Inborn errors in Purine and Pyrimidine metabolism, please don't forget them!

ERNDIM Symposium, September 1st 2015, Lyon

ERNDIM

QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

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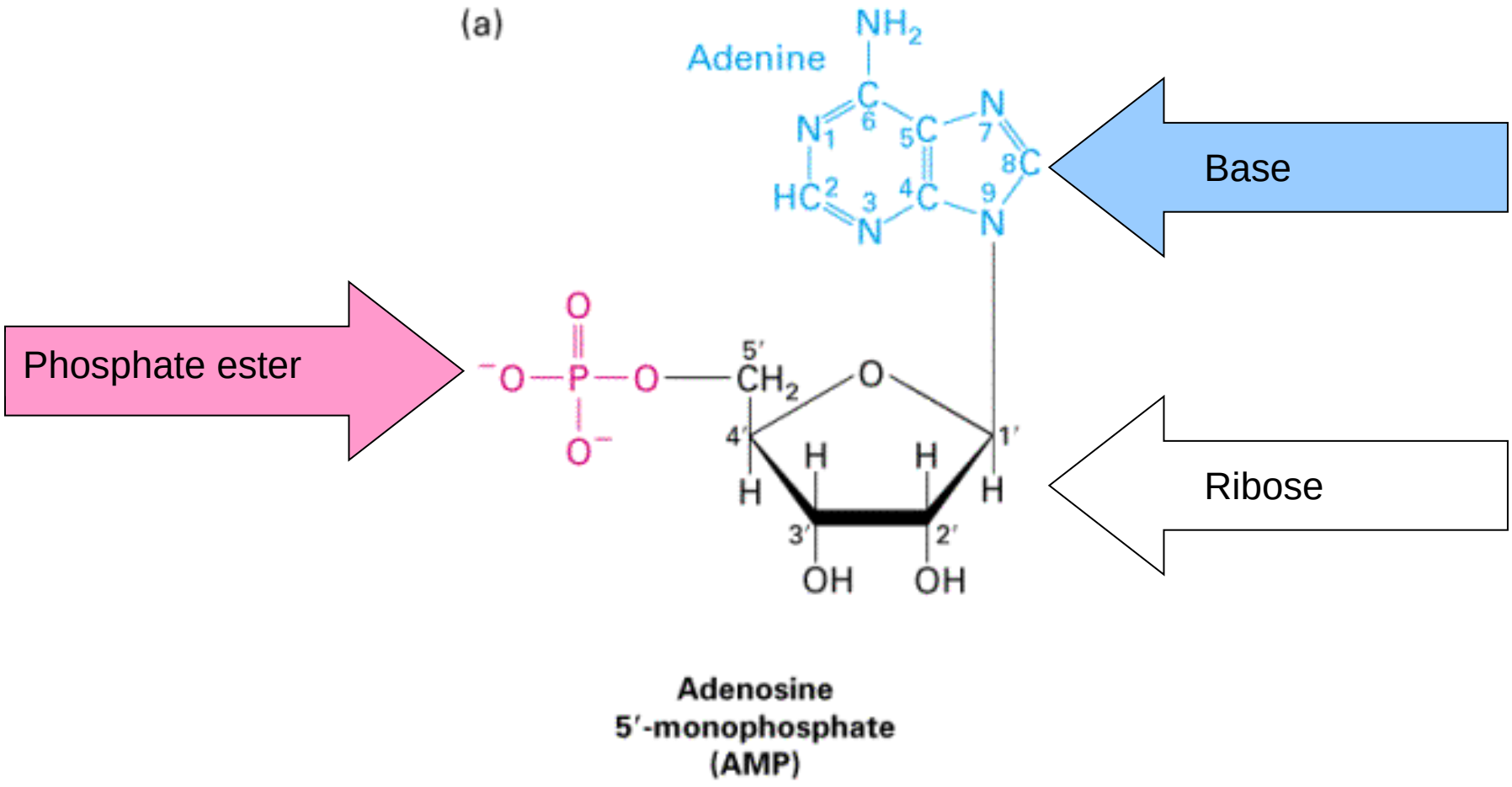
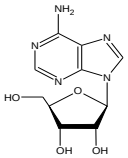
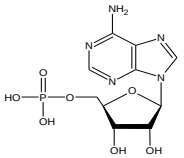
Nucleosides, nucleotides and bases



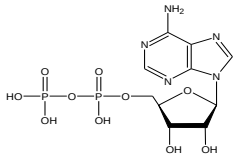
examples of a nucleoside and its nucleotides



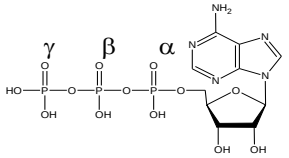
adenosine



adenosine-5'-monophosphate

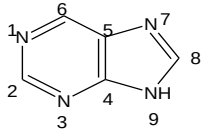


adenosine-5'-diphosphate

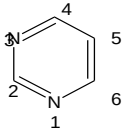


adenosine-5'-triphosphate

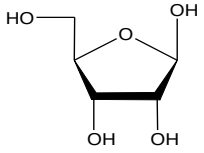
components of natural occuring nucleosides



