ERNDIM

QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

Clinical aspects of the new defects in purine and pyrimidine metabolism

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SALK

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…some thoughts before starting…

- Why are these disorders so rare (or why do I always miss them)?
- Are there any „red flags“/ suggestive clinical findings?
- Which disorders are treatable and (especially) warrant early/quick diagnosis?
- Are Myoadenylate deaminase (AMPD1)-deficiency and DPD-deficiency non-diseases?
- ~~(mitochondrial disorders *DGUOK, TK2, RRM2B*)~~

Known defects in Purine & Pyrimidine metabolism

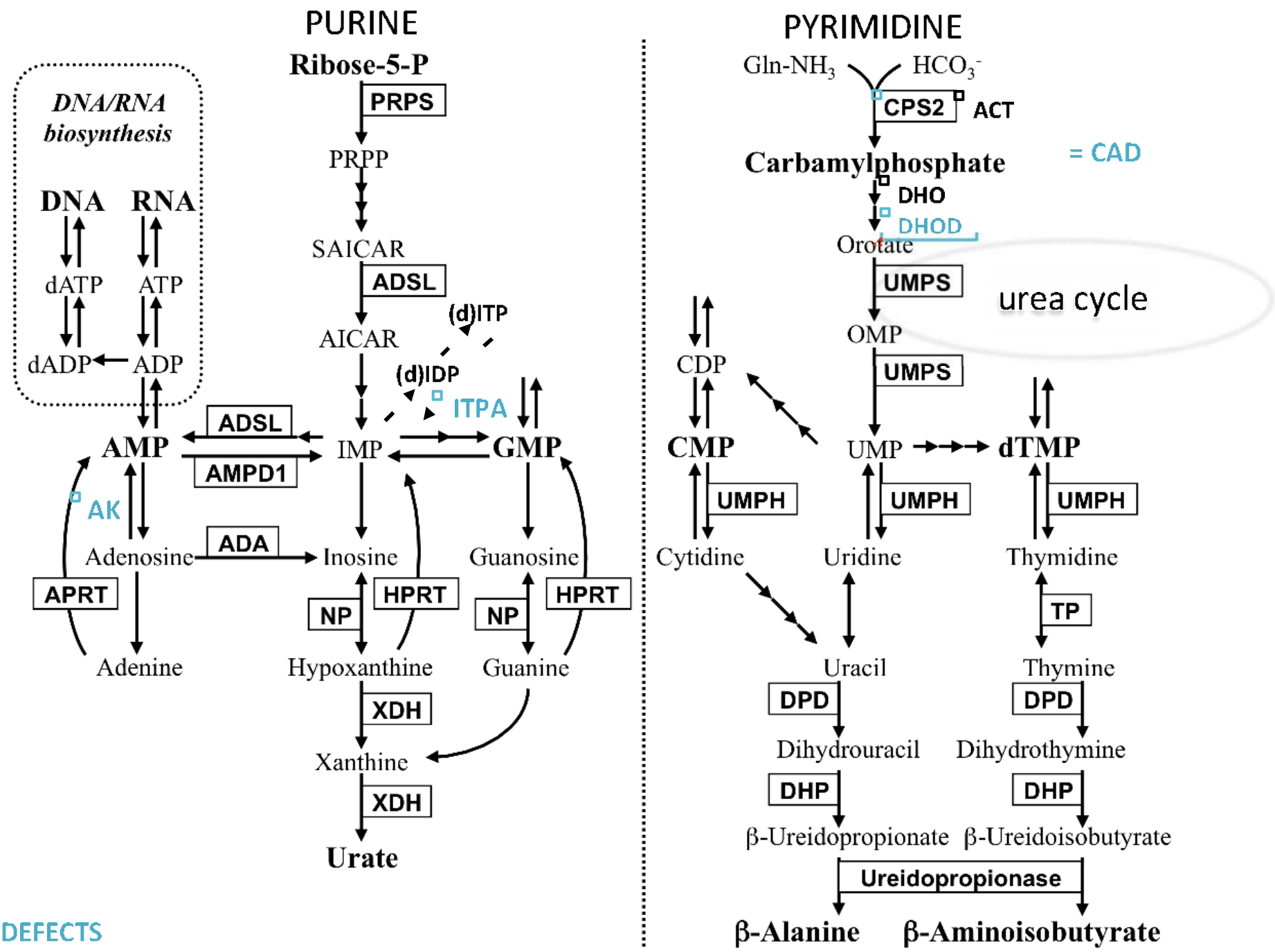
PURINE

- Phosphoribosyl pyrophosphate synthetase (PPRS) superacitivity
- Adenylosuccinate lyase (ADSL) deficiency
- Myoadenylate deaminase (AMPD1) deficiency
- Purine nucleoside phosphorylase (NP) deficinecy
- Xanthinuria (XDH)
- Familial juvenile hyperuricaemic nephropathy
- Lesch-Nyhan syndrome
- Adenine phosphoribosyltransferase (APRT) deficiency
- ITPA encephalopathy
- Adenosine kinase (AK) deficiency

PYRIMYDINE

- UMPS- deficiency
- Pyrimidine 5'-nucleotidase deficiency
- Dihydropyrimidine dehydrogenase (DPD) deficiency
- Dihydropyrimidinase (DHP) deficiency
- Ureidopropionase deficiency
- Dihydro-orotate dehydrogenase (DHODH) deficiency
- CAD-deficiency

