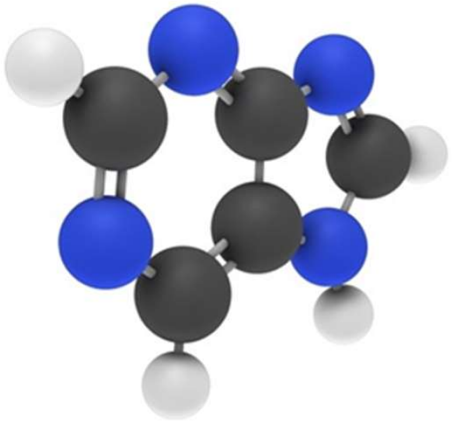
Purines and Pyrimidines
An introduction



SSIEM Academy 2024

Amsterdam

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Clinical Biochemical Geneticist, ErClG

Erasmus MC, Rotterdam,

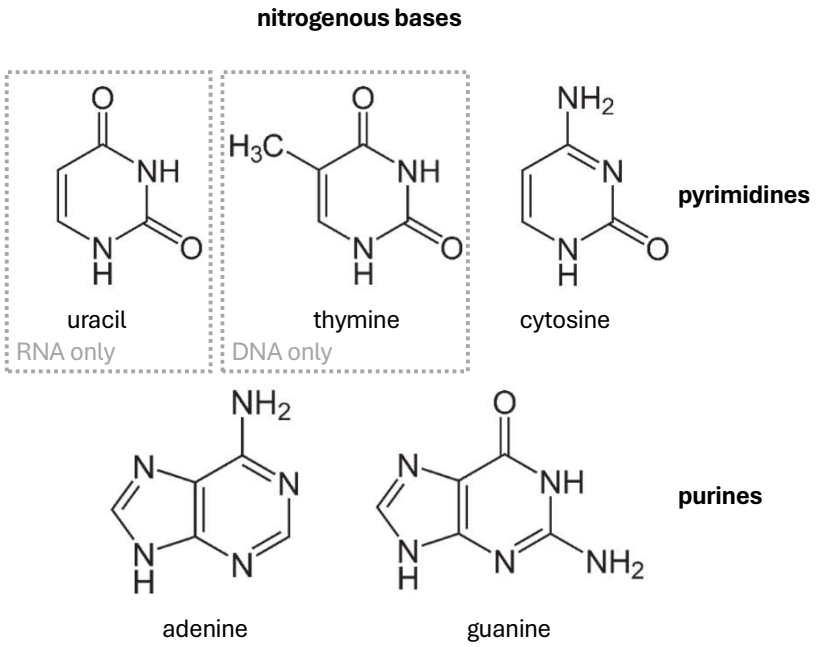
The Netherlands

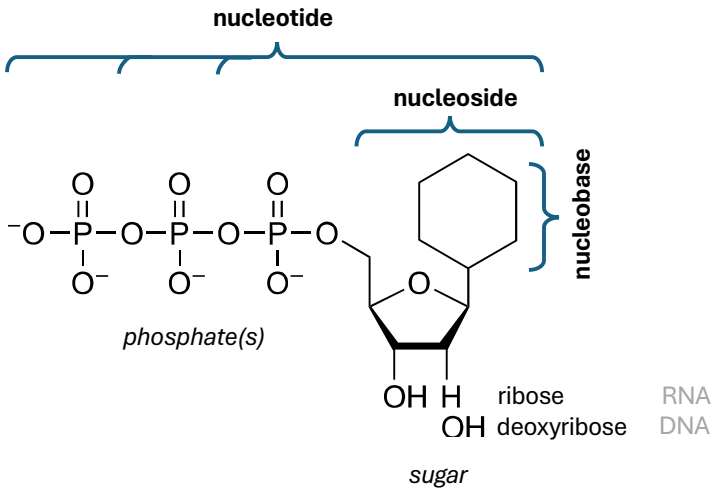
Topics:

- *Introduction*
  - *Biochemistry*
  - *Laboratory diagnosis*

- *Illustrative cases*
  - *Purine metabolism*
  - *Pyrimidine metabolism*

purines and pyrimidines





What's in the name?

Purines and pyrimidines
–ine are nitrogenous bases*
*[except uracil/uridine]

Purines:
–osine are nucleosides/tides
*[except cytosine]

Pyrimidines:
–idine are nucleosides/tides

Naming of bases, nucleosides and nucleotides

Category	**Nitrogeous Base (Nucleobase)**	**Nucleoside (Ribose)**	**Deoxynucleoside (Deoxyribose)**	**Nucleotide (Ribose)**	**Deoxynucleotide (Deoxyribose)** *[only monophosphate]*
Purines	Adenine	Adenosine	Deoxyadenosine	Adenosine Monophosphate (AMP)	Deoxyadenosine Monophosphate (dAMP)
	Guanine	Guanosine	Deoxyguanosine	Guanosine Monophosphate (GMP)	Deoxyguanosine Monophosphate (dGMP)
	Hypoxanthine	Inosine	Deoxyinosine	Inosine Monophosphate (IMP)	-
Pyrimidines	Cytosine	Cytidine	Deoxycytidine	Cytidine Monophosphate (CMP)	Deoxycytidine Monophosphate (dCMP)
	Thymine	-	Thymidine (Deoxythymidine)	-	Thymidine Monophosphate (dTMP)
	Uracil	Uridine	Deoxyuridine	Uridine Monophosphate (UMP)	-

Good to know

Purine & pyrimidine metabolism includes:

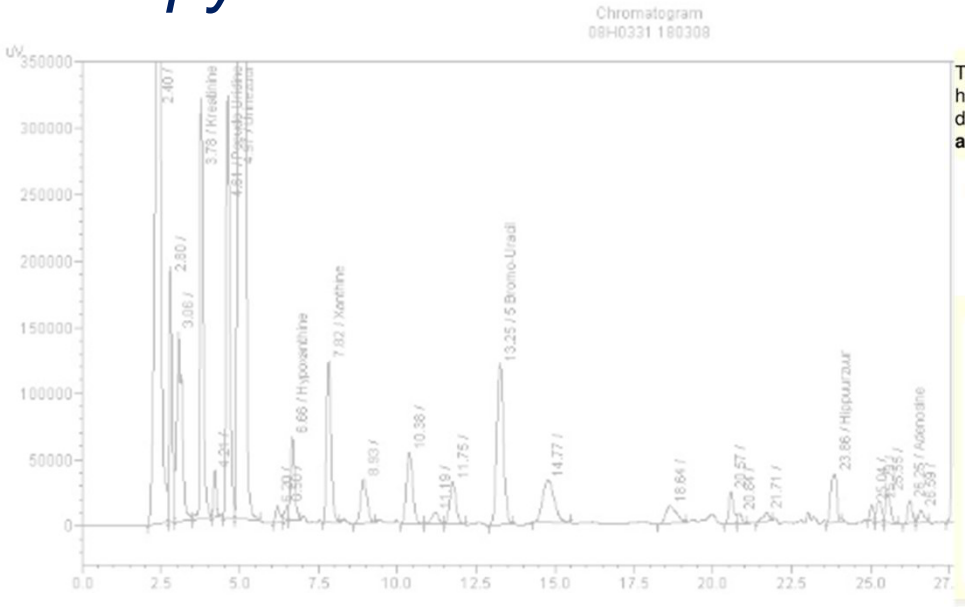
Bases, nucleosides and (deoxy)nucleotides

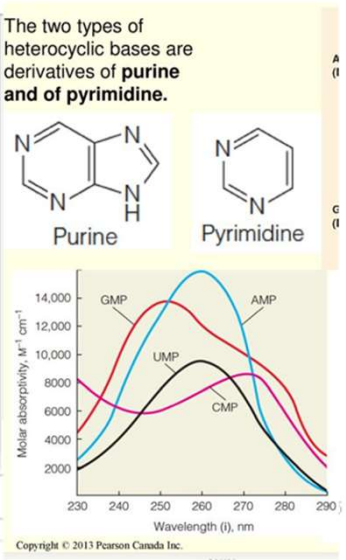
Purine & pyrimidine biosynthesis modalities:

- *De novo* biosynthesis from amino acids, ribose and folate
- Recycling of bases and nucleosides: salvage metabolism

Diagnostic tool kit

- *Urinary (plasma) purines & pyrimidines*
  - *LC-UV*
  - *LC-MS/MS*
- *Genomics*
  - *Functional assays*
  - *Enzyme activity*
    - *Erythrocytes, PBMC, skin fibroblasts*
  - *Fluxomics*



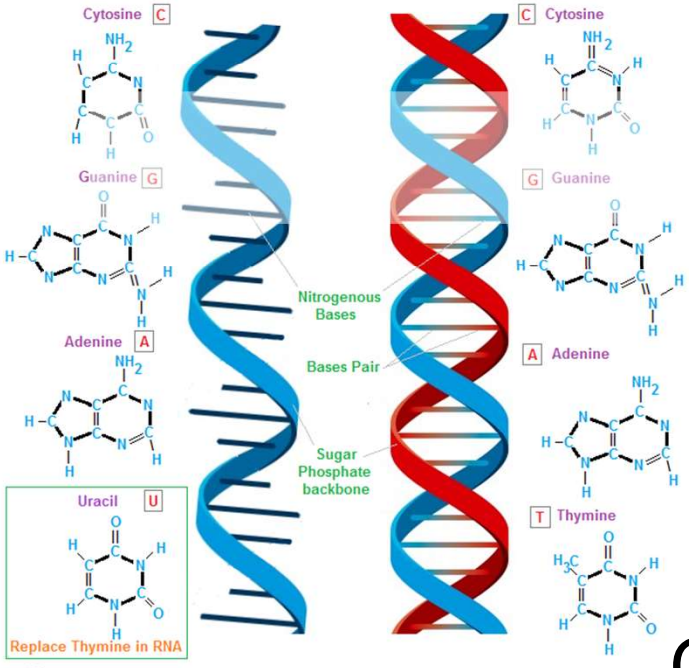


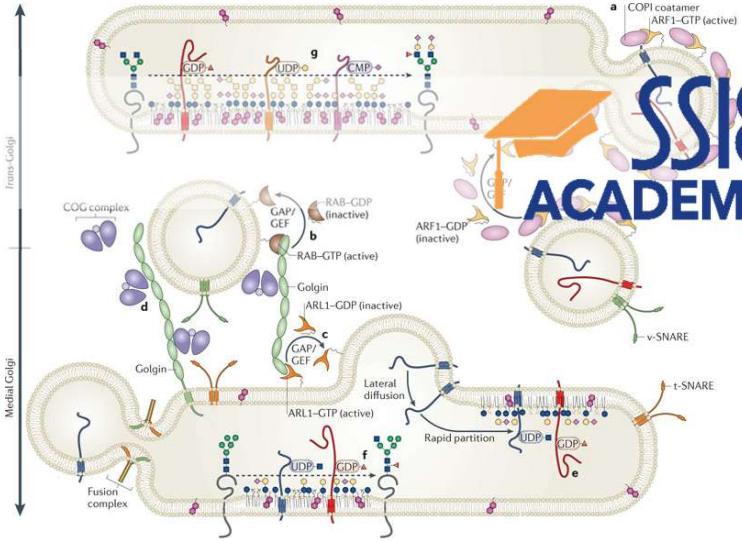
Important notes:

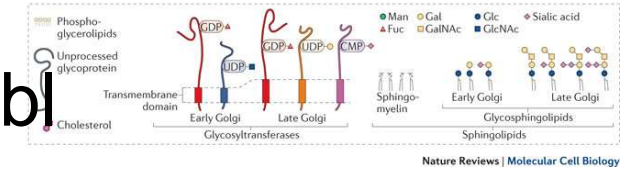
- not all defects have (specific biomarkers)
- not all (predicted) biomarkers are available
- only bases and nucleosides in urine and plasma

When to screen purines & pyrimidines

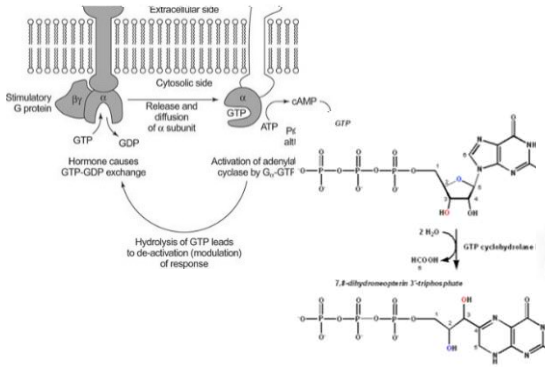
Anaemia (megaloblastic, haemolytic), Arthritis, Autism, Auto mutilation, Cachexia, feeding difficulties, Cerebral palsy, Developmental delay, Dysmorphic features, Encephalopathy, Epilepsy, seizures, fitting, Exercise intolerance, Gout, Haematuria, Hepatomegaly, Hyperactivity, short attention span, Hyperuricaemia,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