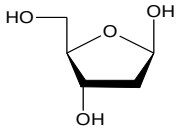
purine



pyrimidine

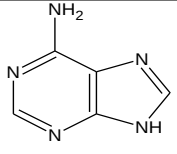


ß-D-ribose

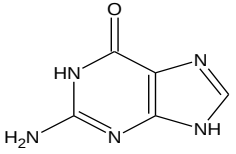


ß-D-deoxyribose

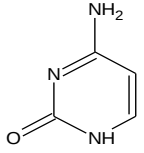
natural occuring bases



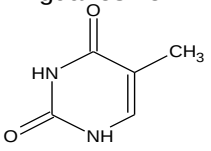
adenine



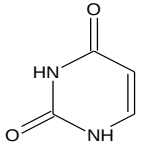
guanosine



cytosine

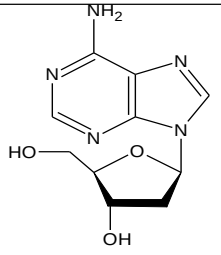


thymine

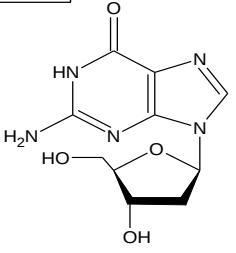


uracil

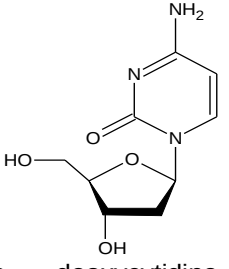
natural deoxynucleosides



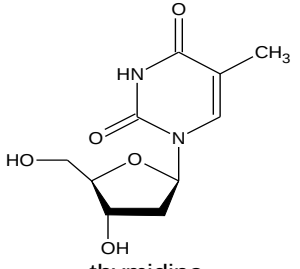
deoxyadenosine



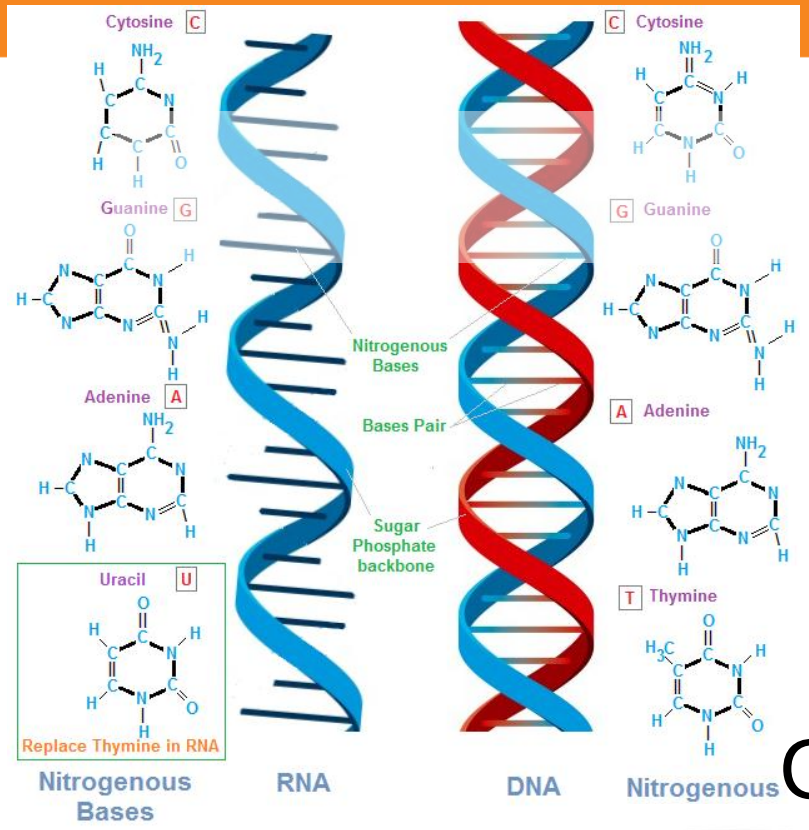
deoxyguanosine

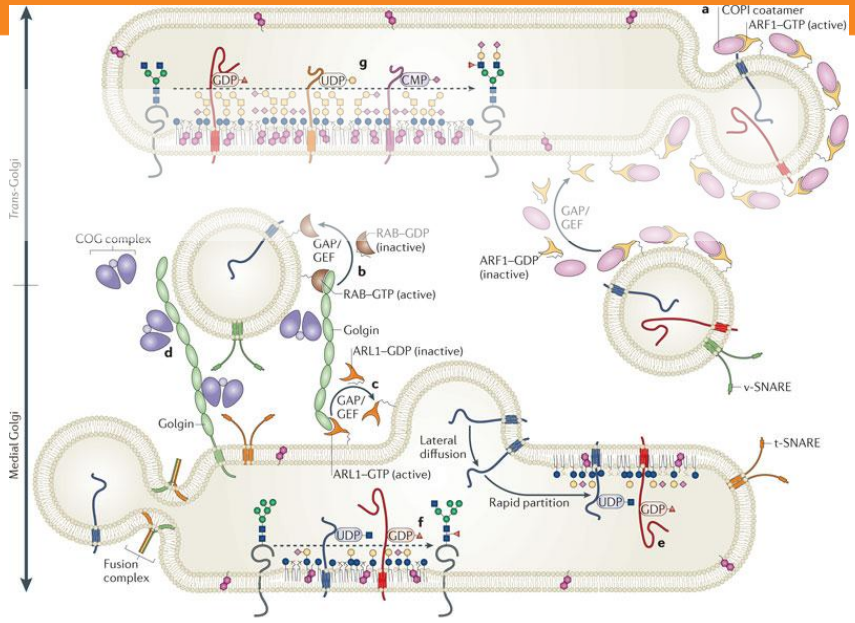


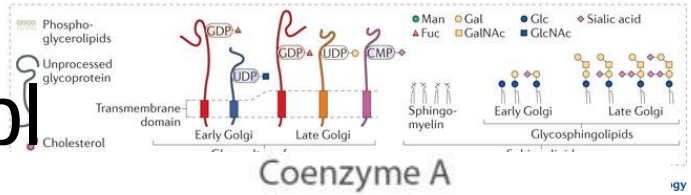
deoxycytidine



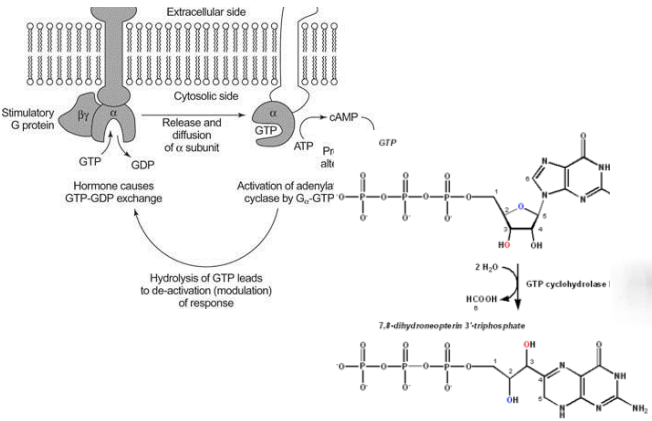
thymidine

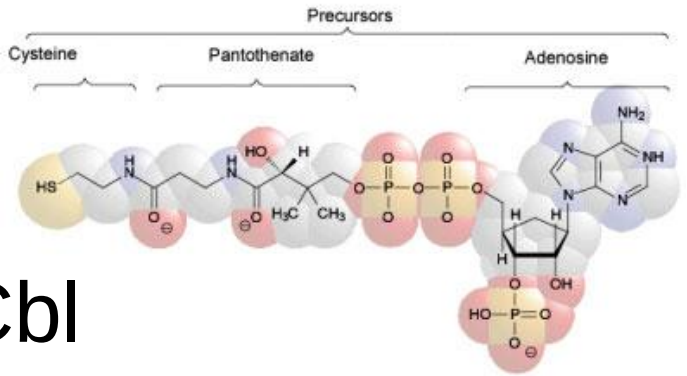






OH-Cbl





Ado-Cbl

Methylmalonyl-CoA → Succinyl-CoA

Symptoms associated with IEM of purine and pyrimidine metabolism

Anaemia (megaloblastic, haemolytic), Arthritism, Autism, Automutilation, Cachexia, feeding difficulties, Cerebral palsy, Developmental delay, Dysmorphic features,Encephalopathy, Epilepsy, seizures, fitting, Exercise intolerance, Gout, Haematuria, Hepatomegaly, Hyperactivity, short attention span, Hyperuricaemia, Hypo-/hypertonia, Immunodeficiency, Impaired hearing, deafness, Lactic acidosis, Lens dislocation, Lymphopaenia, Microcephaly, Mitochondrial DNA-depletion,Muscle weakness, Psychomotor retardation, Nephropathy, Nephro/urolithiasis, Optic atrophy, Renal failure (acute and chronic), Scoliosis, Severe combined immunodeficiency, Spastic diplegia, Splenomegaly, T-cell immunodeficiency, Tetraparesis

What I hear about purines and pyrimidines

Defects in purine and pyrimidine metabolism are rare, it's not worthwhile screening for

Purine and pyrimidine metabolism is complex and hard to understand, let alone remember