BALASUBRAMANIAM, DULEY and CHRISTODOULOU
JIMD 2014

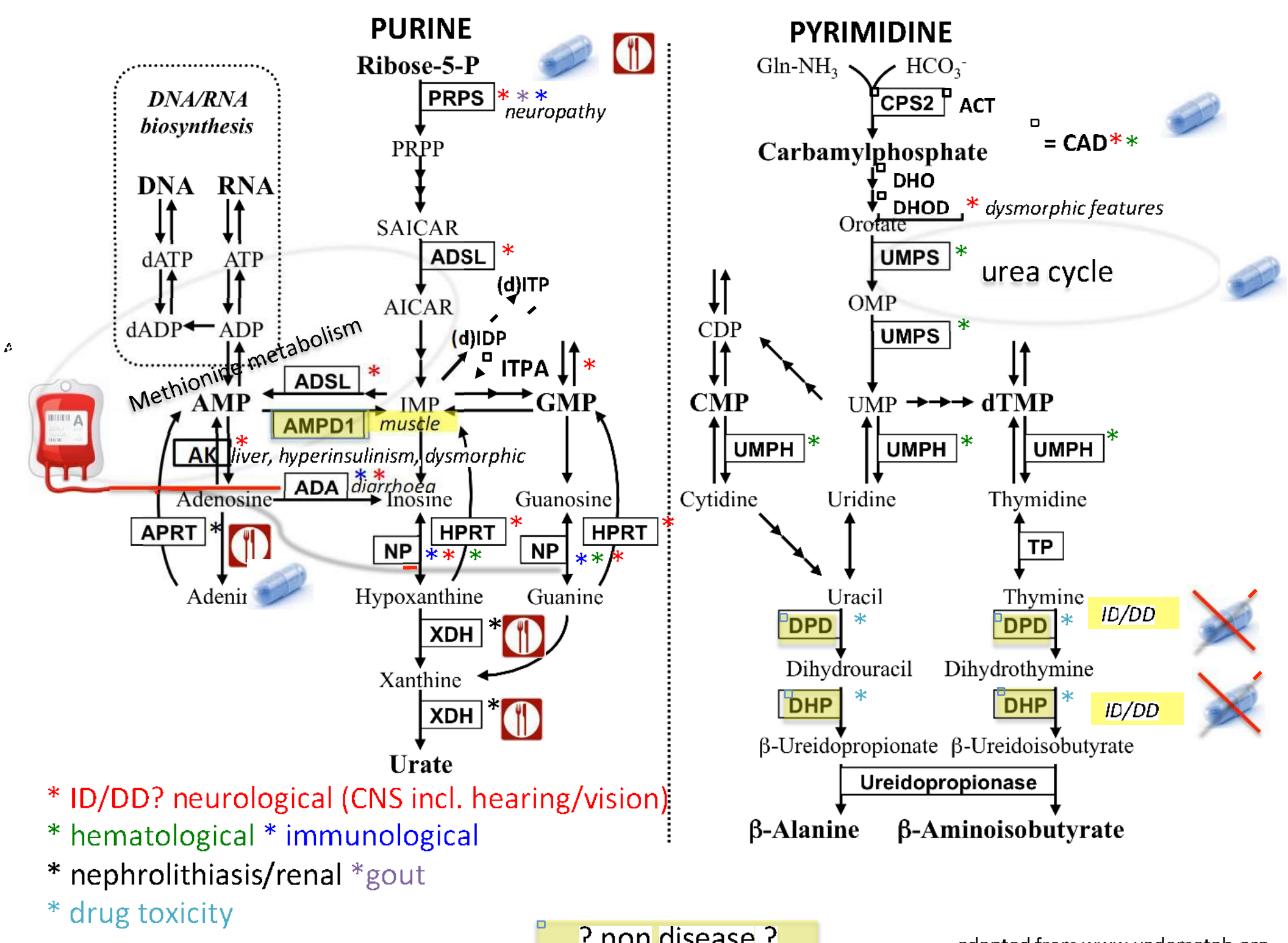


NEW DEFECTS

Inosine triphosphate pyrophosphatase (ITPA) encephalopathy
Adenosine kinase (AK) deficiency

Dihydro-orotate dehydrogenase (DHODH) deficiency
CAD-deficiency

adapted from www.vademetab.org