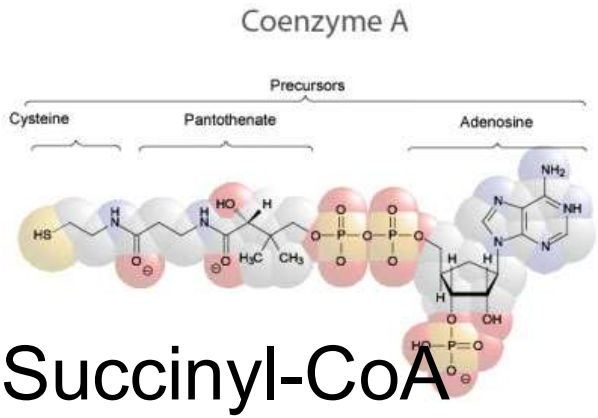
Nature Reviews | Molecular Cell Biology

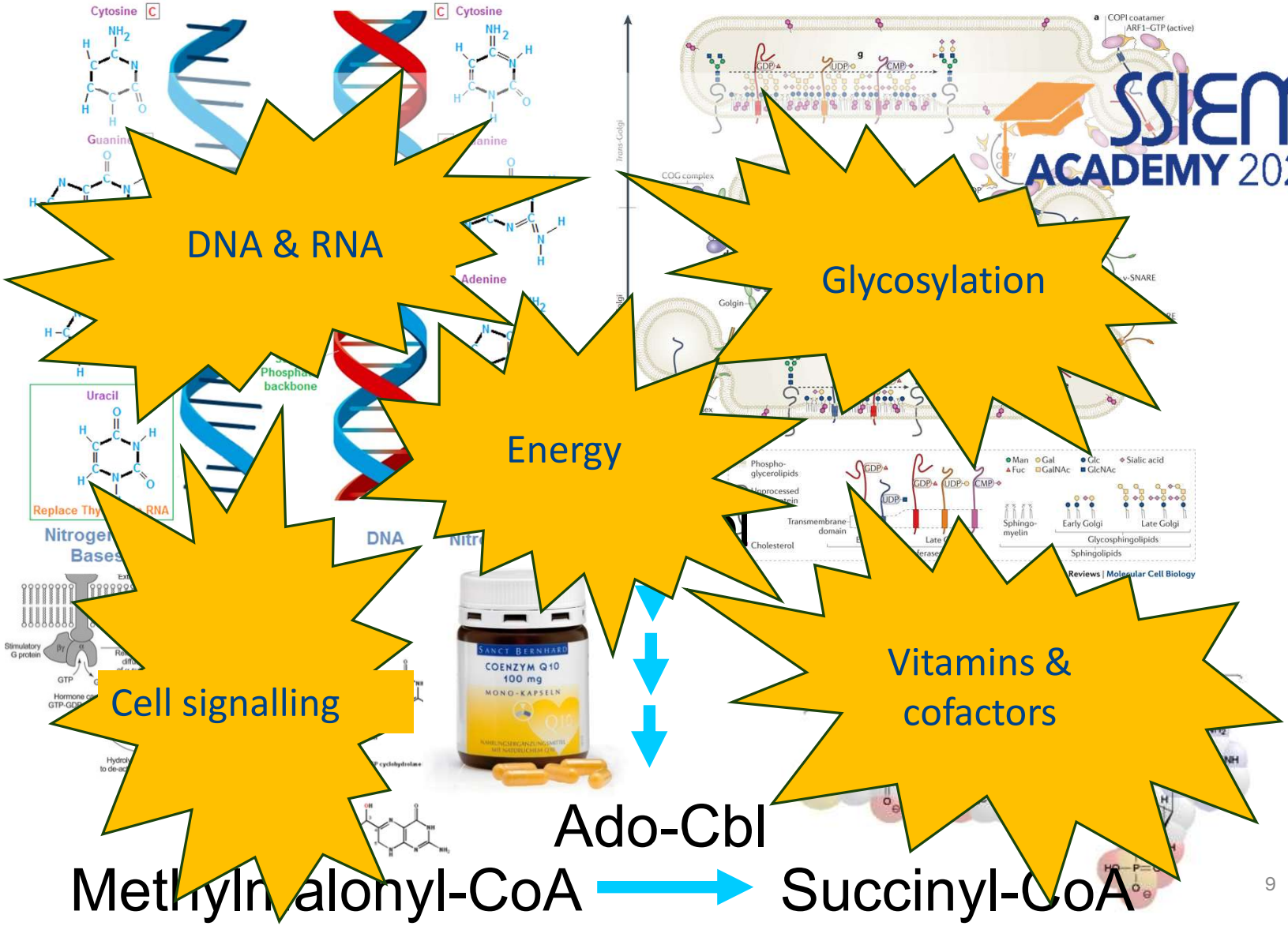


OH-Cbl

Ado-Cbl

Methylmalonyl-CoA → Succinyl-CoA




DNA & RNA
Glycosylation
Energy
Vitamins & cofactors
Cell signalling
Ado-Cbl
Methylmalonyl-CoA
Succinyl-CoA
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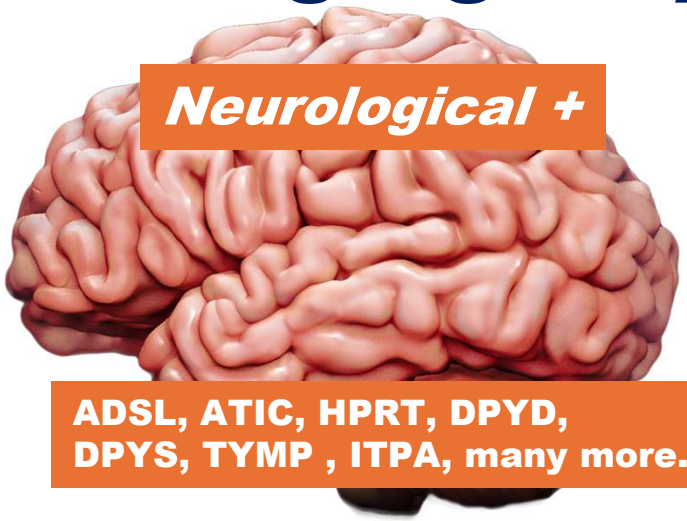
Rough groups of symptoms



Neurological +

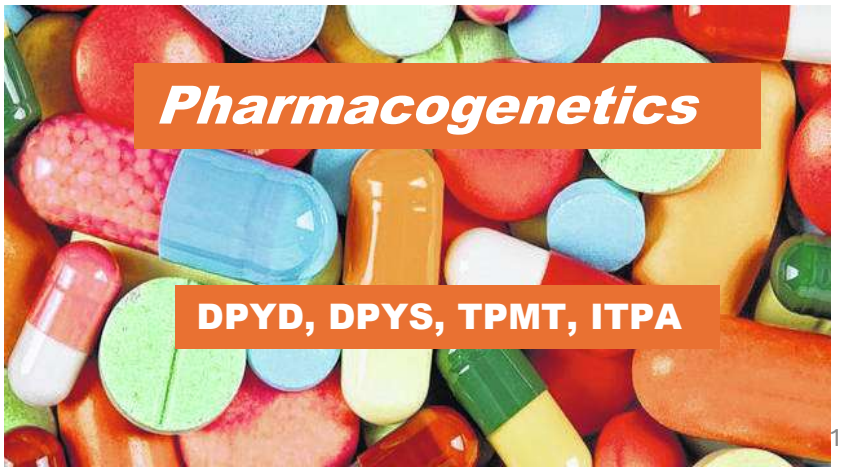
ADSL, ATIC, HPRT, DPYD, DPYS, TYMP , ITPA, many more.

Gout, stones & sediment

APRT, Xanthinuria, HRPT, PRPSs, FJHN, UMPS

Haematological/ immunological

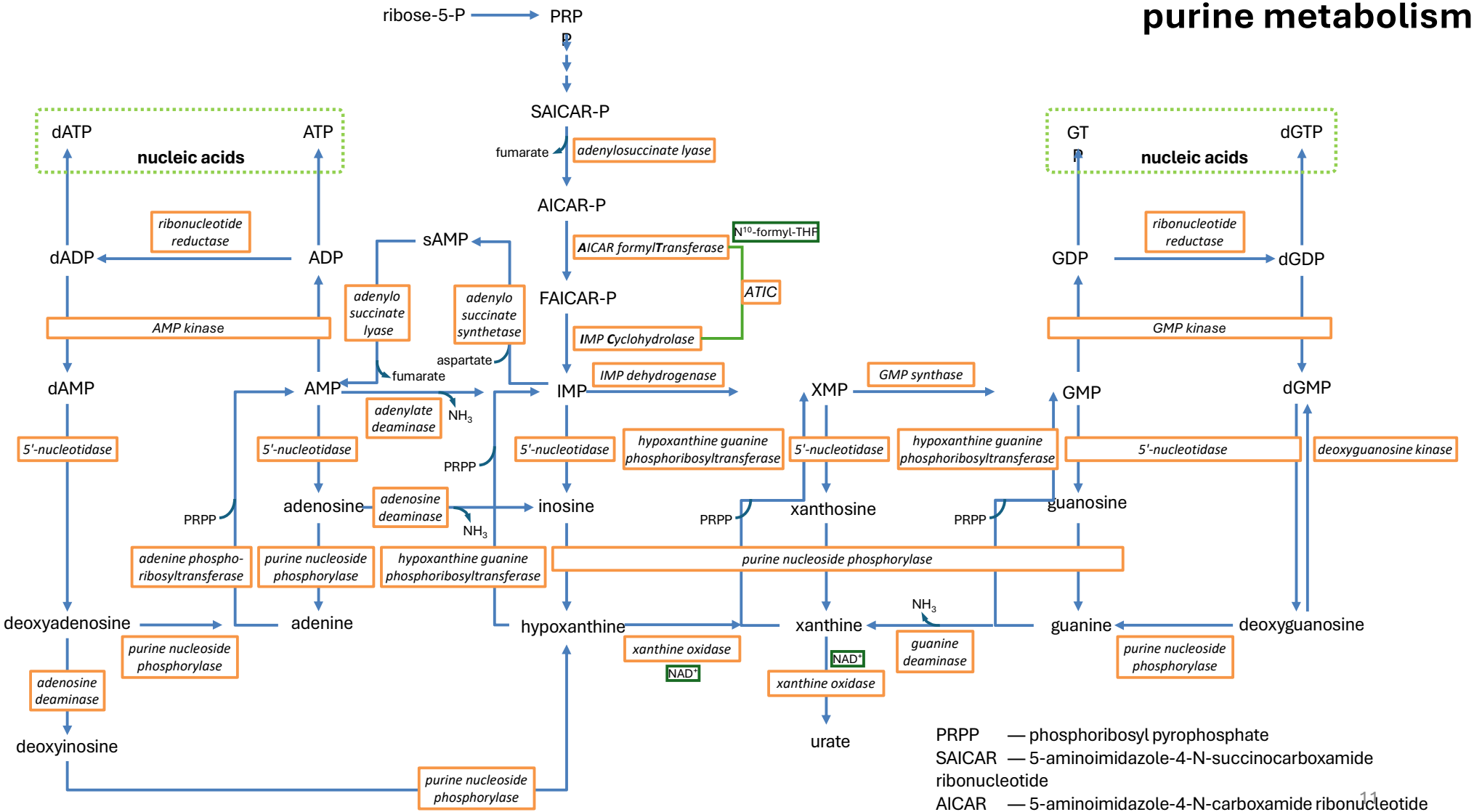
SCID-ADA, PNP, UMPS, CAD



Pharmacogenetics

DPYD, DPYS, TPMT, ITPA

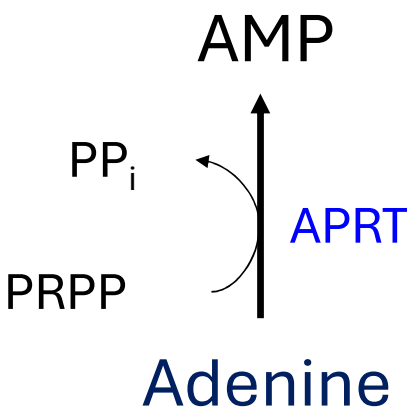
purine metabolism



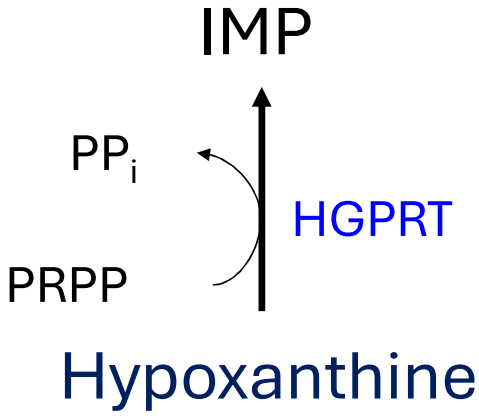
PRPP — phosphoribosyl pyrophosphate
SAICAR — 5-aminoimidazole-4-N-succinocarboxamide ribonucleotide
AICAR — 5-aminoimidazole-4-N-carboxamide ribonucleotide
FAICAR — 5-formamidoimidazole-4-N-carboxamide ribonucleotide

Two phosphoribosyl transferases

Adenine
Phosphoribosyl Transferase

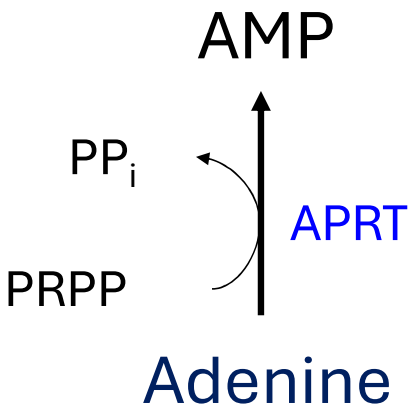


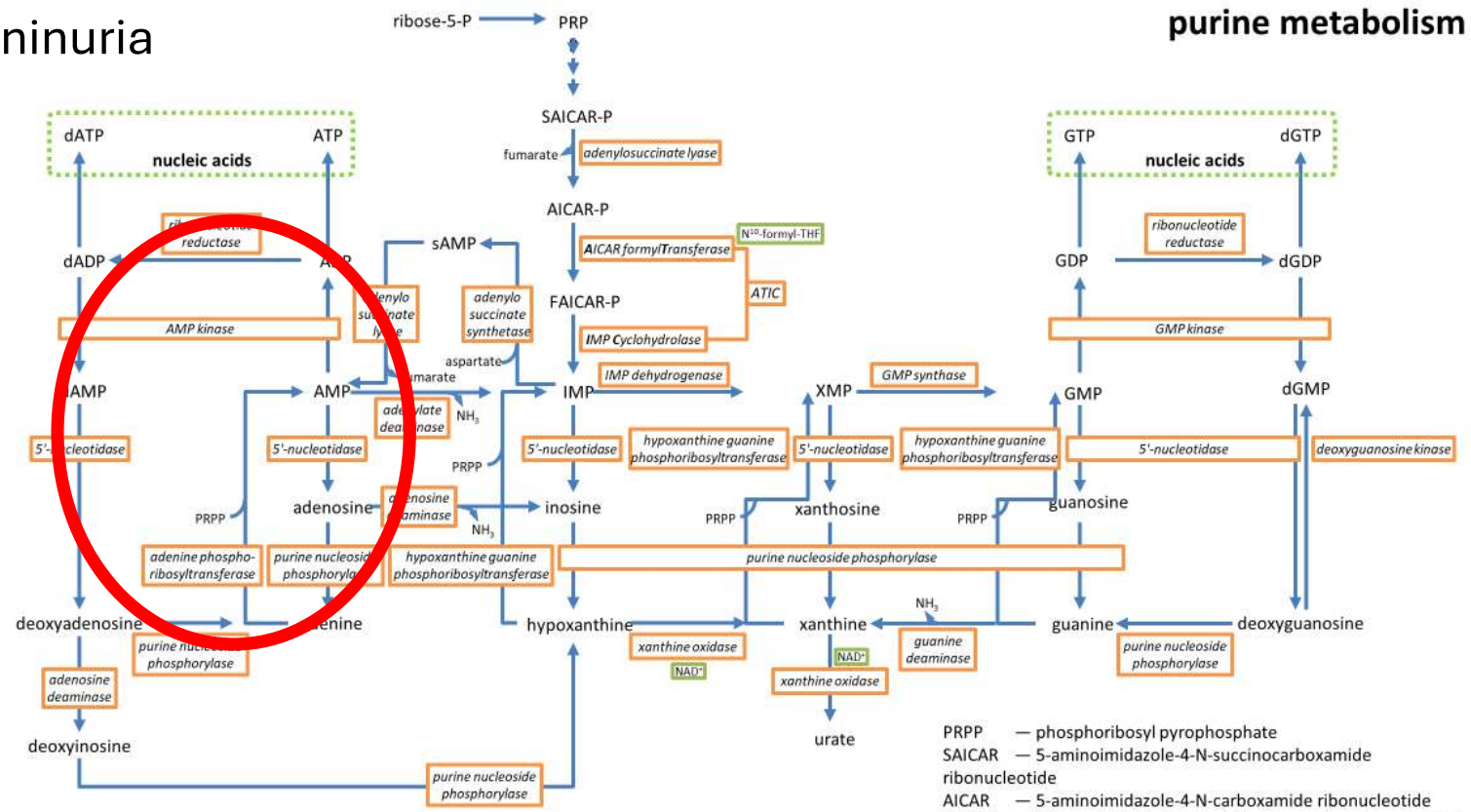
Hypoxanthine Guanine
Phosphoribosyl Transferase