The analysis of purine and pyrimidine is difficult

- Nothing happens in purines and pyrimidines

What I hear about purines and pyrimidines

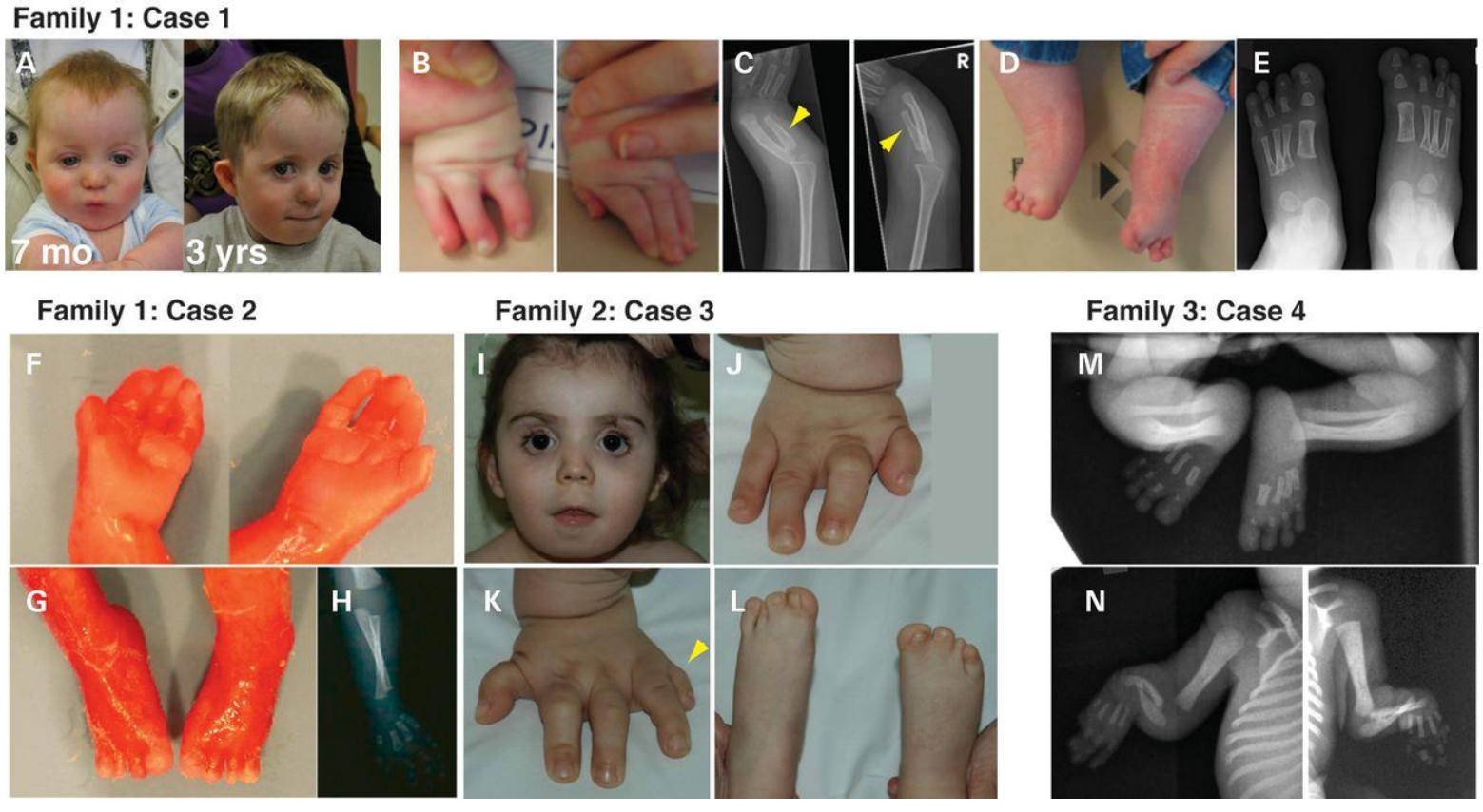
These patients have a right to be diagnosed, too!

That's relative, and good overviews are available

- **No more than other analyses**

- **??????????????????????????**

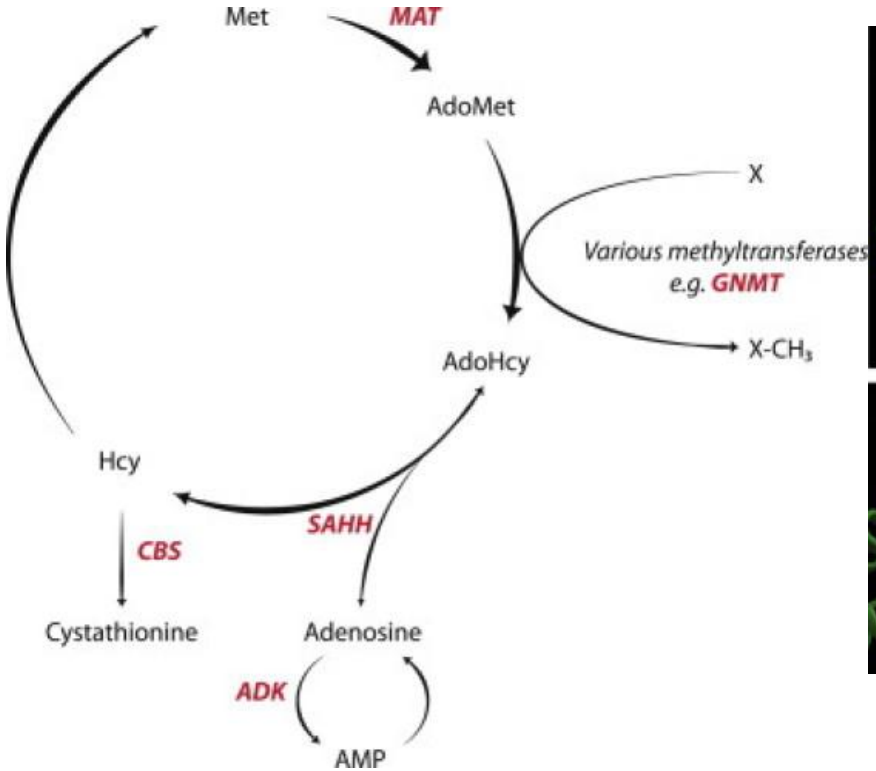
Miller syndrome

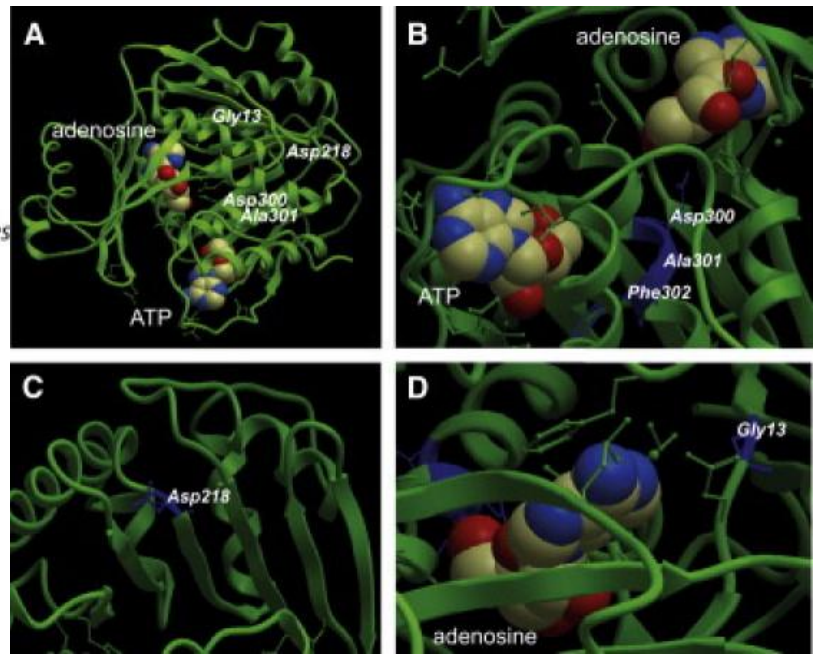


DHODH mutation-positive POADS cases.