* ID/DD? neurological (CNS incl. hearing/vision)

* hematological * immunological

* nephrolithiasis/renal *gout

* drug toxicity

? non disease ?

adapted from www.vademetab.org

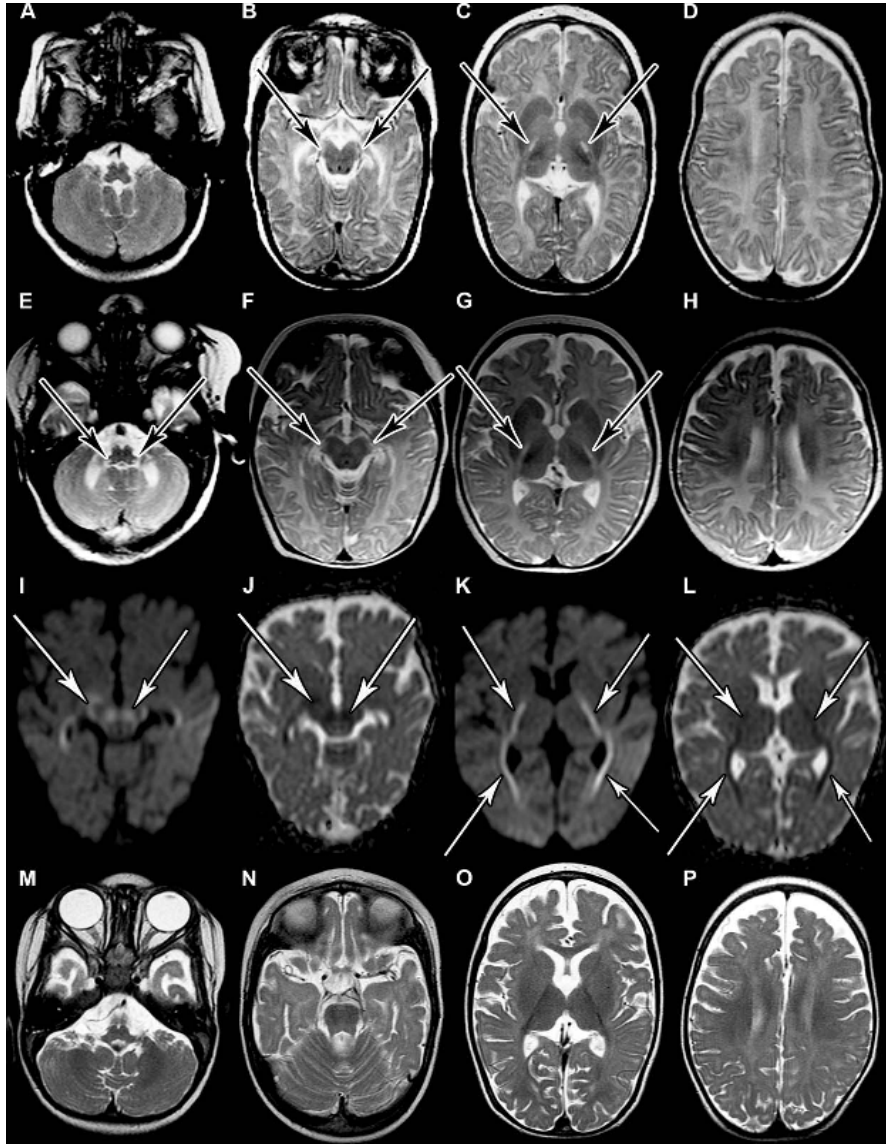
ITPA encephalopathy (Kevelam et al., Ann Neurol. 2005)