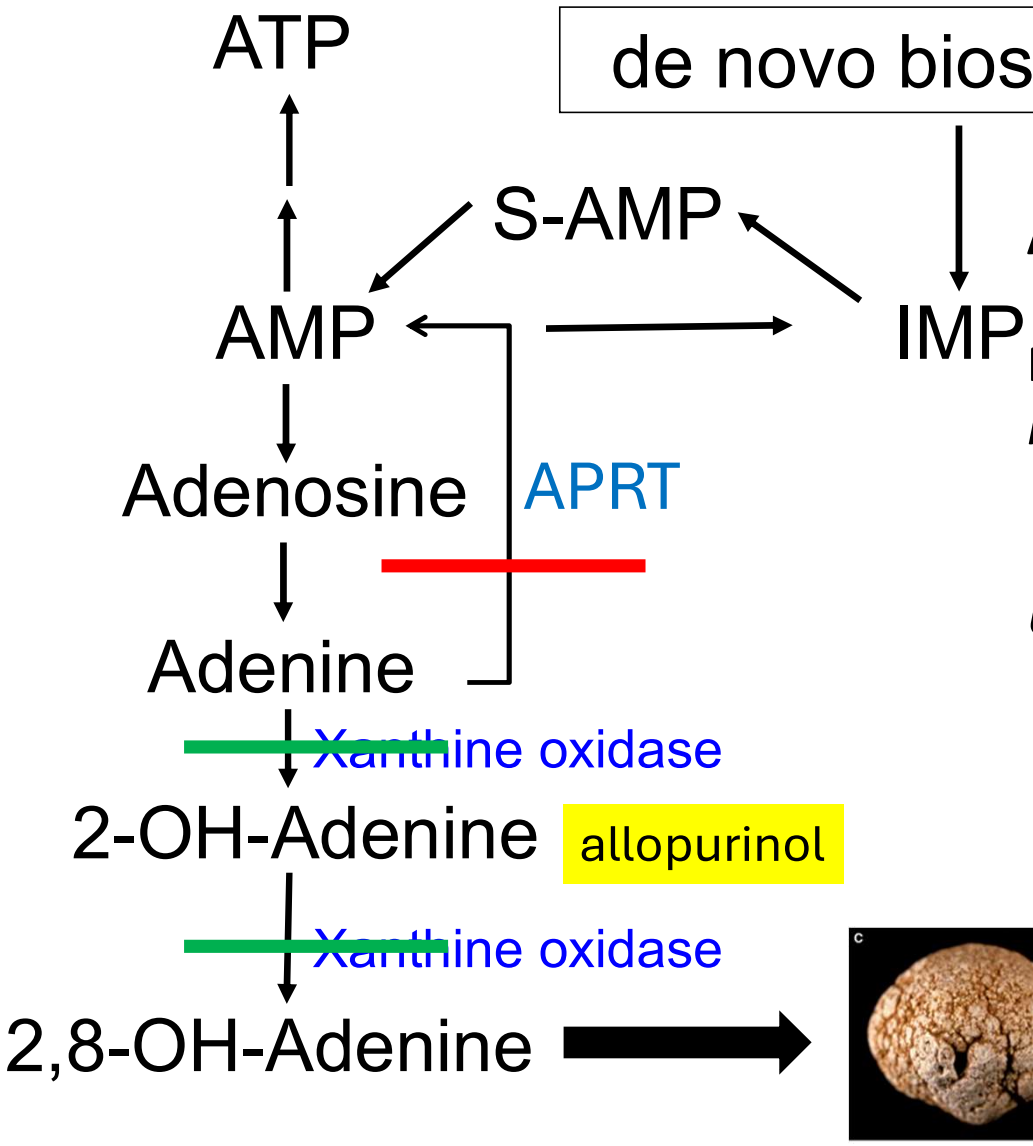


Adenine phosphoribosyl transferase

APRT-deficiency
2,8-dihydroxy adeninuria







Adenine Phosphoribosyl Transferase deficicency

Main symptoms:

Kidneys:

-stones

- renal failure

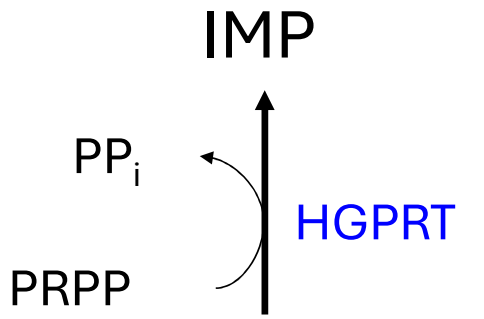
Ureters:

-urolithiasis

Treatment:

- Purine restriction
- Allopurinol: inhibitor of xanthine oxidase

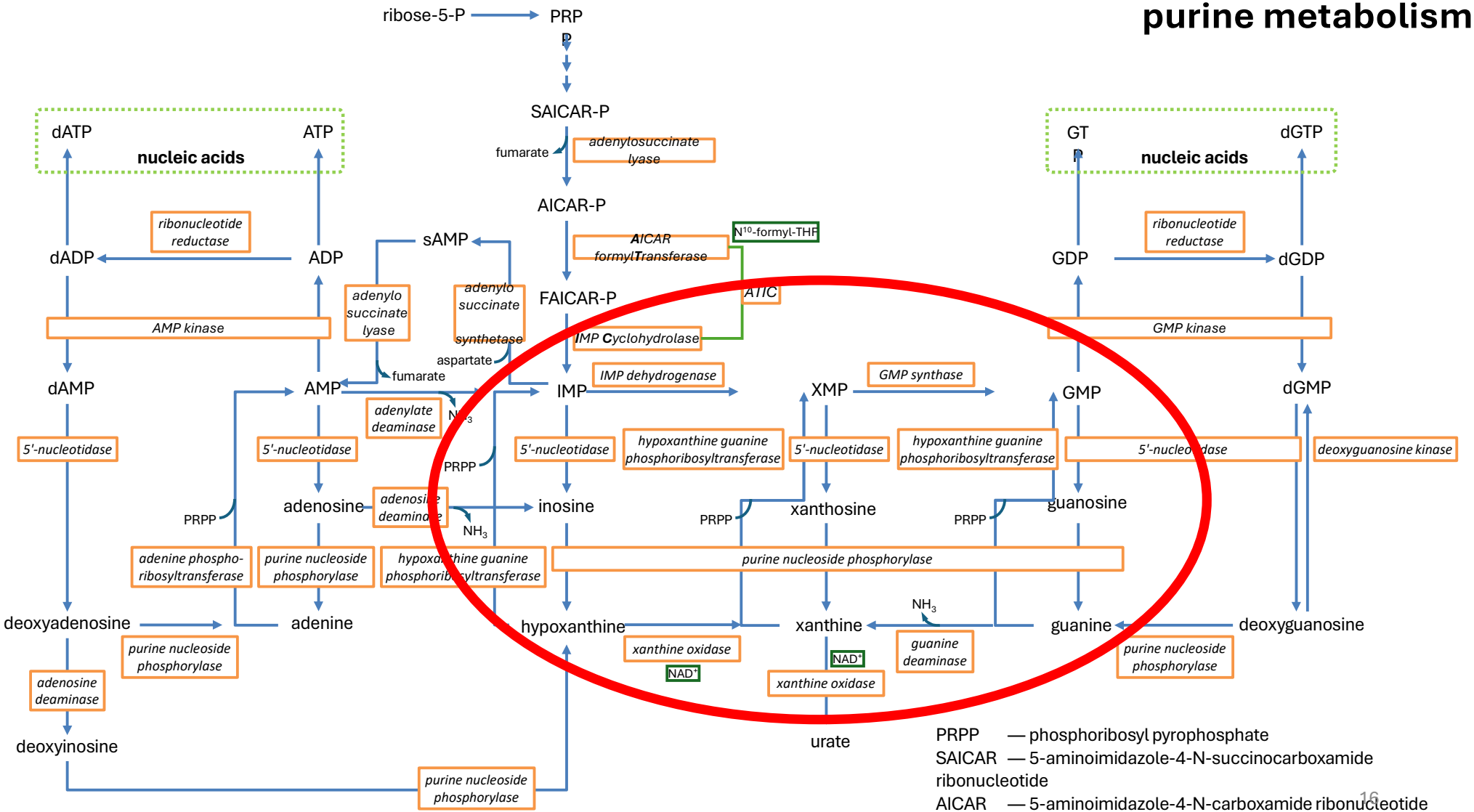
Hypoxanthine Guanine Phosphoribosyl Transferase



Hypoxanthine

A completely different story

purine metabolism



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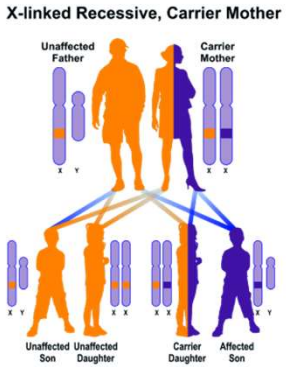
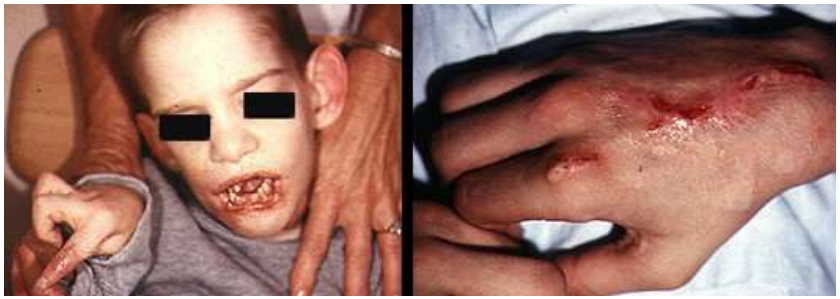
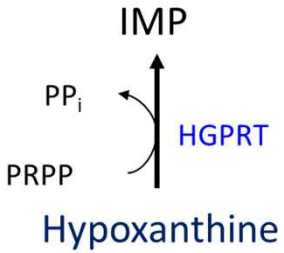
HGPRT-deficiency - Lesch-Nyhan Disease

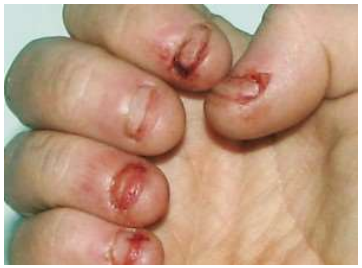
X-linked disorder
Hyperuricuria and hyperuricaemia



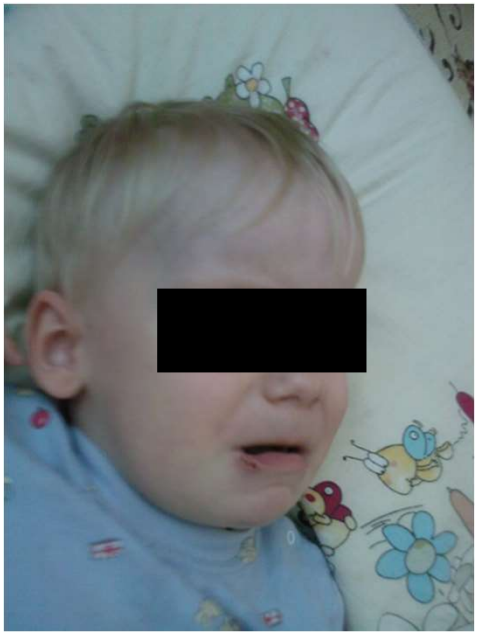


Above all neurological disease
Developmental delay
Hypotonia
Failure to reach milestones
Abnormal involuntary movements
Dystonia, choreoathetosis
Cerebral palsy
Inability to walk





Asymptomatic female carriers HPRT-related gout — Lesch-Nyhan Disease



Domynikas

8-year-old boy
Severe psychomotor development delay
Muscle hypotonia
Insensitivity to pain
Internal hydrocephalus
Kidney ultrasound - normal

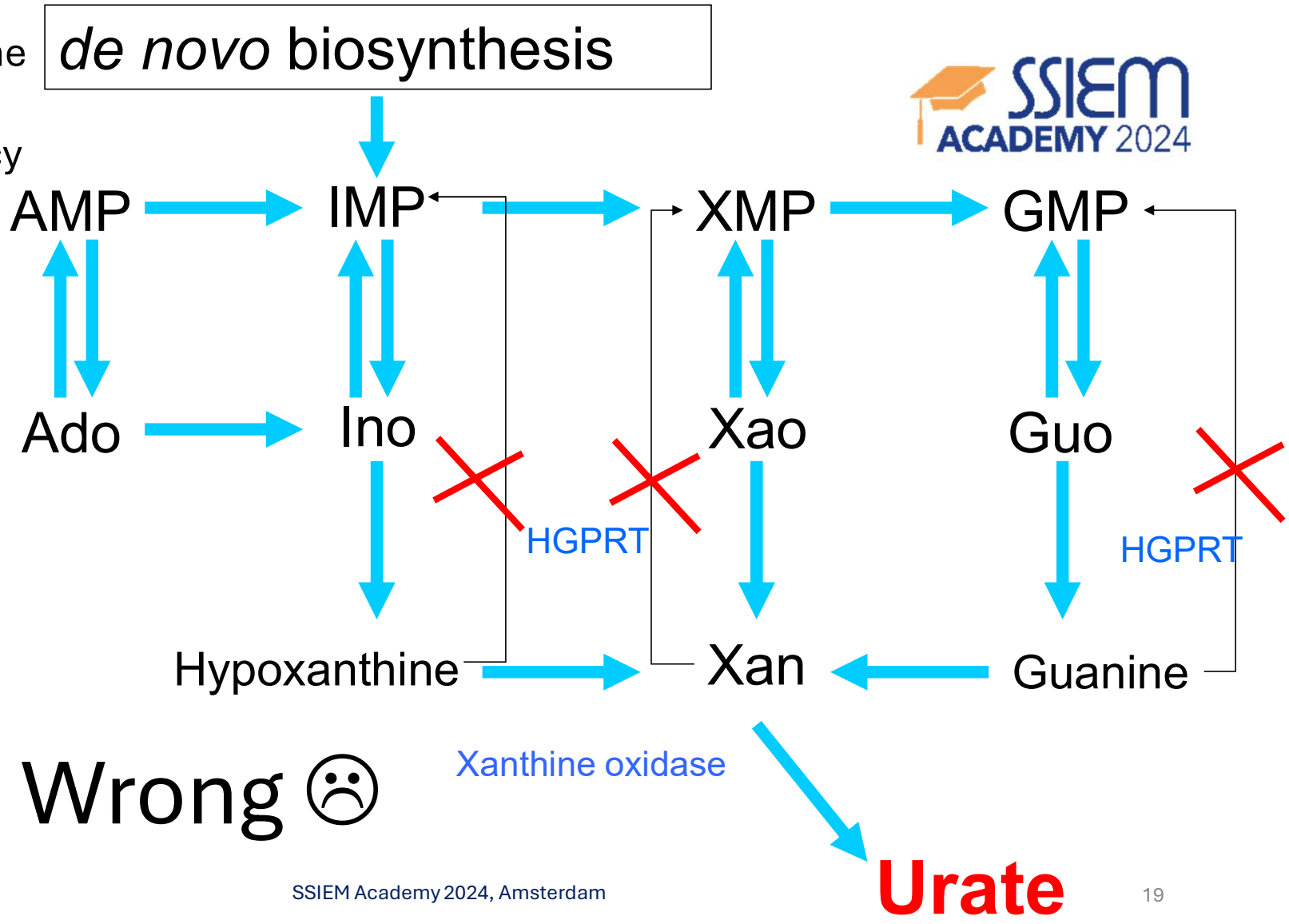
Urine purines	µmol/mmol creatinine
Uric acid	6300 (ref. < 2100)
Hypoxanthine	195 (ref 2-37)
Xanthine	109 (ref 6-31)
AICAriboside	13 (ref <5)