Rainger J et al. Hum. Mol. Genet. 2012;21:3969-3983

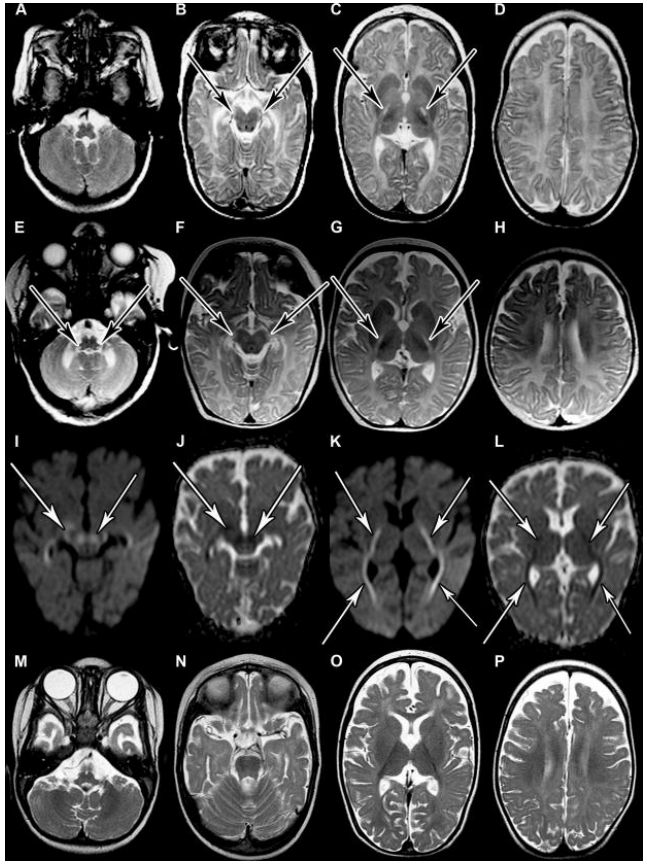
Adenosine kinase deficiency

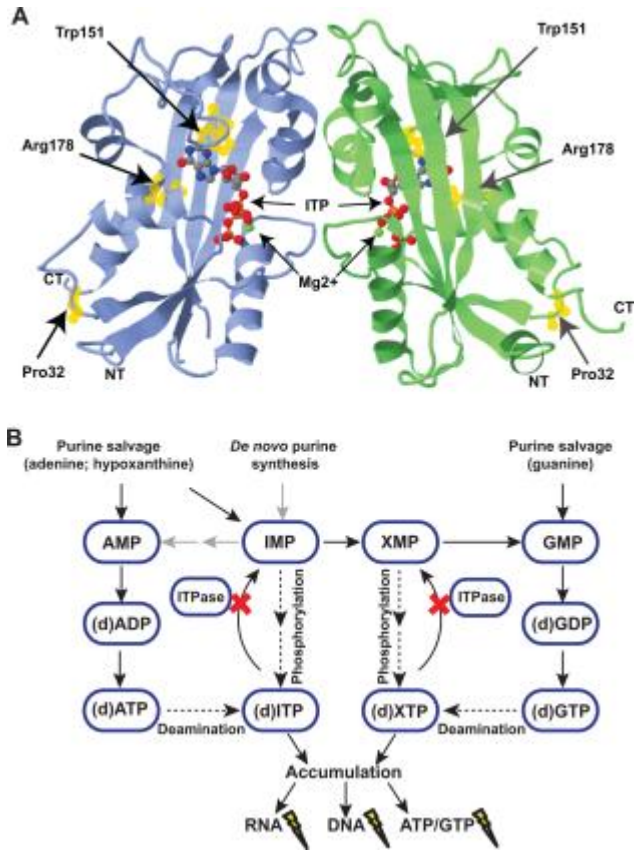




Am J Hum Genet. 2011 Oct 7; 89(4): 507–515.

ITPA early infantile encephalopathy





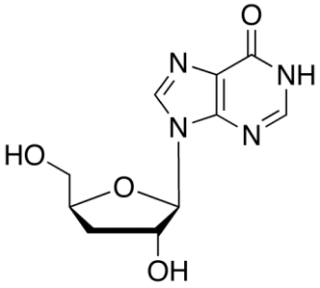
Annals of Neurology
21 AUG 2015 DOI: 10.1002/ana.24496
http://onlinelibrary.wiley.com/doi/10.1002/ana.24496/full#ana24496-fig-0001

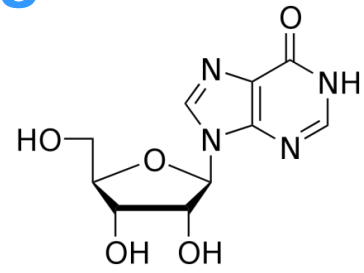
Specialised purine and pyrimidine assay

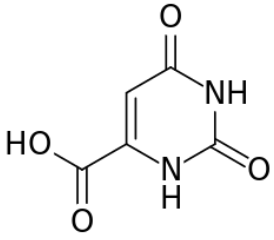
- State of the art: LC-MS/MS method
  - Comprehensive screening for all known biomarkers is possible

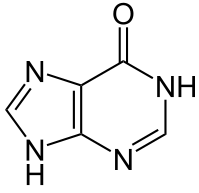
- HPLC with UV fixed wavelength UV or DAD detector
  - Non-UV compounds are missed
  - More prone to interference

Minimal essential Purine and Pyrimidine screening in urine







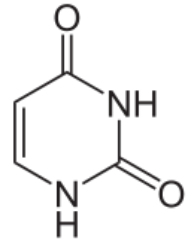


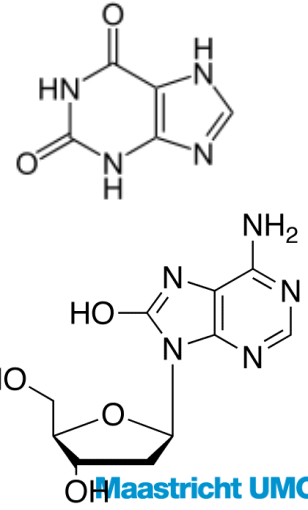
Purines

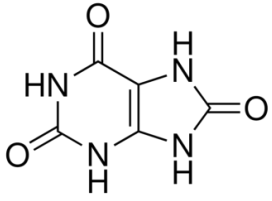
- Inosine
- Deoxyinosine
- Hypoxanthine
- Xanthine
- Deoxyadenosine
- Uric acid

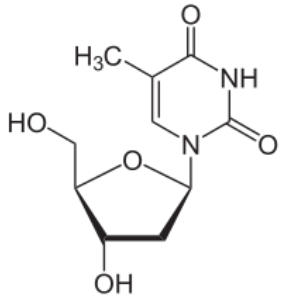
Pyrimidines

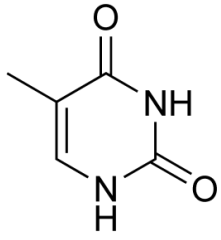
- Orotic acid
- Uracil
- Thymine
- Thymidine











Purines and Pyrimidines in ERNDIM

Quantitative Purines and Pyrimidines in urine

Include defects in Diagnostic Profiency Testing (not all DPT schemes)

Purines and Pyrimidines (urine)

Aim: Comparison of Purine and Pyrimidine analysis in a lab with respect to median and target values

Status: Full ERNDIM EQA scheme since 2000

Geographic area: Worldwide

No. of samples/year: 8

Eligibility Requirements: None

Sample matrix: Lyophilised, spiked human urine

lo. of registrations (2015): 51

Volume/sample: 5 ml/vial

Analytes (2016):

5-OH methyluracil	Adenine	Adenosine	AICAR
Creatinine	Deoxy-adenosine	Deoxy-guanosine	Deoxy-inosine
Deoxy-uridine	Dihydro-thymine	Dihydro-uracil	Guanosine
Hypoxanthine	Inosine	Orotic acid	Orotidine
Pseudo-uridine	Thymidine	Thymine	Uracil
Uric acid	Xanthine		

No. of shipments/year: One shipment of 8 samples in January- March

Submission deadlines: 8 deadlines at 6-8 weeks intervals from March to November

Submission of results: Via Results website

Reports: Interim reports available from the Results website during the scheme year
Annual report published in January-March of the following year