- 7 patients from 4 families

- identified based on MRI pattern recognition

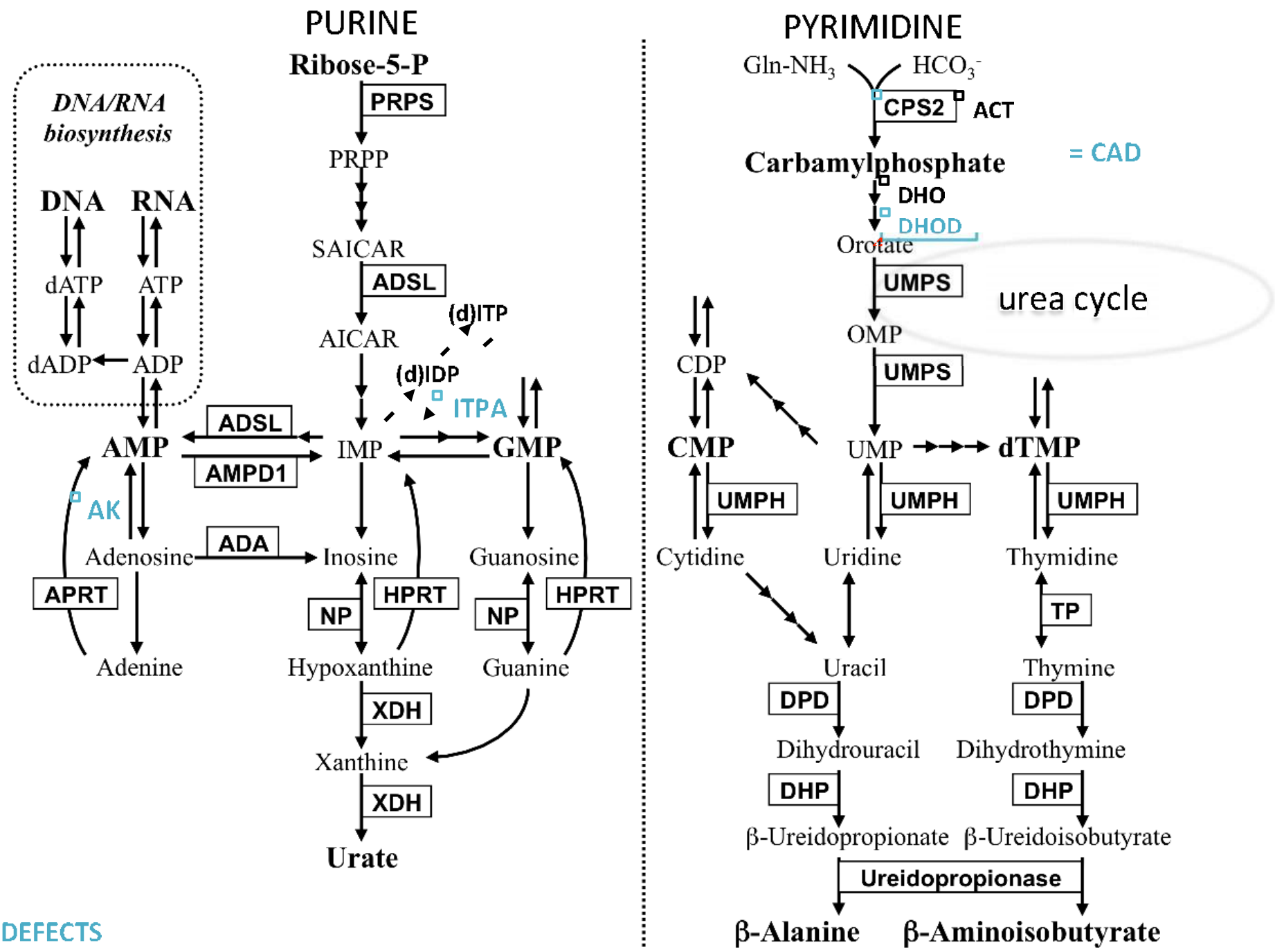
- small for gestational age
- presented shortly after birth
- severe and progressive microcephaly
- seizures, in 2 patients the epilepsy became refractory
- failure to achieve developmental milestones
- 3 patients: cardiac involvement (dilated cardiomyopathy or electrocardiographic abnormalities)
- early death

ITPA encephalopathy MRI pattern



- T2 signal abnormalities and diffusion restriction
  - in the posterior limb of the internal capsule
  - often also optic radiation
  - brainstem tracts
  - acerebellar white matter,

- delayed myelination and
- progressive brain atrophy.