Plasma uric acid:
0.5 mmol/l (ref. 0.013 -0.23)

HGPRT activity <1% residual activity

HPRT c.601G>T (p.Asp201Tyr)

Hypoxanthine guanine phosphoribosyl transferase deficiency



No salvage of
- Hypoxanthine
- Xanthine

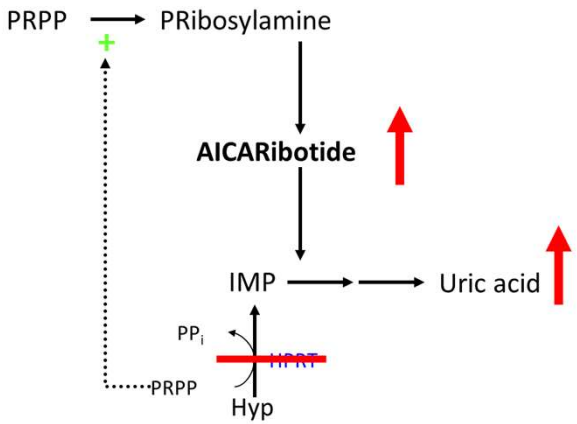
Leading to *hyperuricemia*

Treatment with allopurinol….

Wrong ☹

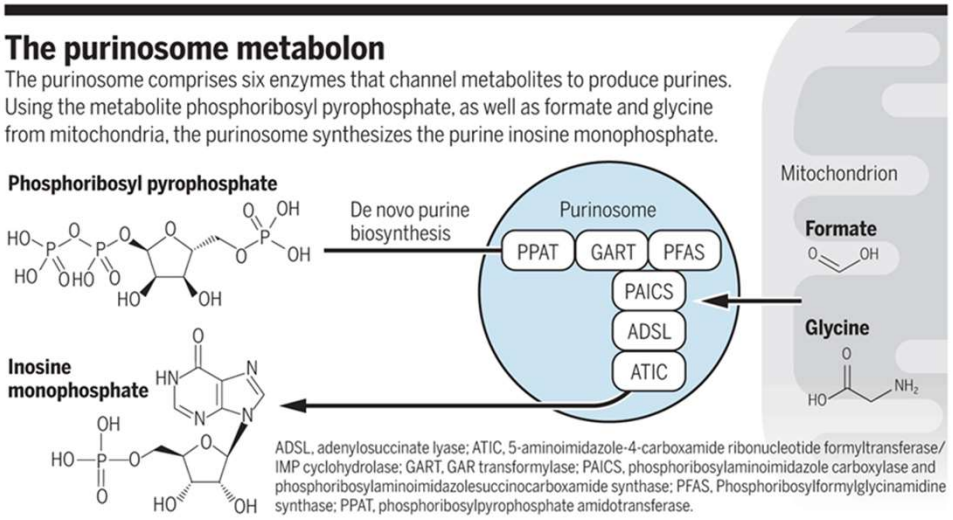
Simple, right?

What else happens in Lesch-Nyhan Disease?



Purine *de novo* biosynthesis mainly occurs in the purinosome.

Purinosomes assemble when needed
No formation in PNDS defects.
No degradation in LND.

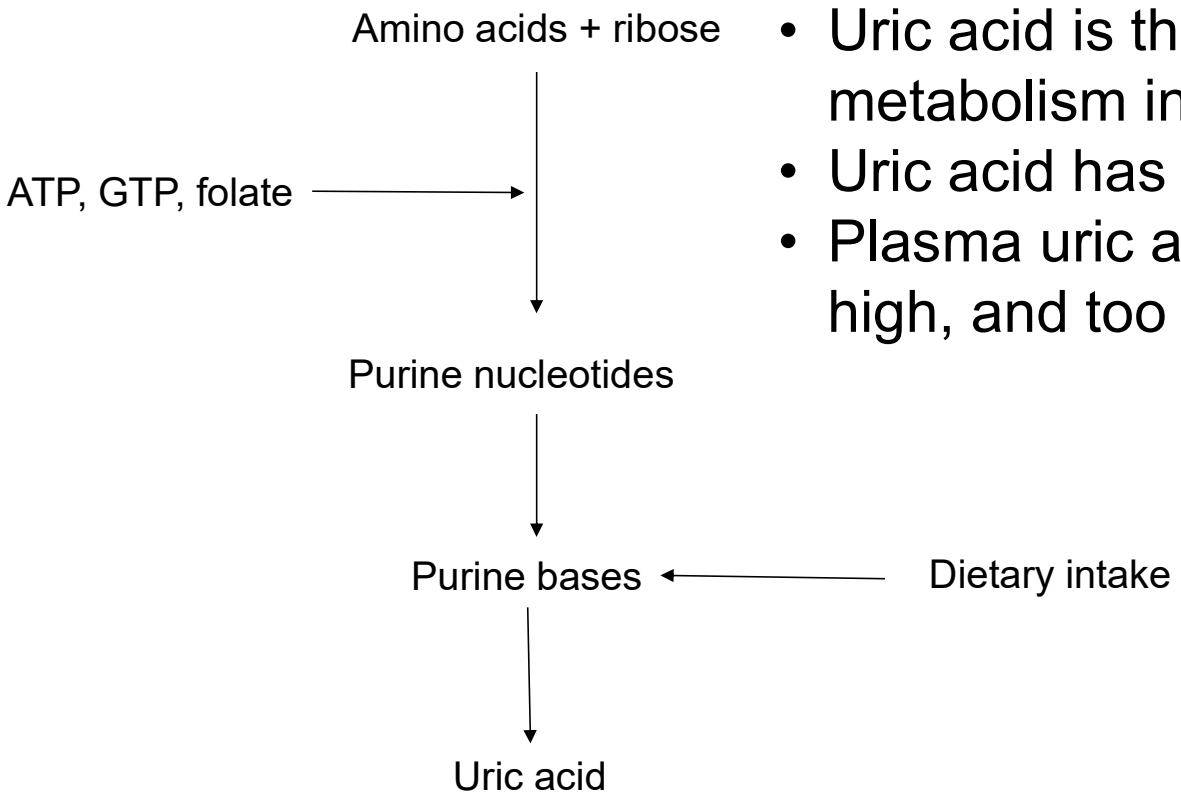


To make things worse:

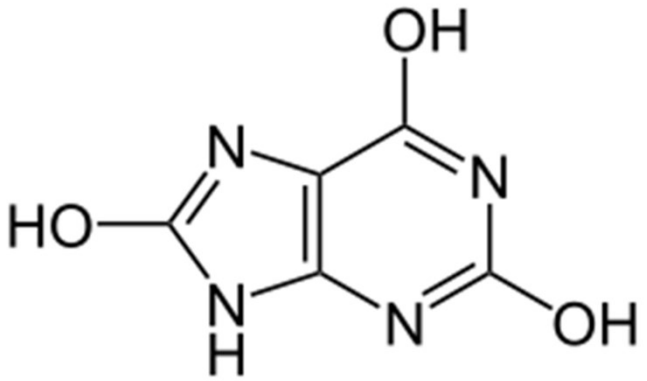
Midbrain dopaminergic cells have:

- energy deficit
- Impaired dopamine synthesis

Uric acid: a crucial biomarker



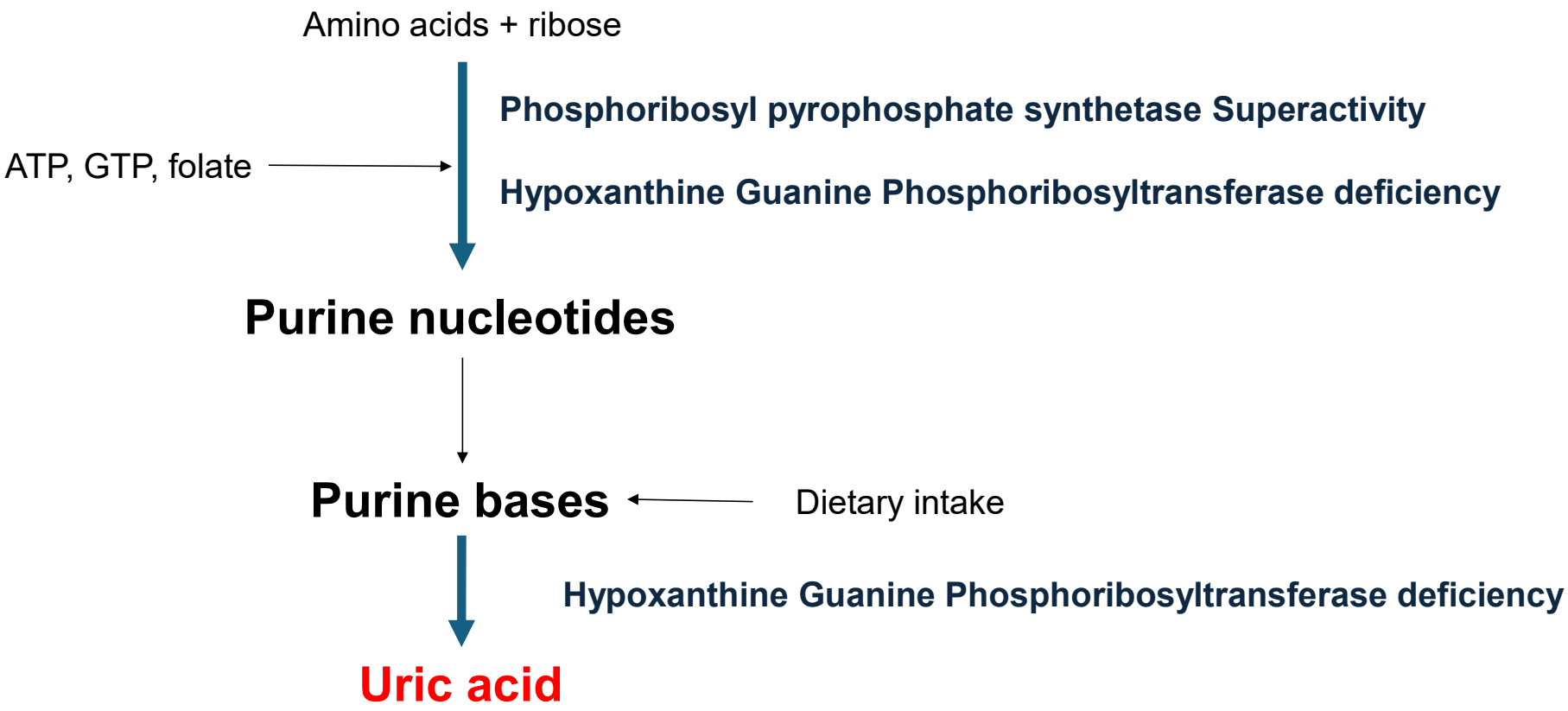
- Uric acid is the end-product of purine metabolism in humans
- Uric acid has poor solubility
- Plasma uric acid concentration can be too high, and too low



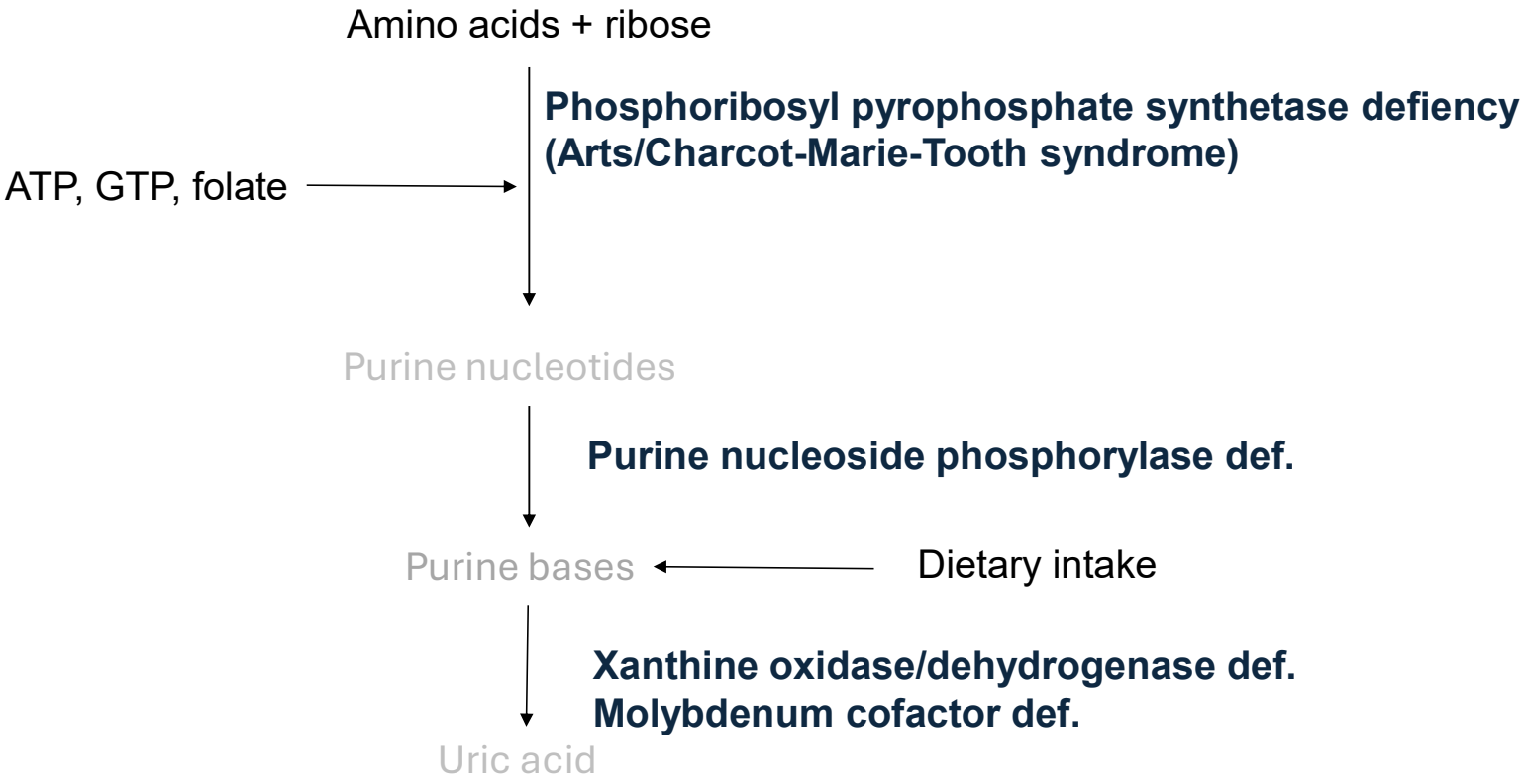
Genetic causes of altered uric acid

Increased	Decreased
Lesch-Nyhan disease (Hypoxanthine Guanine Phosphoribosyl Tranferase deficiency)	Purine Nucleoside Phosphorylase deficiency.
Phosphoribosyl pyrophosphate synthetase super activity	Isolated xanthine dehydrogenase deficiency.
Familial Juvenile Hyperuricaemia and Nephropathy (FJHN)	Combined xanthine dehydrogenase/sulphite oxidase deficiency.
Fructose-1,6-bisphosphatase deficiency Aldolase B deficiency, Glucose-6-phosphatase deficiency, Glucose-6-phosphatase deficiency; Muscle phosphofructokinase deficiency, organic acidurias and more	Molybdenum cofactor deficiency. Phosphoribosyl pyrophosphate synthetase deficiency