Scientific Advisor: Dr. Jörgen Bierau, jorgen.bierau@mumc.nl

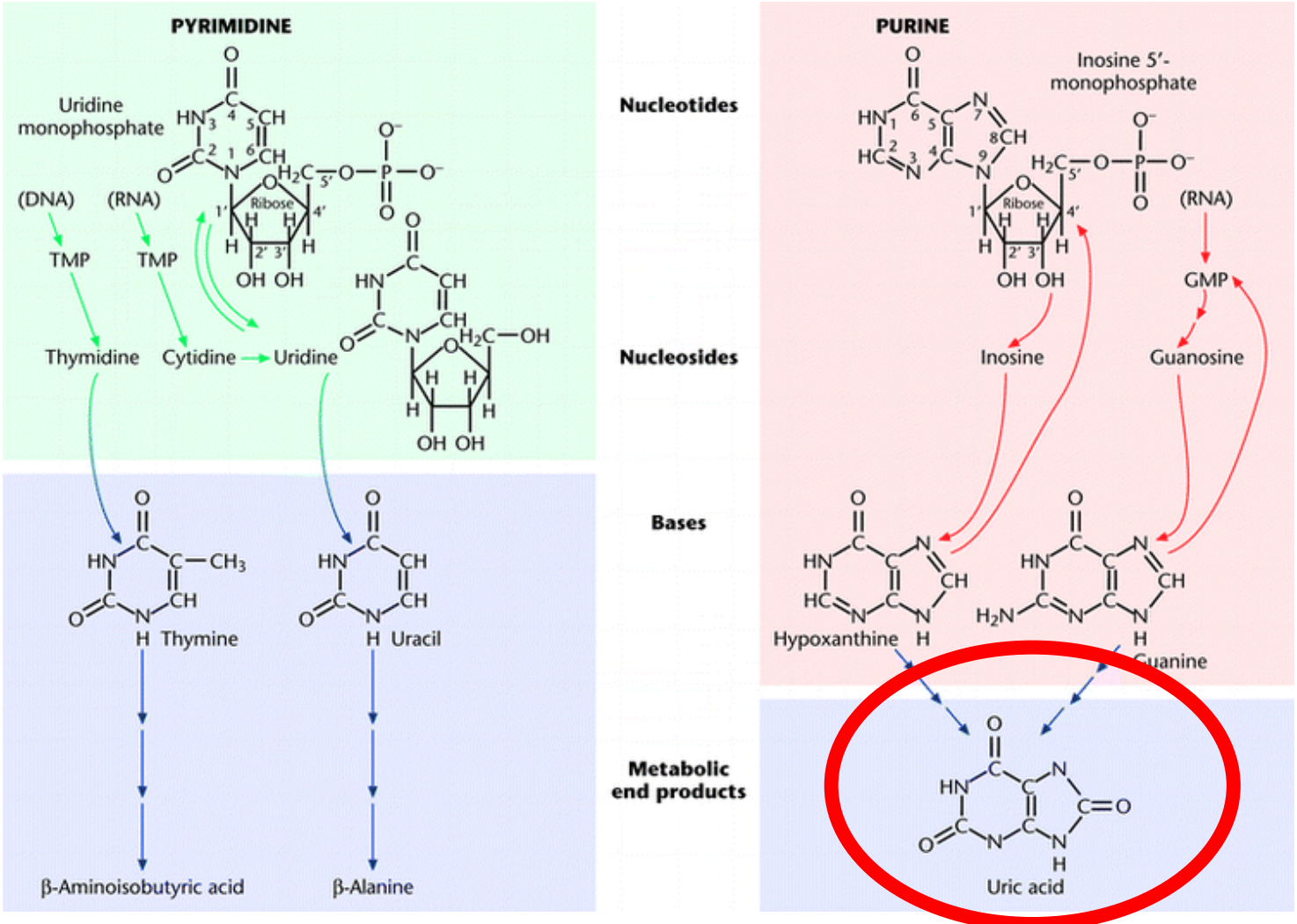
Scheme Organiser: Dr. Cas Weykamp (SKML), c.w.weykamp@skbwinterswijk.nl

Can I identify patients with a possible defect in Purine or Pyrimidine metabolism without a specialist assay?

Yes, not all but many!

Uric acid, thymine, uracil en orotic acid are very informative

Usefull Biomarkers



Serum/plasma Uric acid

Increased	Decreased
Lesch-Nyhan disease	Purine Nucleoside Phosphorylase def.
Familial Juvenile Hyperuricaemia and Nephropathy (FJHN)	Isolated xanthine dehydrogenase def.
Phosphoribosyl pyrophosphate synthetase superactivity	Combined xanthine dehydrogenase/sulfite oxidase def.
	Molybdenum cofactor def.
	Pyrimidine 5'-nucleotidase superactivity Phosphoribosyl pyrophosphate synthetase def.

Increased urinary pyrimidines

Thymine	Uracil
Dihydropyrimidine dehydrogase deficiency	
Dihydropyrimidinase deficiency	
Thymidine phosphorylase deficiency Hyperammonaemia	
	Degradation of Pseudo-uridine

Increased urinary orotate

Orotate phosphoribosyl transferase def. (orotic aciduria I) Orotidine monophosphate decarboxylase def. (orotic aciduria II)
Dihydroorotate dehydrogenase def. (Miller syndrome) Hyperammonaemia

Defects detectable with special assay only

Defect	Marker metabolite(s)
Adenine phosphoribosyl transferase	2,8-dihydroxyadenine
Adenosine deaminase def.	Deoxyadenosine
Adenylosuccinate lyase def.	Succinyladenosine and SAICAr
AICAR transformylase/IMP cyclohydrolase (ATIC) def.	AICAr
Beta-ureidopropionase def.	N-carbamoyl-ß-alanine and N-carbamoyl-ß-aminoisobutyric acid
Adenosine kinase def.	Methionine

Defects without specific biomarkers

Phosphoribosyl pyrophosphate synthetase def.
AMP deaminase def.
Inosine triphosphatase def.
Pyrimidine 5’-nucleotidase deficiency
Deoxyguanosine kinase def.
Thymidine kinase 2 def.
Mitochondrial ribonucleotidase subunit 2 def.

HGPRT deficiency

Sample A: Hypoxanthine-Guanine Phosphoribosyltransferase Deficiency (Lesch-Nyhan Disease) OMIM No. 300332

Patient details provided
12 months old boy with muscular hypotonia, dystonia and slight global retardation; the sample was taken at 5 years while under treatment.

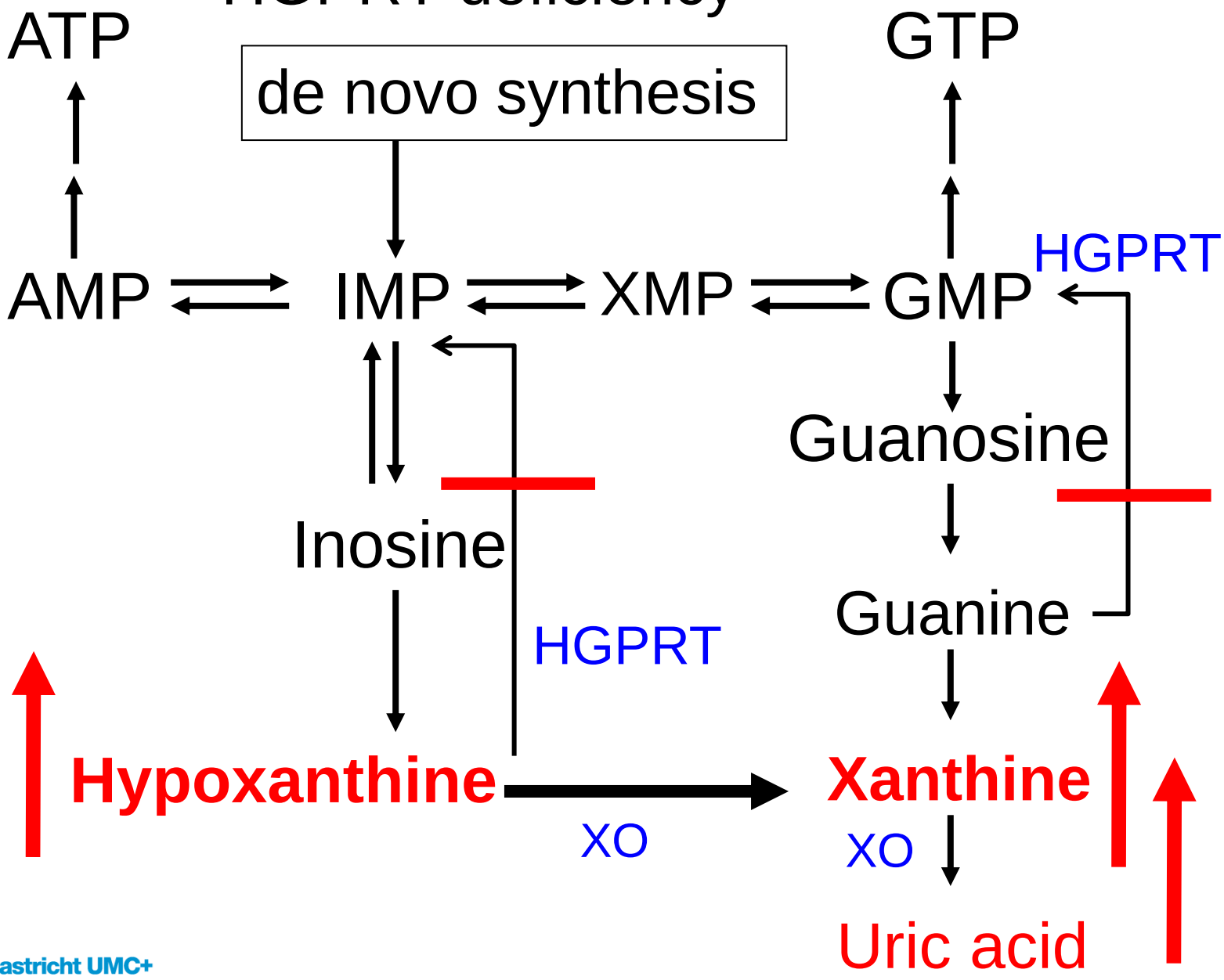
Further information
The patient was found to have elevated xanthine, hypoxanthine and uric acid in urine and plasma uric acid at 12 months of age. HPRT activity in lymphocytes was deficient (Amsterdam) confirming Lesch-Nyhan syndrome. The diagnosis was confirmed by DNA analysis. Treatment has been undertaken with allopurinol.
The sample came from Zürich Children's Hospital.

