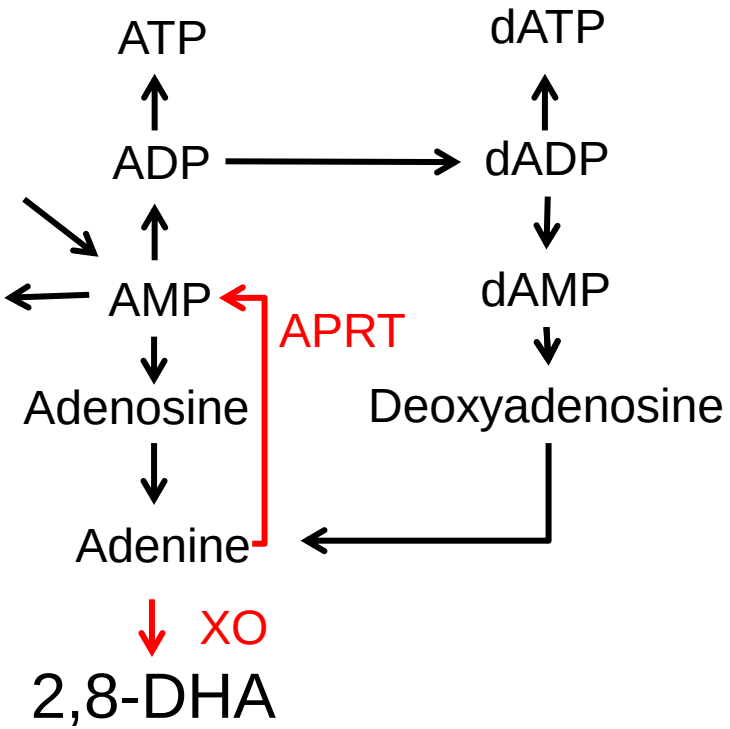
All other compounds undetectable

Case 4 - APRT deficiency

PLASMA ENDOGENOUS PURINES				
Urate (HPLC)	196		umol/L	(100-260)
Blood 2-8di-OH-Adenine	1		umol/L	(0-1)
ENZYMES - nmol/mg Hb/h				
HPRT (rbc)	160	*		(80-130)
APRT (rbc)	2	*		(16-32)

Comment:
The red cell lysate APRT activity was low, indicating APRT deficiency.
APRT mutation analysis is available if required.

- Defect in the recycling pathway leading to accumulation of adenine.
- This is converted to 2,8-dihydroxadenine (2,8-DHA) by xanthine oxidase, 2,8-DHA is very insoluble and is renally excreted.
- Typically presents in adulthood as renal stones.
- Stones can lead to recurrent UTIs and eventually renal transplant.
- This patient has been stone-free in the 3 years since starting allopurinol.



Allopurinol and monitoring

- Allopurinol is used to treat many of the Purine disorders
  - To reduce urate concentration in urate-overproducing disorders (HPRT deficiency, PRPS superactivity)

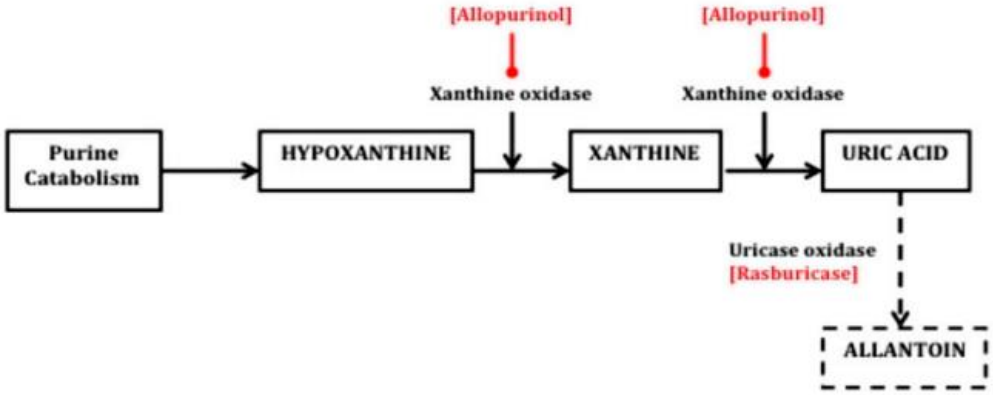
    Mechanism: allopurinol metabolite oxipurinol inhibits xanthine oxidase
  - To minimise 2,8-DHA production in APRT deficiency

    Mechanism: inhibition of xanthine oxidase, which is also responsible for converting adenine to 2,8-DHA
  - To minimise SAICAR in ADSL deficiency

    Mechanism: allopurinol ribonucleosides inhibit production of SAICAR

Allopurinol and monitoring

- In urate over-producing disorders, allopurinol reduces urate concentration but increases hypoxanthine and xanthine
- Monitoring required to:
  - Ensure adherence
  - Ensure correct balance of urate reduction with xanthine increase
  - Aim is to maintain urate in the upper third of the reference range



Summary

- Purine and pyrimidine disorders can present with a wide spectrum of symptoms and are often not considered in a differential diagnosis
  - Neurological
  - Immunological
  - Haematological
  - Renal (stones)
- Uric acid can be a useful marker for purine disorders
- Organic acid analysis can identify a number of pyrimidine disorders
- Dedicated methods needed for most disorders
- Allopurinol is used to help treat several purine disorders and monitoring is important

Thank you for listening