Purine high urate IMD



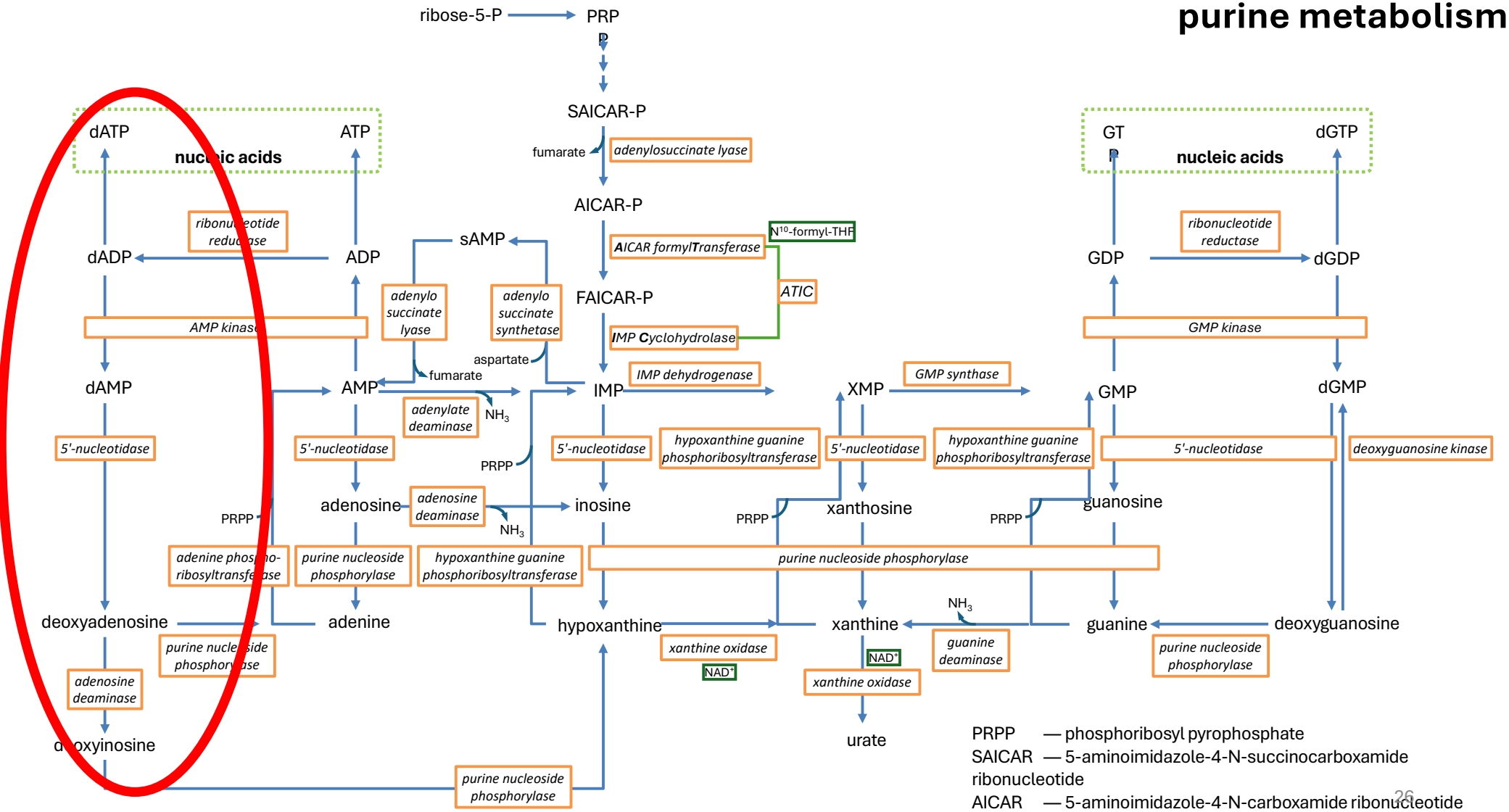
Purine low urate IMD



Somatic causes of altered uric acid

Increased	Decreased
Tumour lysis syndrome Reduced renal clearance Excessive purine intake	Treatment with uricase

purine metabolism



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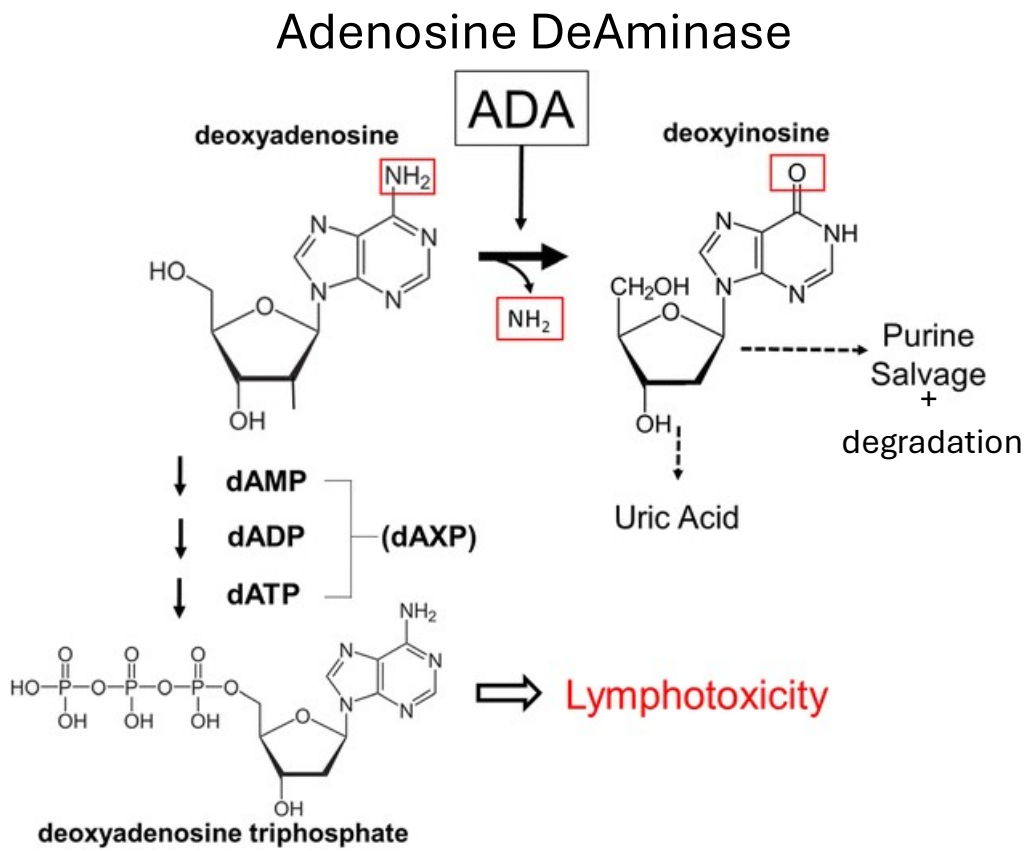
Newborn screening: SCID-ADA



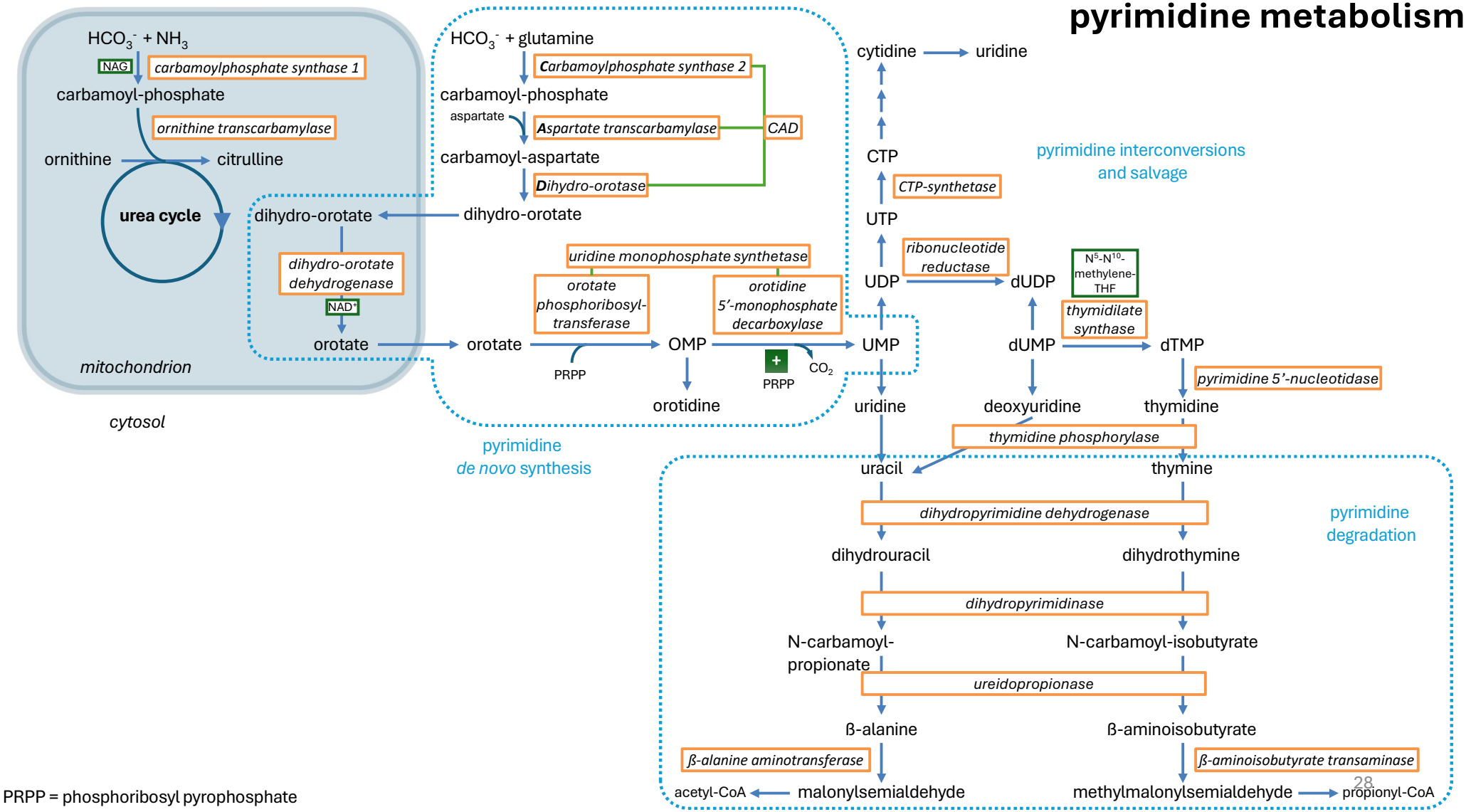
Dutch newbown screening program:
TRECs (T-cell receptor excision circles)
Gene panel

(Curative) treatment: Allogeneic hematopoietic stem cell transplantation (HSCT)

Biochemistry:

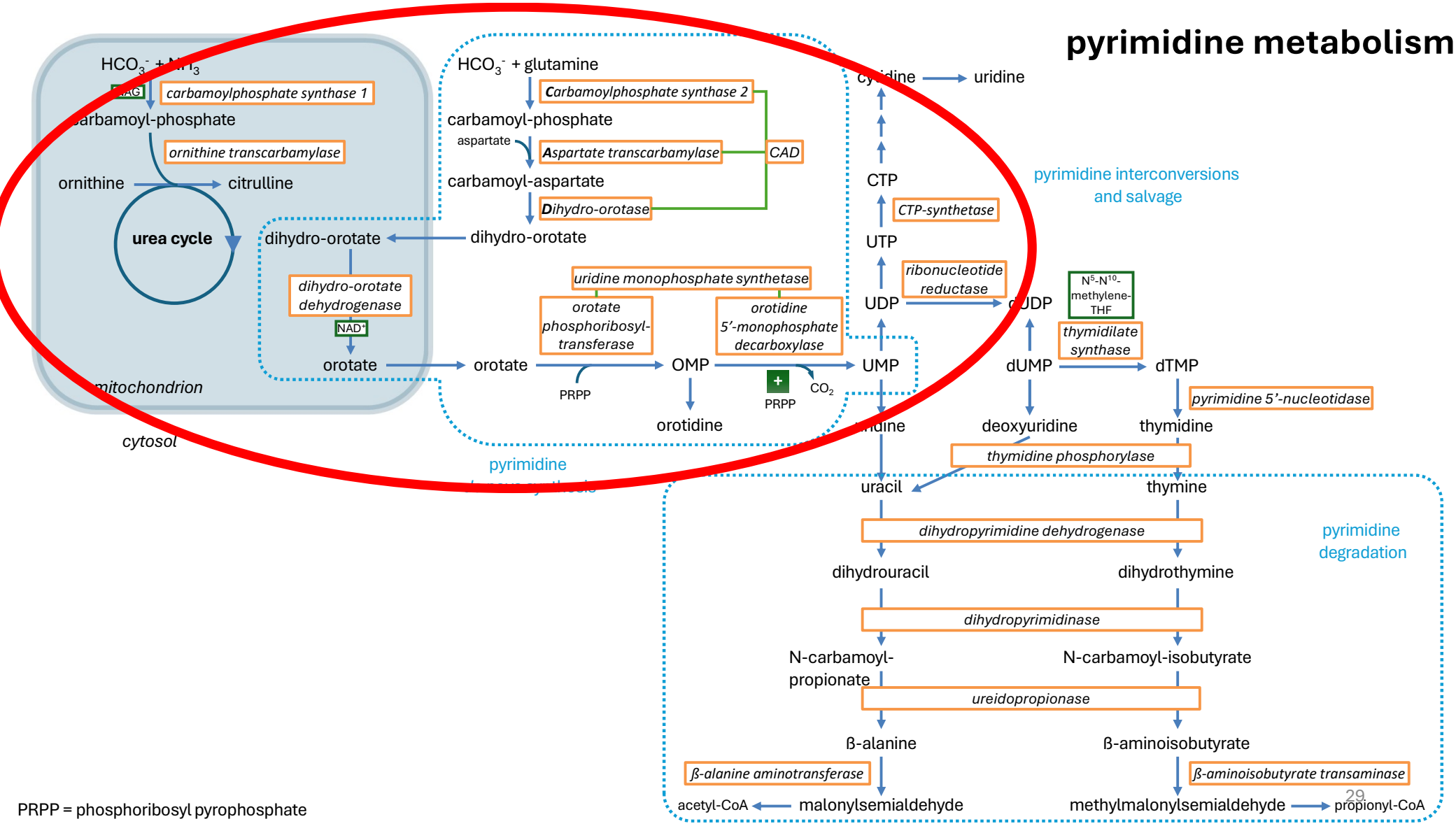
Deoxyadenosine accumulates.
ADA activity in erythrocytes deficient.