Analytical performance: Labs performing purine and pyrimidine analysis were able to detect increased hypoxanthine, xanthine and when investigated uric acid (12 of 19) whilst one lab reported upper normal values of hypoxanthine and xanthine but elevated uric acid.

Interpretative proficiency
All but two labs correctly interpreted the abnormal metabolites as HPRT deficiency (11 of 19).

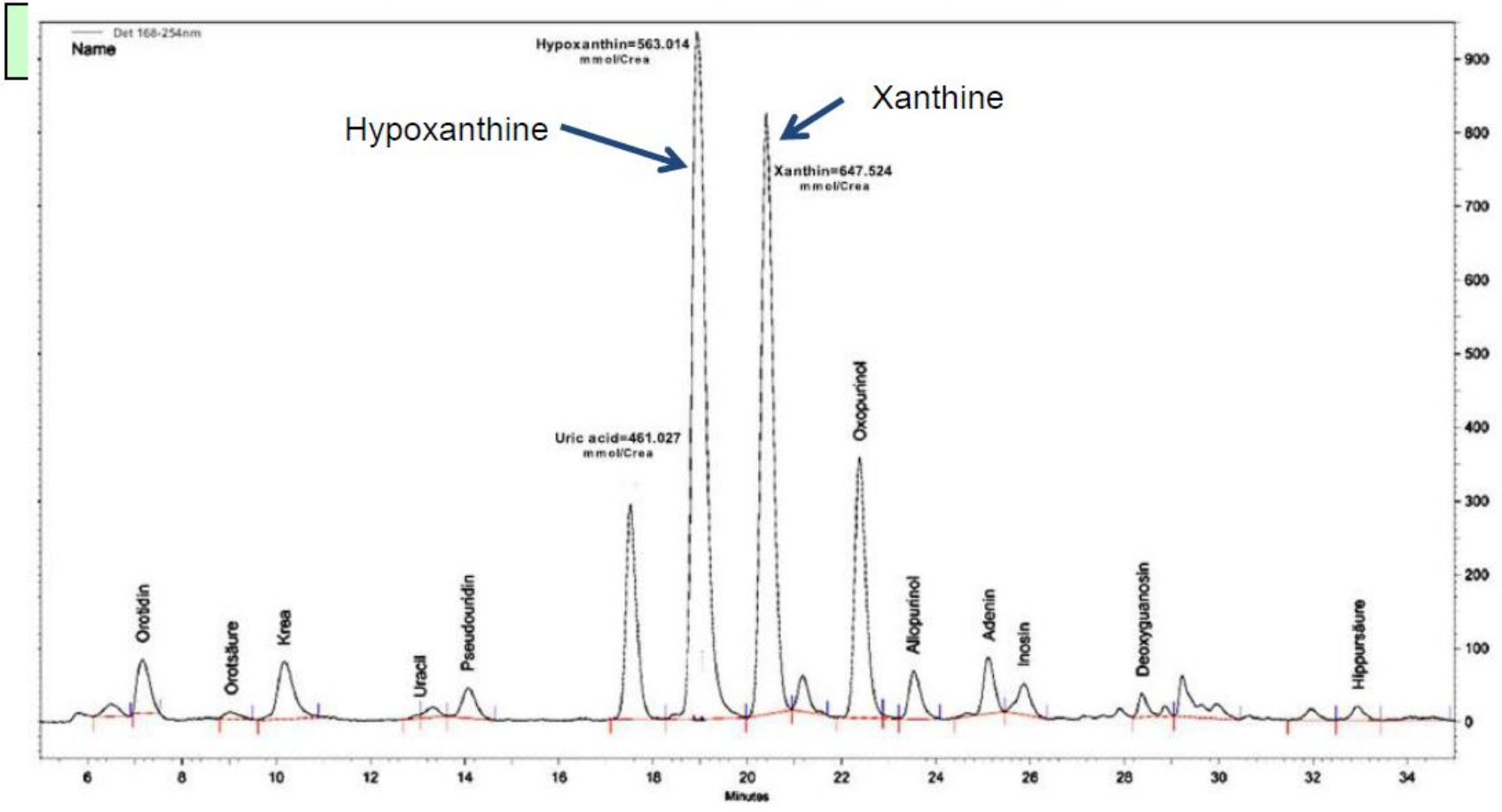
Overall impression:
Diagnosis required purine and pyrimidine analysis allowing correct diagnosis (11 labs) with overall efficiency of 59%.

HGPRT deficiency



P & P chromatogram HGPRT deficiency

P & P HPLC (courtesy O. Sass, Zürich Children's Hospital)



PNP deficiency

Sample B: Purine nucleoside phosphorylase deficiency, OMIM No. 613179.

Patient details provided:
Mental retardation noted at 9 months of age. At 2 yrs there were frequent infections and stomatitis + persisting mental retardation Urine collected at 2y. 10m. on treatment.

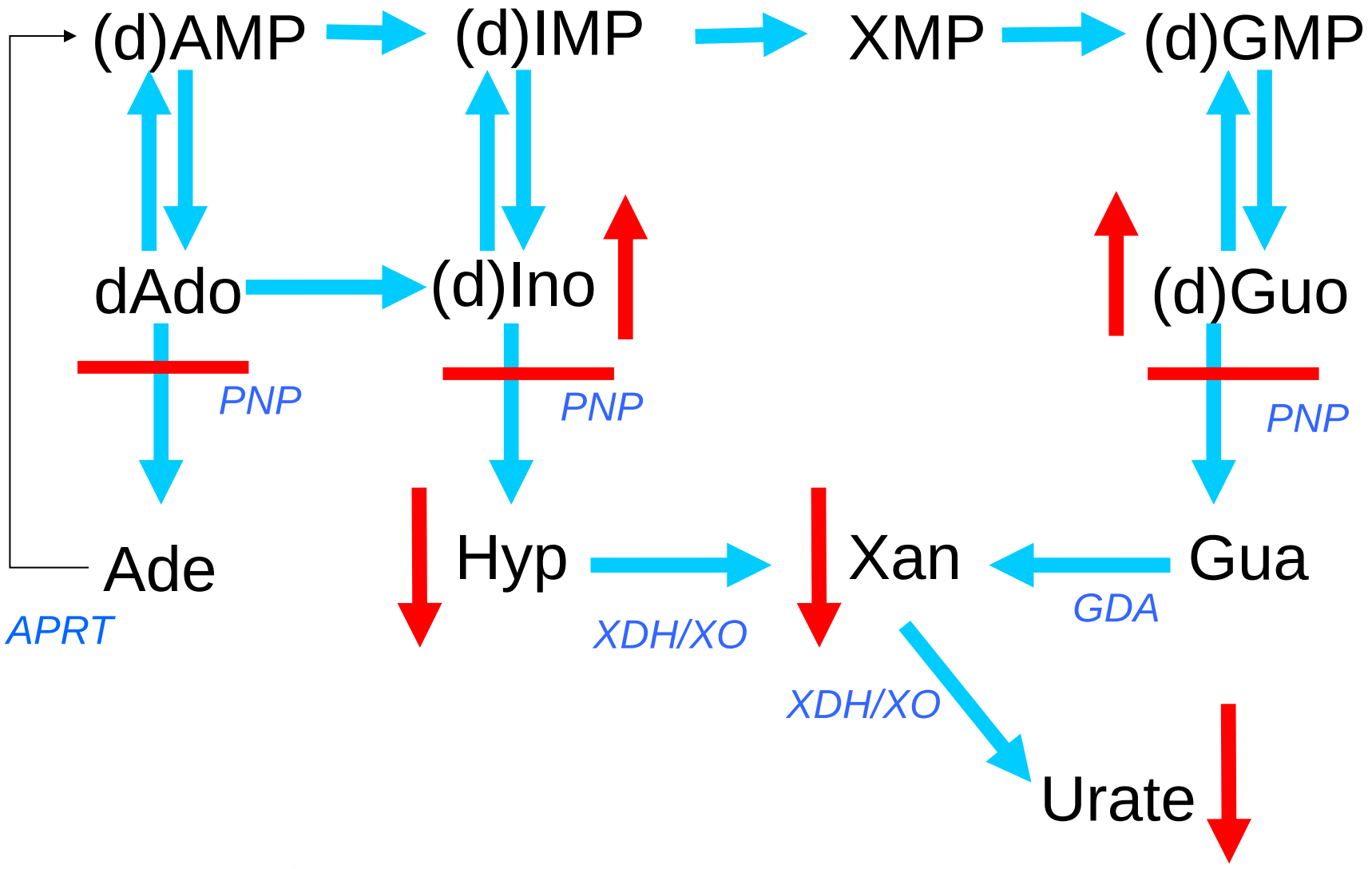
Further information
This patient excreted massively increased purine metabolites (deoxyinosine,guanosine and inosine) and low urate pointing to purine nucleoside phosphorylase deficiency. The patient was picked up initially by urinary organic acid analysis and the diagnosis has been confirmed by DNA analysis. At 2 yrs 10 mo the boy was hospitalised in preparation for transplantation. The sample was kindly provided by Prof. Elisabeth Holme, Gothenburg.

Analytical performance:
Purine and pyrimidine analysis was key to making the diagnosis with detection of abnormal metabolites (13 labs) whilst one lab found the abnormalities on organic acid analysis.

Interpretative proficiency:
14 of 19 labs correctly interpreted the abnormal findings as purine nucleoside phosphorylase deficiency.

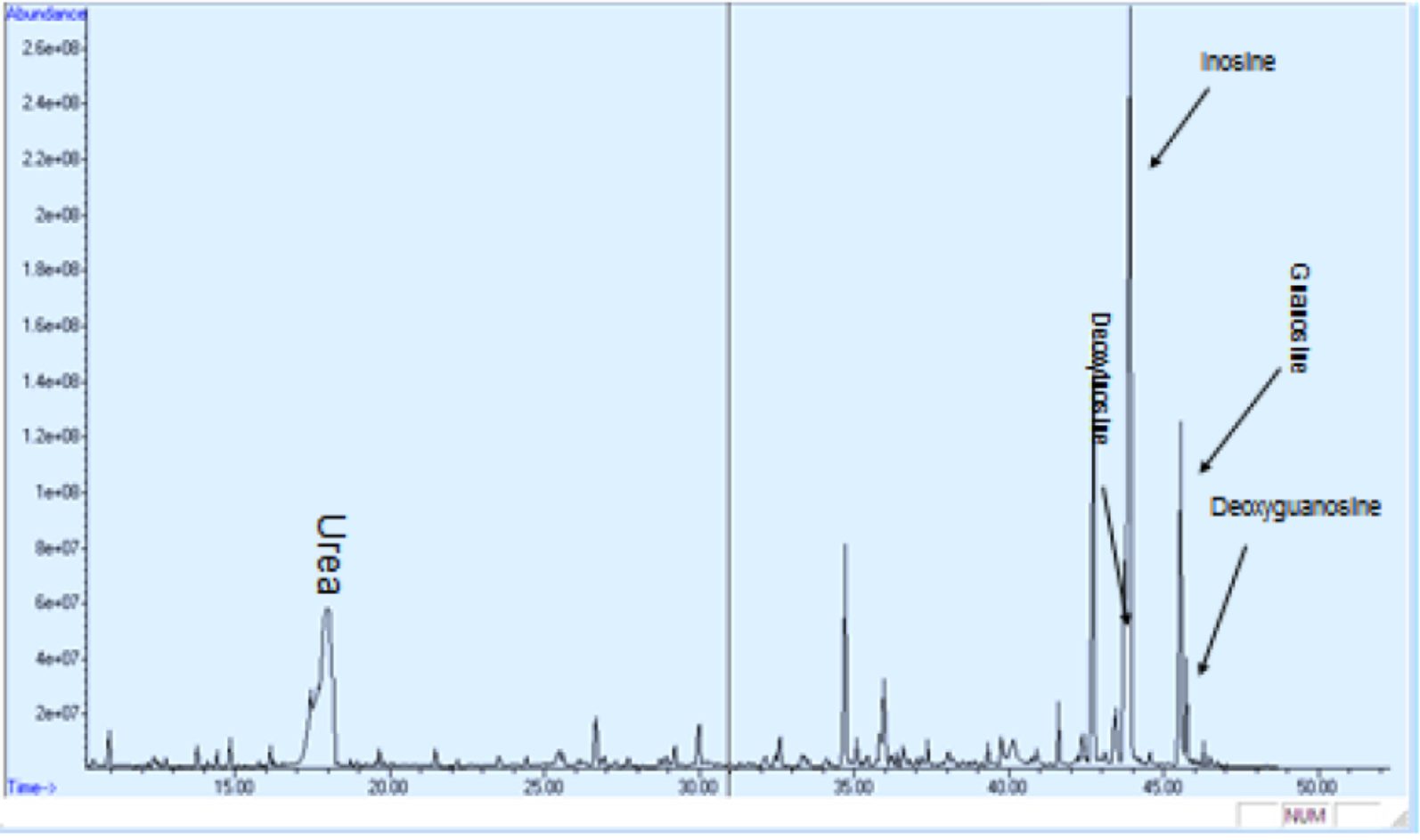
Overall impression
14 of 19 labs were able to make the correct diagnosis, with overall proficiency of 75%.