pyrimidine metabolism



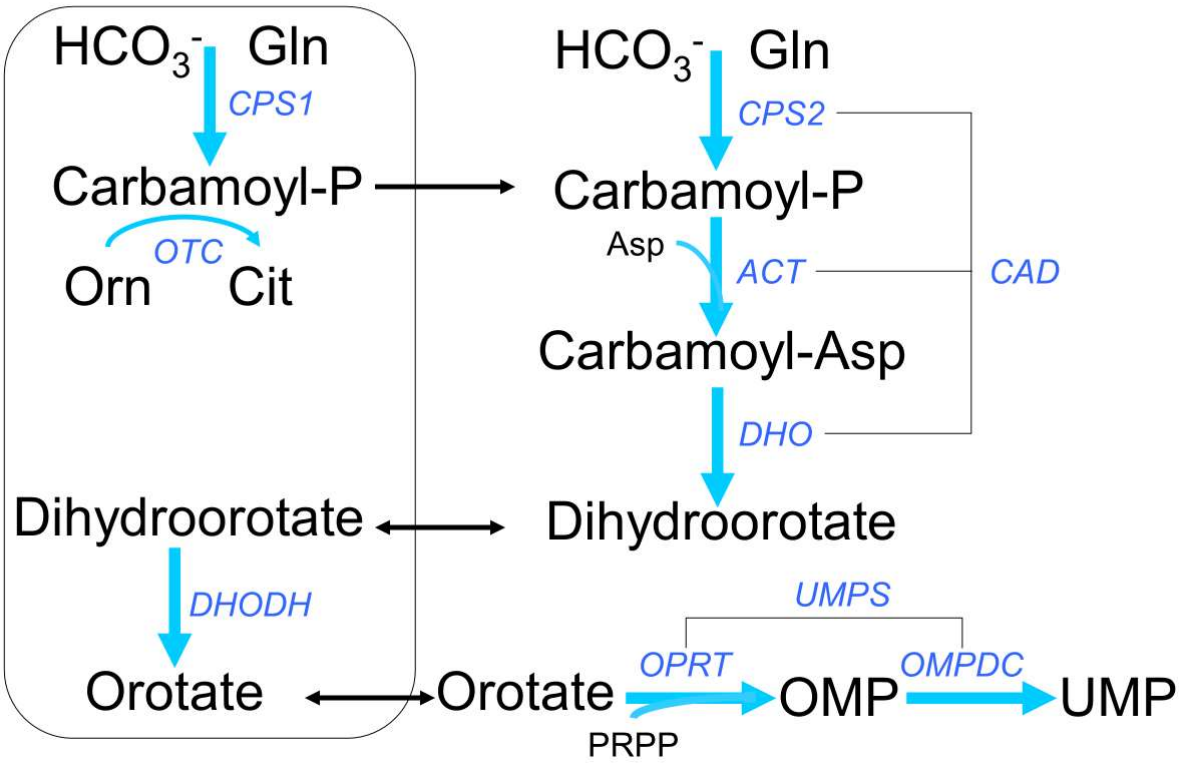
PRPP = phosphoribosyl pyrophosphate

pyrimidine metabolism



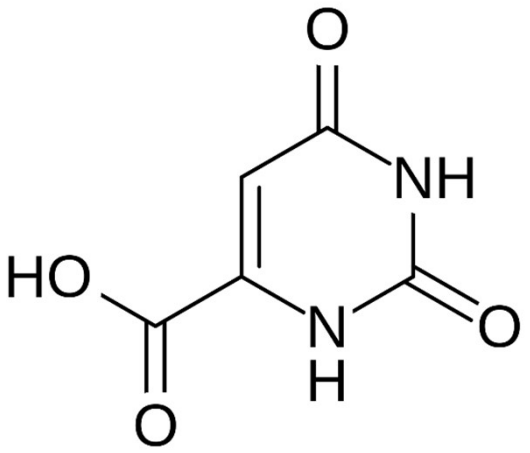
PRPP = phosphoribosyl pyrophosphate

otic acid: a versatile biomarker



Detection of orotic acid:

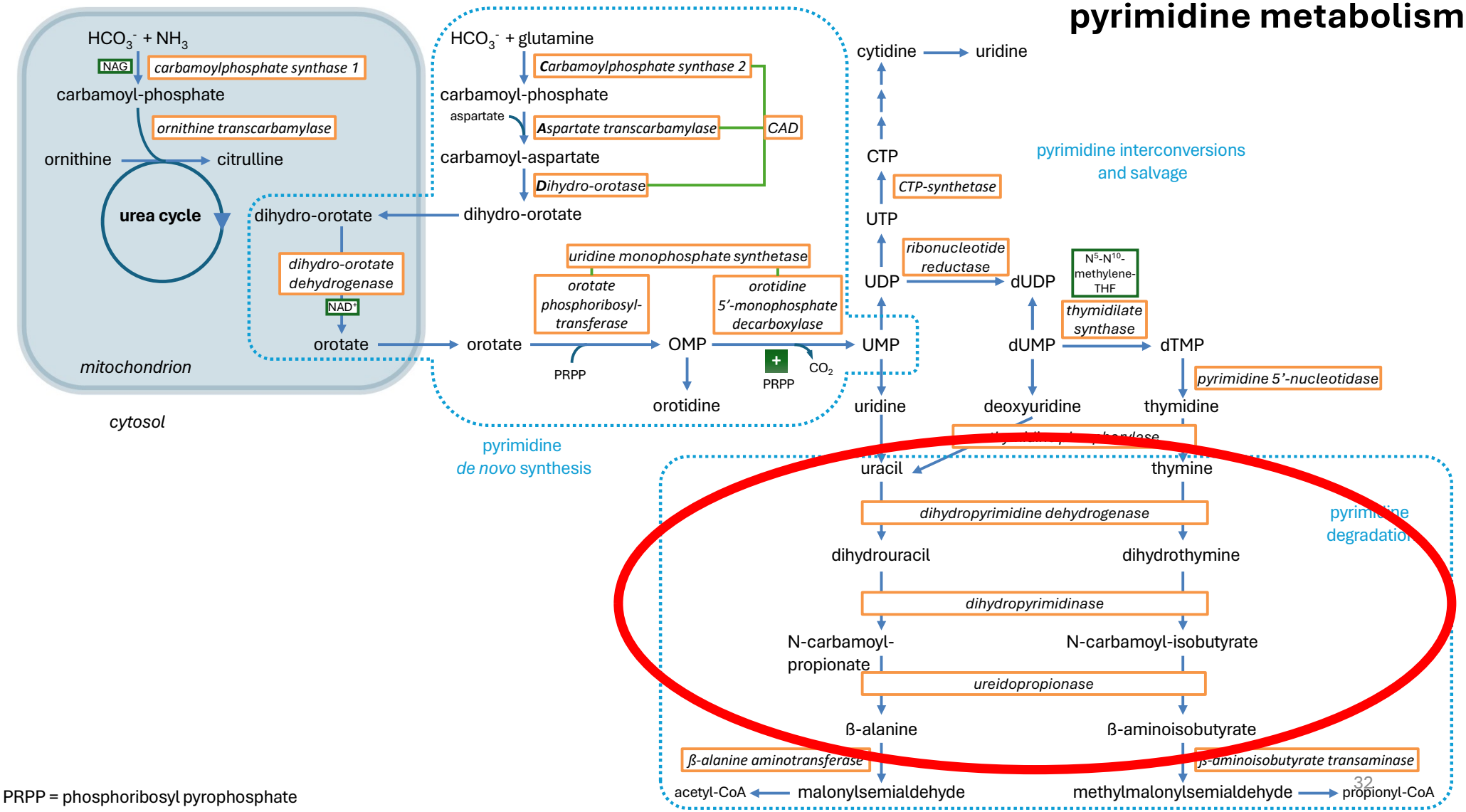
- LC-MS/MS
- GC-(MS)
- LC-UV



Orotic acid in diagnostics

Increased	
Hyperammonaemia	Urea cycle defects. Also orotidine and other pyrimidines.
Dihydro-orotate dehydrogenase deficiency (DHODH, Miller/Genee-Wiedemann syndrome)	severe micrognathia, cleft lip and/or palate, hypoplasia or aplasia of the postaxial elements of the limbs, coloboma of the eyelids, and supernumerary nipples. + Orotidine
Uridine monophospate synthetase deficiency (UMPS)	Crystalluria, megaloblastic anaemia, anisocytosis, poikilocytosis, hypochromia, development delay, T-cell immune def.
Treatment with allopurinol	Inhibition of decarboxylase activity of UMPS. Also orotidine.
Normal (not detectable)	
Carbamoyl phospate synthetase 1 deficiency N-acetyl glutamate synthetase deficiency	Hyperammonaemia without orotic aciduria
CAD-trifunctional enzyme deficiency	delayed psychomotor development, early-onset refractory seizures, severe developmental regression, anisocytosis, poikilocytosis, normocytic anaemia.

pyrimidine metabolism



PRPP = phosphoribosyl pyrophosphate

Dihydropyrimidine dehydrogenase deficiency?

Boy born from non-consanguineous Dutch parents

Irritability and hypertonia from birth, transient respiratory problems and feeding difficulties

Course: hypertonia and hyperreflexia changed into hypotonia and areflexia

Growth p50 for height and p97 for head circumference.

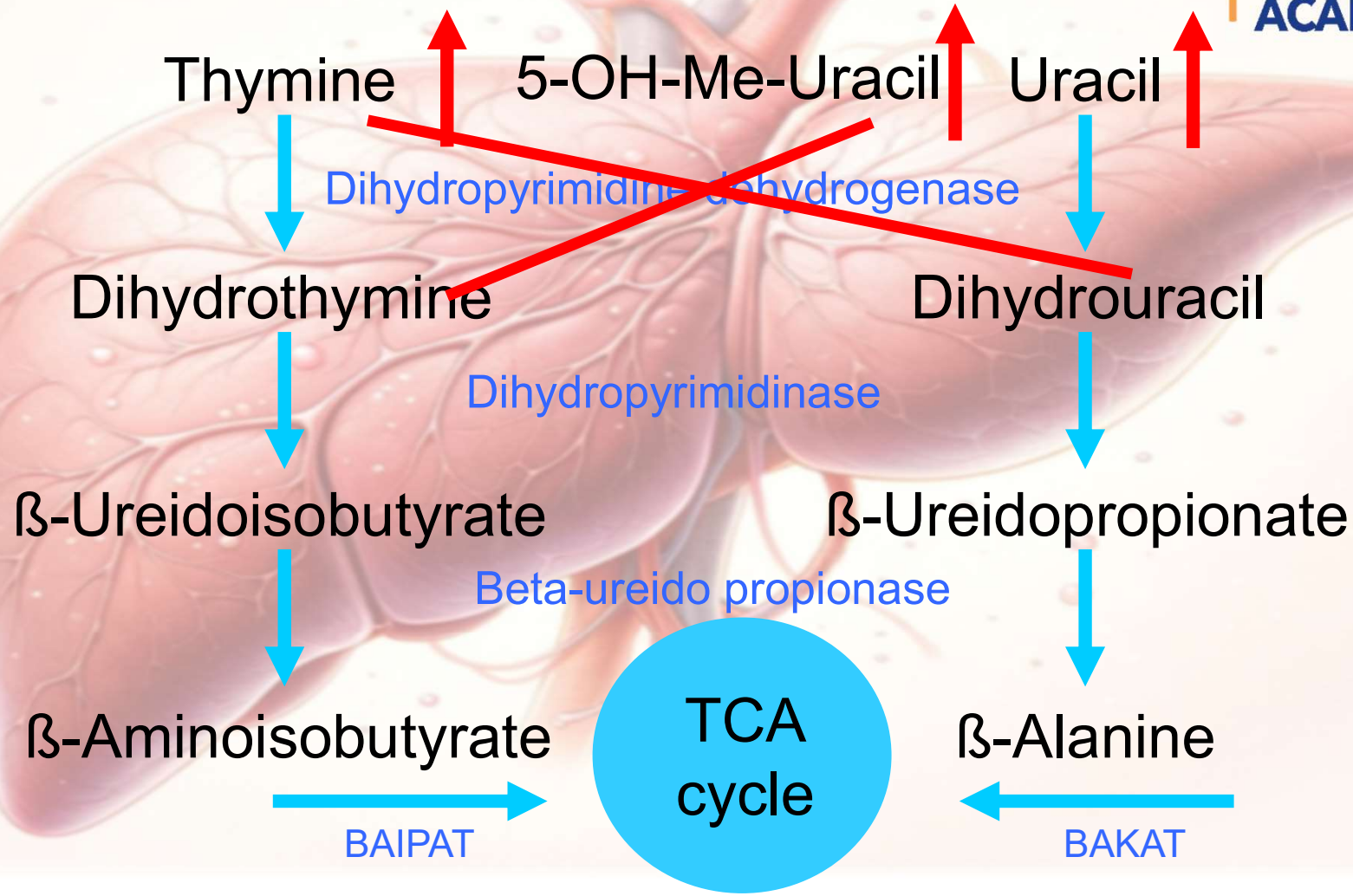
Severe mental retardation

Multiple dysmorphic features

Deceased at 10 years of age

Van Kuilenburg et al. Hum Genet 2009

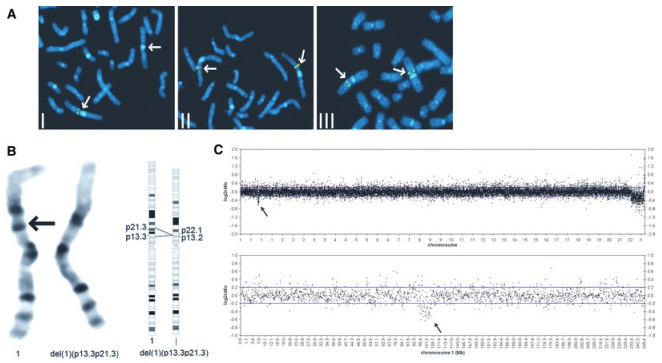
Pyrimidine *degradation*



Molecular analysis

- Homozygosity for DPYD c.299_302delTCAT
- Mother heterozygosity for DPYD c.299_302delTCAT
- Father no DPYD mutation
- Clinical phenotype could not be explained solely by DPD deficiency

MLPA: allele of DPYD missing



Gene	(putative) function
NTNG1	Axon guidance
GPSM2	Neuroblast renewal
PRG-1 (LPR4)	Axon growth
COL1A1	Connective tissue, bones

Deletion of 14 Mb between 1p13.3 and 1p21.3
57 genes are located in this region.

Molecular Genetics and Metabolism 137 (2022) 62–67

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

The relationship between beta-ureidopropionase deficiency due to *UPB1* variants and human phenotypes is uncertain

Sarah Righetti [a], Richard J.N. Allcock [b], Joy Yaplito-Lee [c], Louisa Adams [d], Carolyn Ellaway [d], Kristi J. Jones [d,e], Arthavan Selvanathan [d], Janice Fletcher [f], James Pitt [g], André B.P. van Kuilenburg [h,i], Martin B. Delatycki [g], Nigel G. Laing [j], Edwin P. Kirk [a,d,f,*]

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Hum Genet (2009) 125:581–590
DOI 10.1007/s00439-009-0653-6

ORIGINAL INVESTIGATION

Analysis of severely affected patients with dihydropyrimidine dehydrogenase deficiency reveals large intragenic rearrangemen of *DPYD* and a de novo interstitial deletion del(1)(p13.3p21.3)

André B. P. van Kuilenburg · Judith Meijer · Adri N. P. M. Mul · Raoul C. M. Hennekam · Jan M. N. Hoovers · Christine E. M. de Die-Smulders · Peter Weber · Andrea Capone Mori · Jörgen Bierau · Brian Fowler · Klaus Macke · Jörn Oliver Sass · Rutger Meinsma · Julia B. Hennermann · Peter Miny · Lida Zoetekouw · Raymon Vijzelaar · Joost Nicolai · Bauke Ylstra · M. Estela Rubio-Gozalbo

Received: 27 January 2009 / Accepted: 5 March 2009 / Published online: 19 March 2009

SSIEM ACADEMY 2024

Dihydropyrimidine Dehydrogenase Deficiency: Metabolic Disease or Biochemical Phenotype?

Research Report

M. Fleger, J. Willomitzer, R. Meinsma, M. Alders, J. Meijer, R. C. M. Hennekam, M. Huemer & A. B. P. van Kuilenburg

Chapter | First Online: 09 March 2017

537 Accesses | 8 Citations

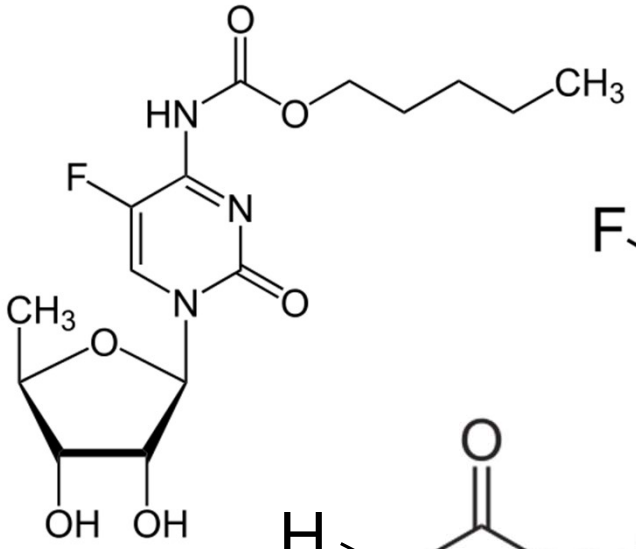
Part of the JIMD Reports book series (JIMD,volume 37)

Dihydropyrimidine dehydrogenase and anti-cancer therapy
Pharmacogenetics of fluoropyrimidines

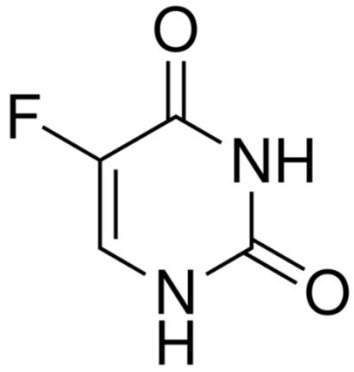
Worldwide the most used chemotherapy for solid tumours:

- Breast cancer
- Colon cancer
- Head and neck cancer

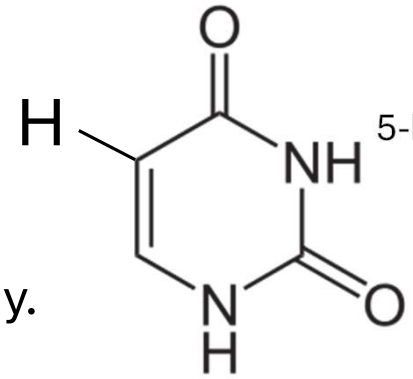
2 million people per year!



capecitabine



5-FU, 5-Fluorouracil



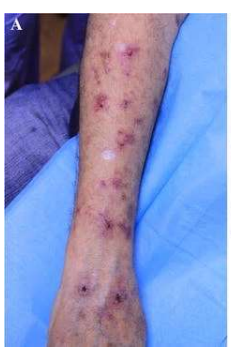
uracil

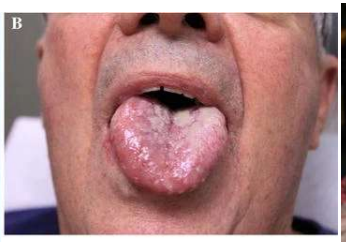
- 10 - 40% of patients develop severe, sometimes life-threatening toxicity including neutropenia, nausea, vomiting, severe diarrhea, stomatitis, mucositis, hand-foot syndrome and neuropathy.

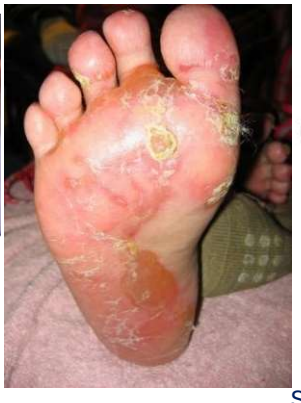
5-FU is degraded by the pyrimidine degradation pathway

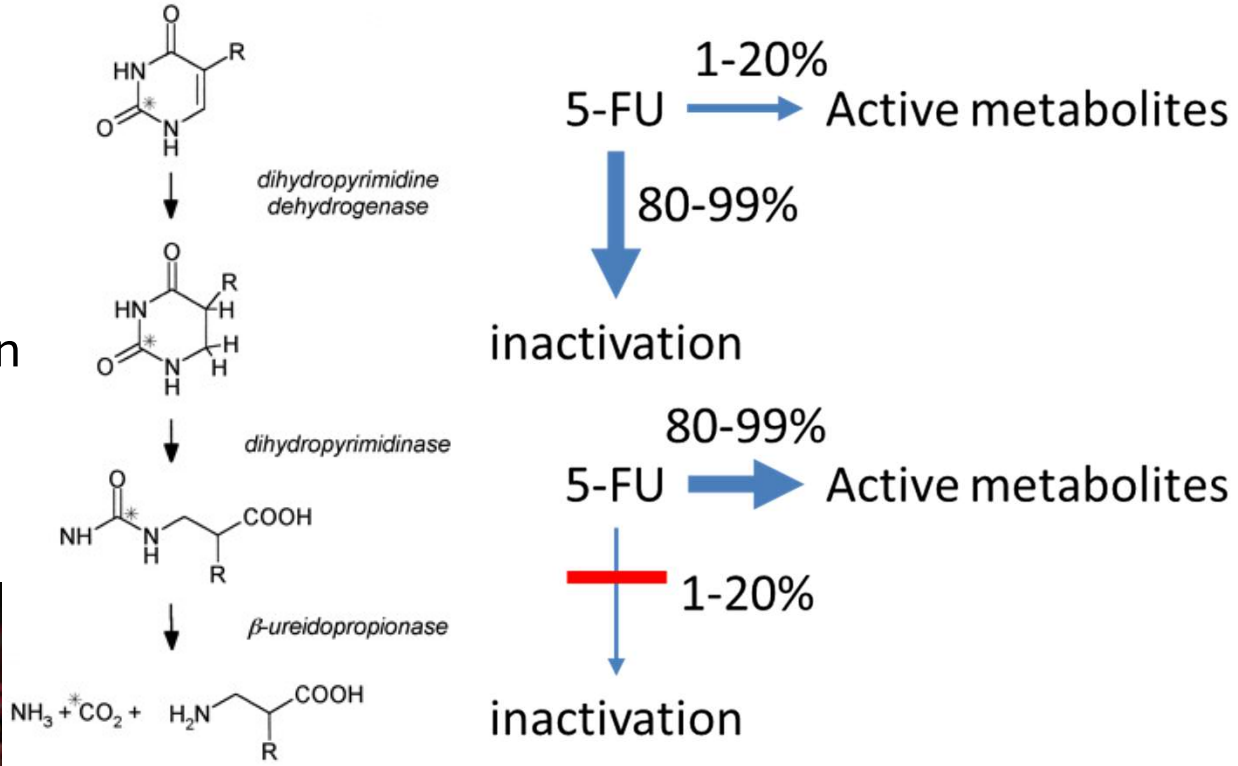
2 - 5% of the Caucasian population have a low DPD activity, depending on subpopulation

Over 80% of them develop severe toxicity within days to a few weeks on standard 5FU treatment, 10% die because of toxicity



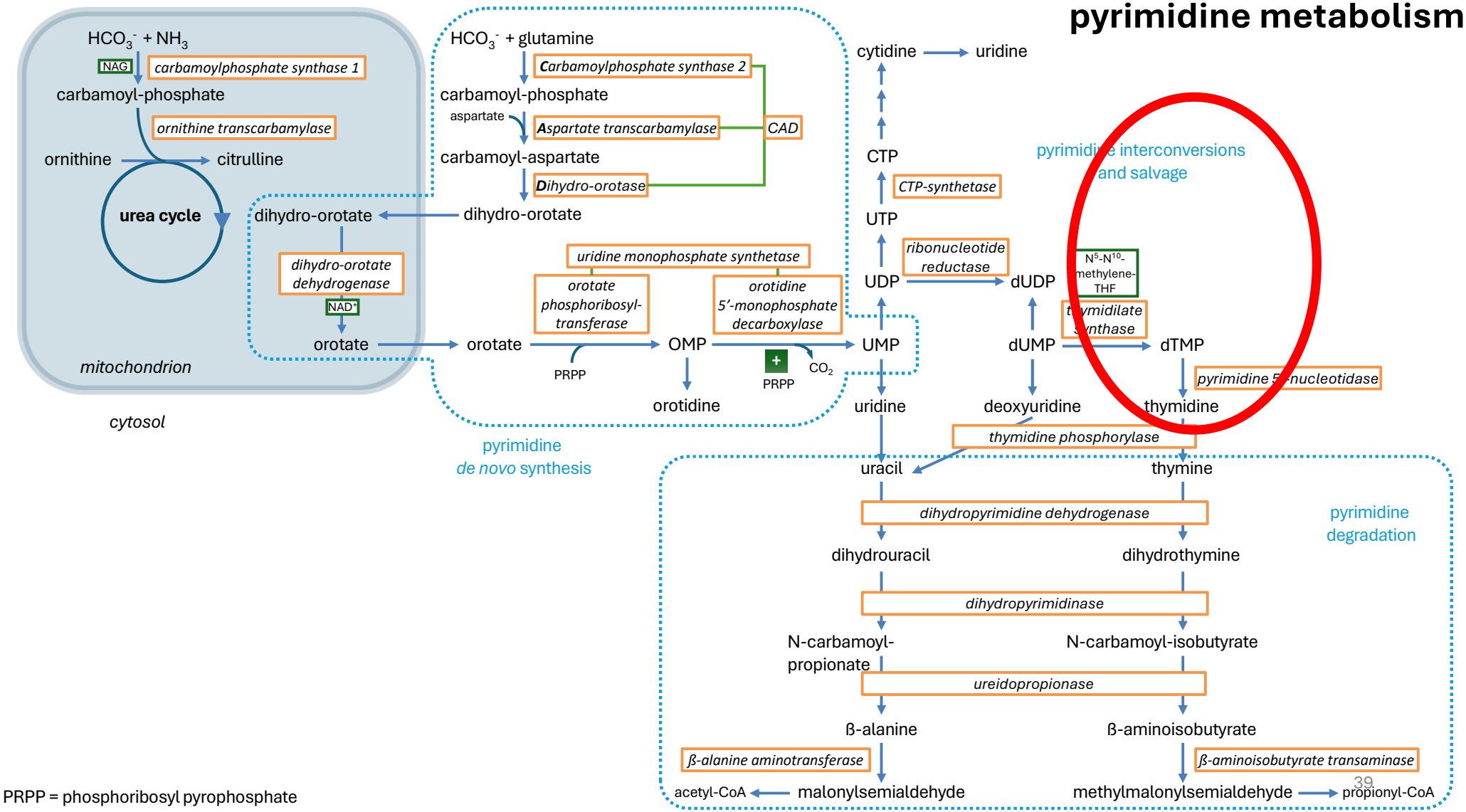






Screening for decreased DPD-activity and DPYD variants saves lives!

pyrimidine metabolism

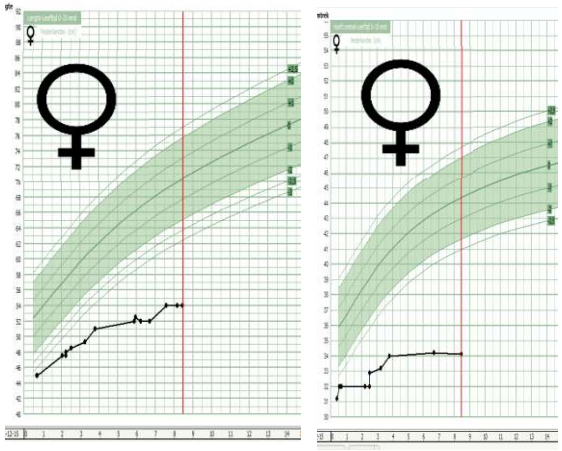


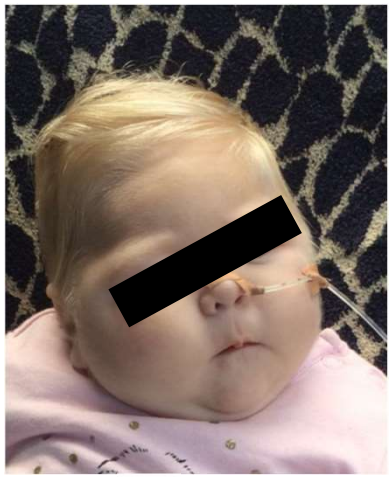
PRPP = phosphoribosyl pyrophosphate

Thymidylate kinase (DTYMK) deficiency: not all defects have biomarkers

- **severe microcephaly**
- no developmental progress
  - no eye contact
- seizures
- severe growth retardation
  →puffy body
- Metabolic screening normal!!!