PNP deficiency



GC-MS of metabolites

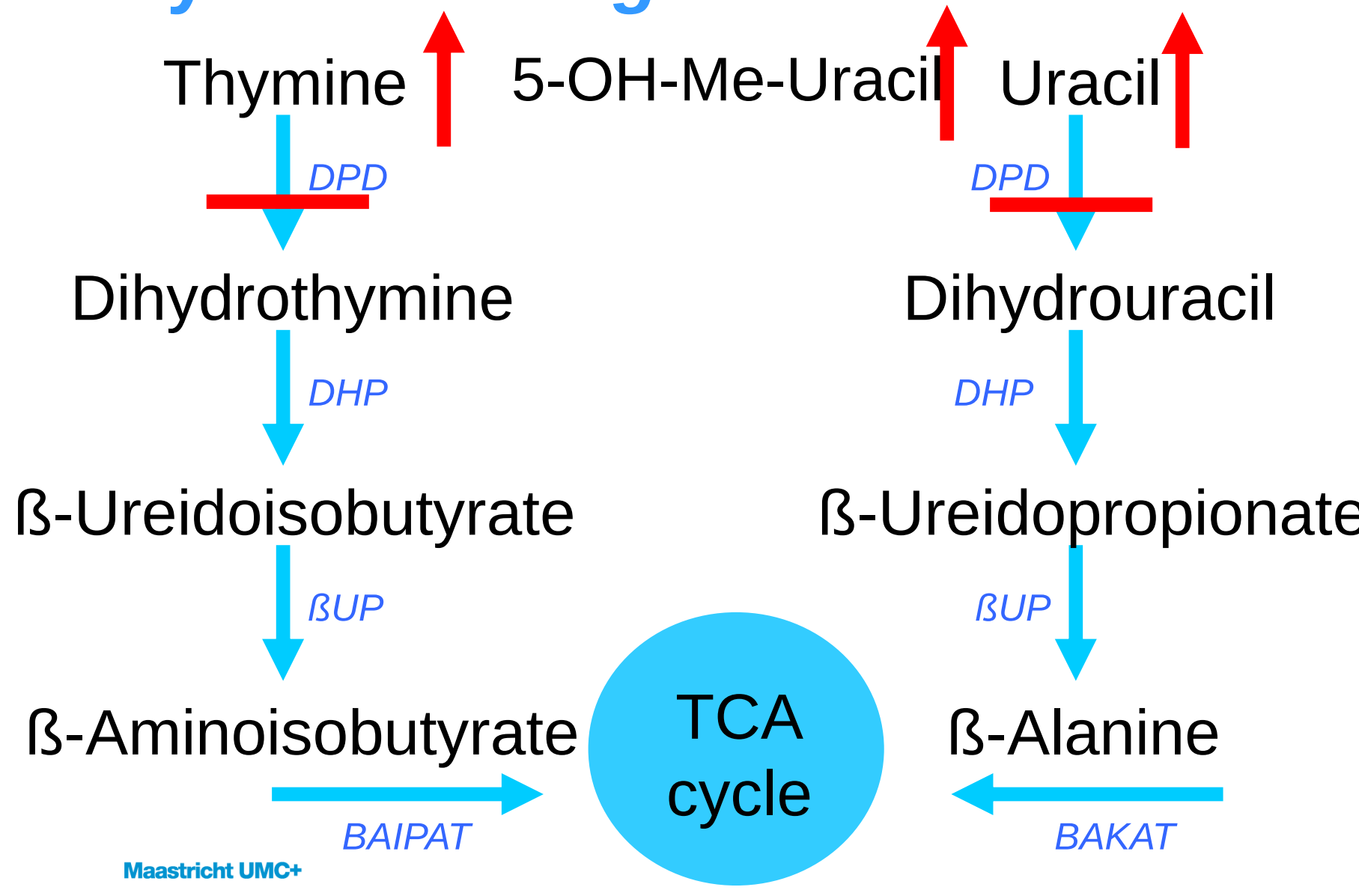
Purine nucleoside phosphorylase deficiency

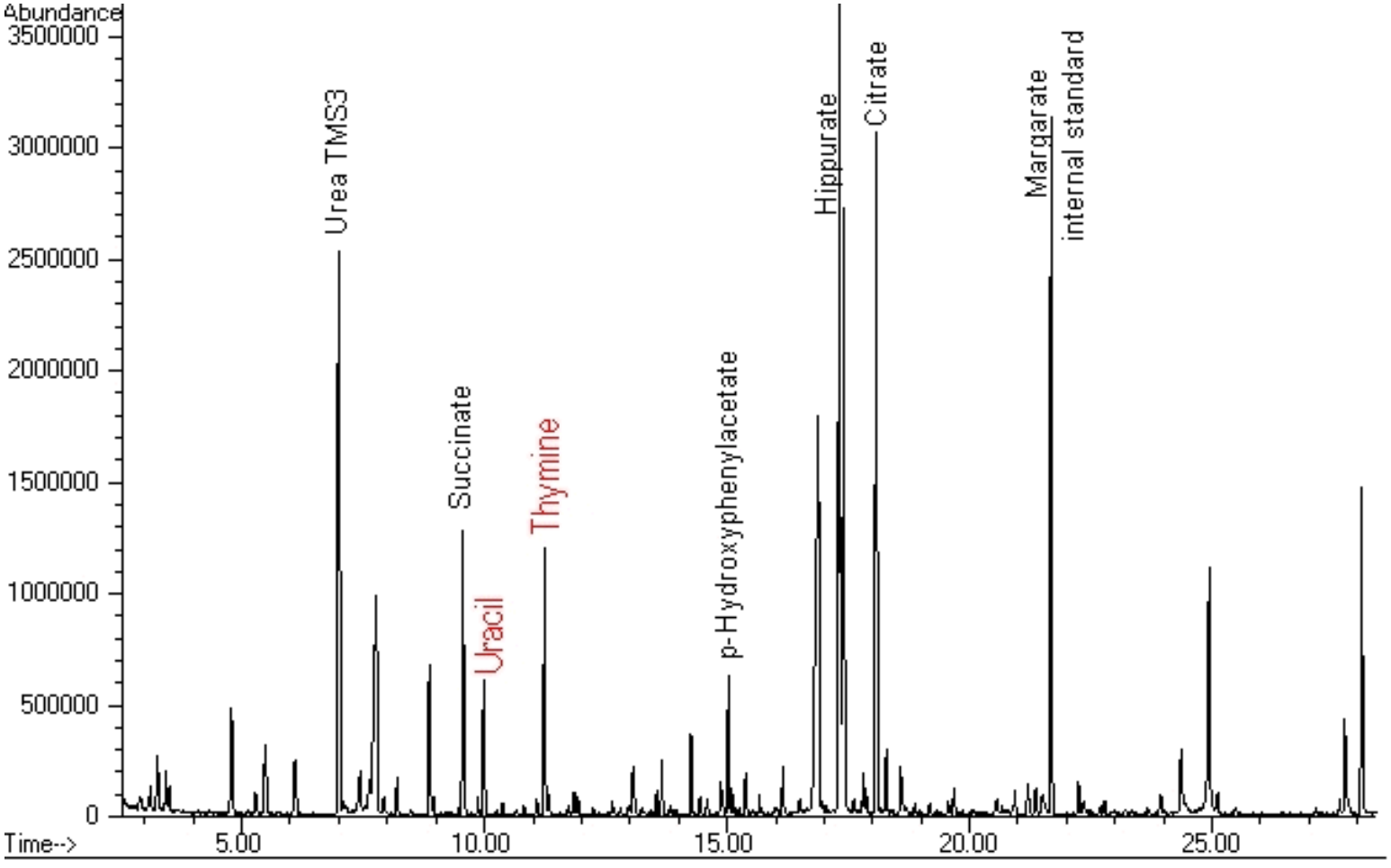


DPD deficiency

Variable clinical picture, ranging from no (apparent) symptoms to severe neurological disease with mental and motor retardation and epilepsy.

Pyrimidine *degradation*





ERNDIM urine # 59: Dihydropyrimidine dehydrogenase deficiency

Summary

- Uric acid and thymine and uracil in routine urinary organic acid analysis are helpful biomarkers
- Orotic acid is important!
- Feel free to contact the P&P specialists
- More labs should analyse purines and pyrimidines

PURINES AND PYRIMIDINES ARE THE COMPONENTS THAT GIVE FLAVOUR TO INBORN ERRORS OF METABOLISM