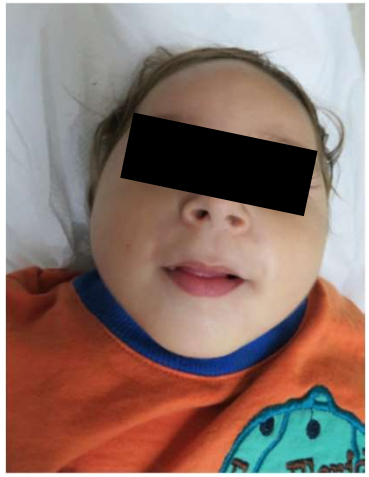
Height **OFC**





patient I; ♀; The Netherlands

Unrelated Dutch parents
First child
Polyhydramnios



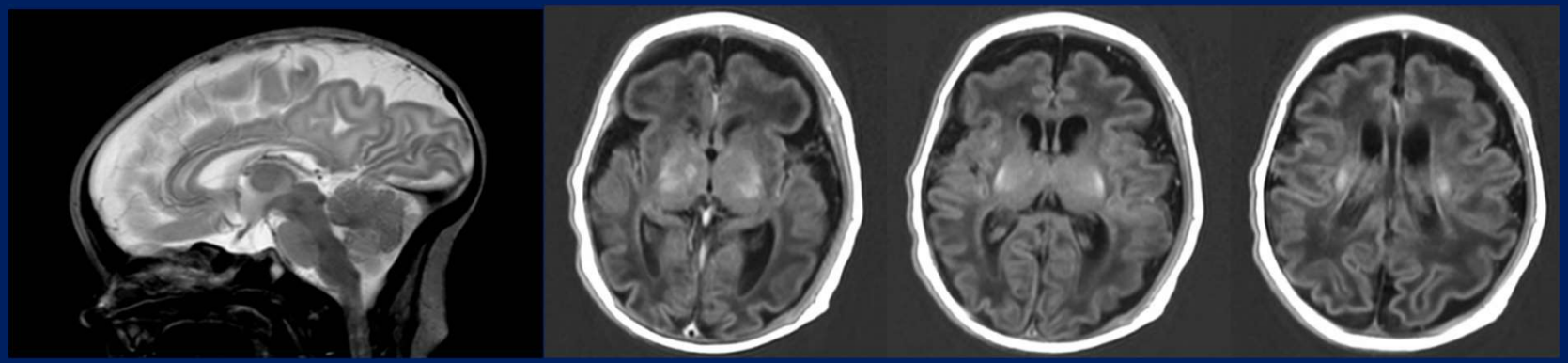
patient II; ♂; Egypt

Egyptian parents, 1st cousins
Second child, healthy elder sister
Uncomplicated pregnancy

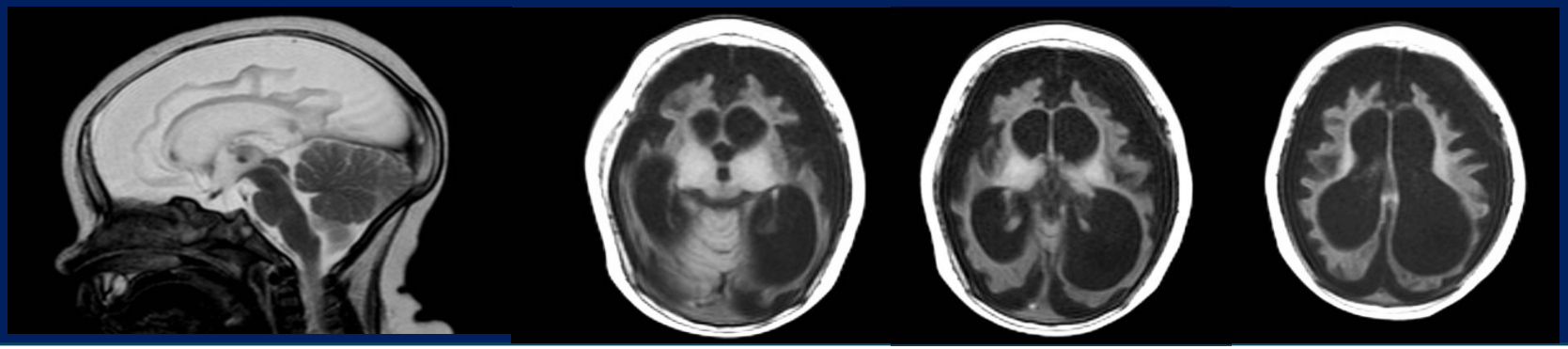
Vanoevelen, J.M., Bierau, J., Grashorn, J.C. et al. DTYMK is essential for genome integrity and neuronal survival. Acta Neuropathol 143, 245–262 (2022). https://doi.org/10.1007/s00401-021-02394-0

Images of brain patient I

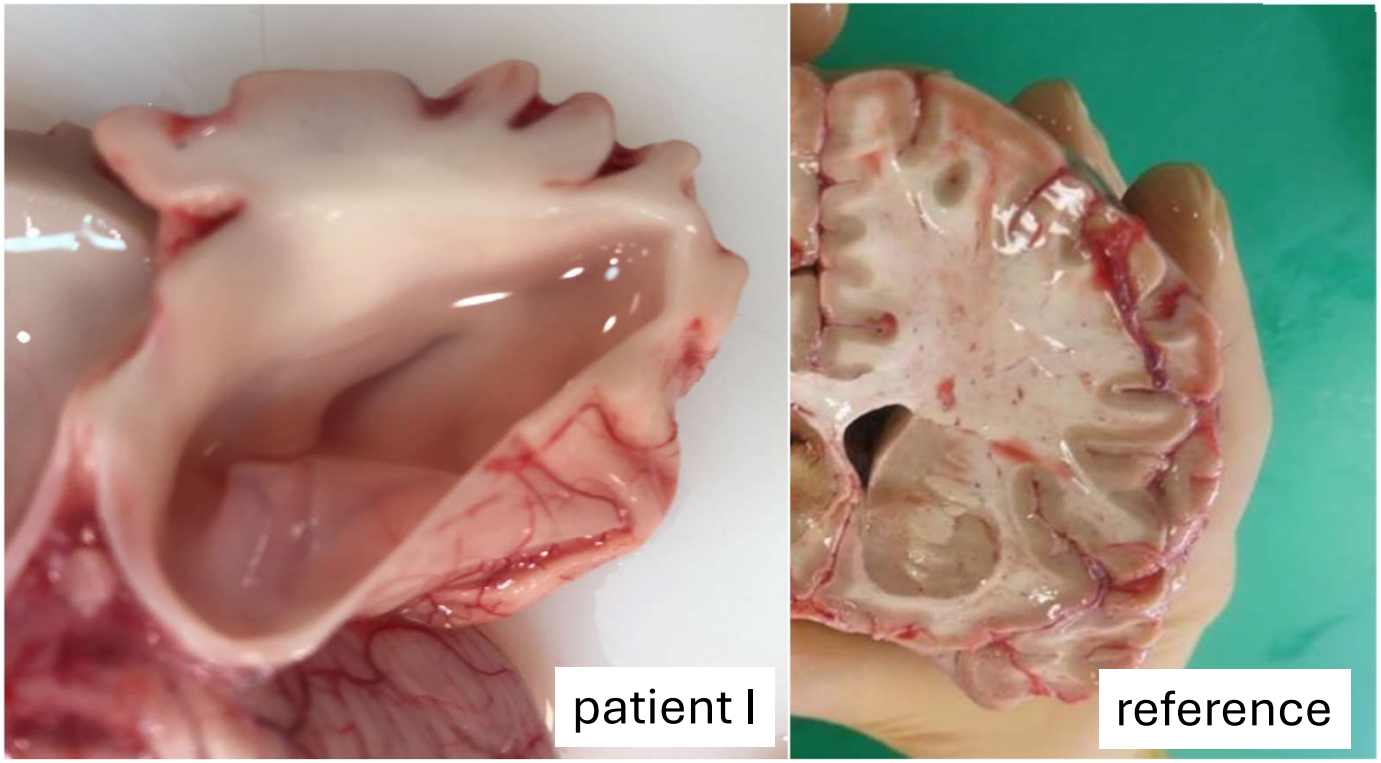
3 weeks



6 months

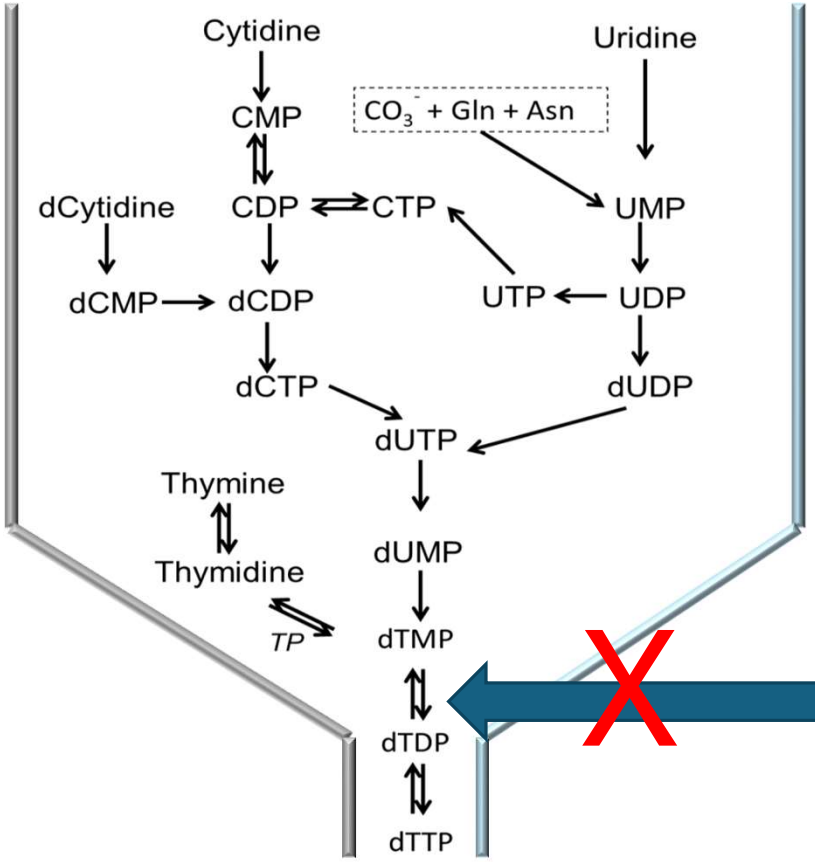


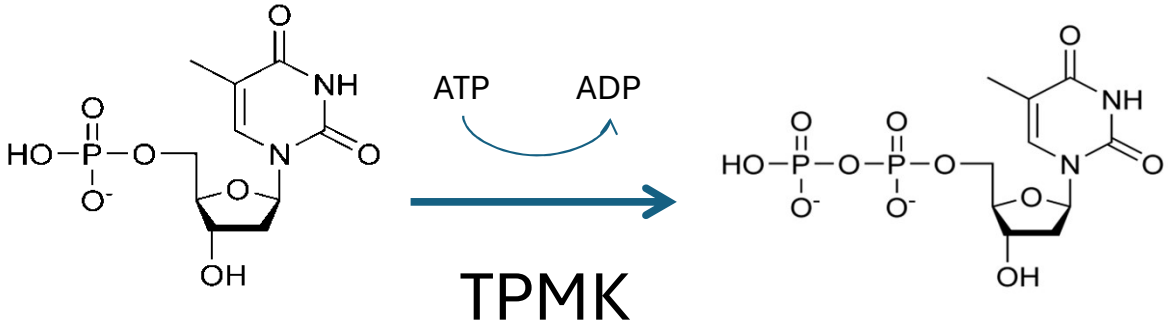
Images of brain patient I



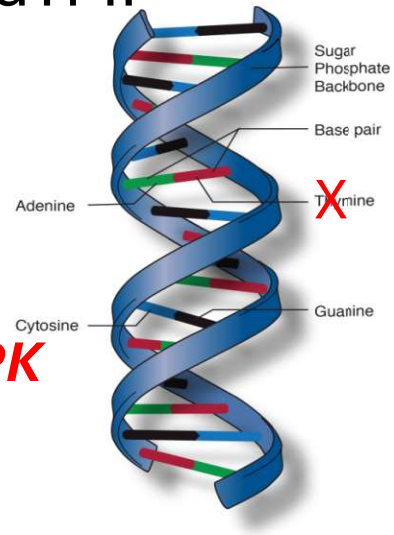
Cerebral cortex largely vanished

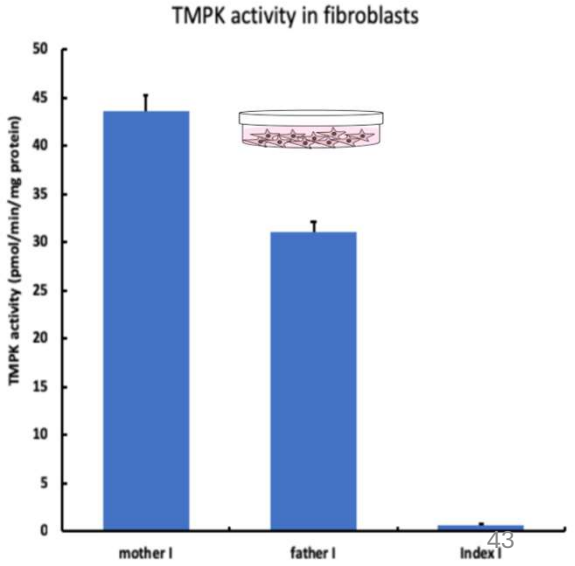
Thymidylate kinase and dTTP metabolism





dTMPK





Thank you for your attention!

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