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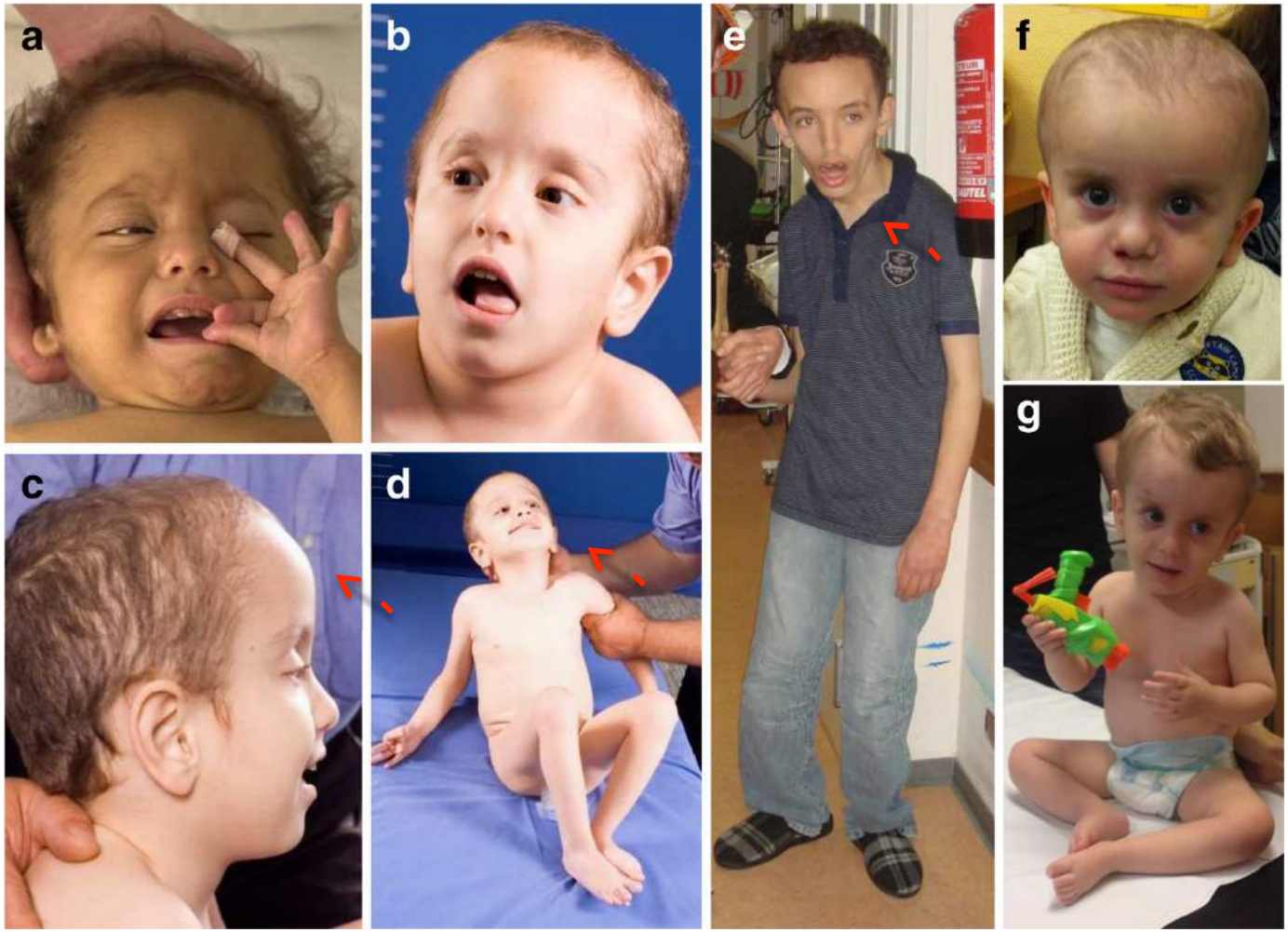
Adenosine Kinase Deficiency (Staufner et al, JIMD 2015)

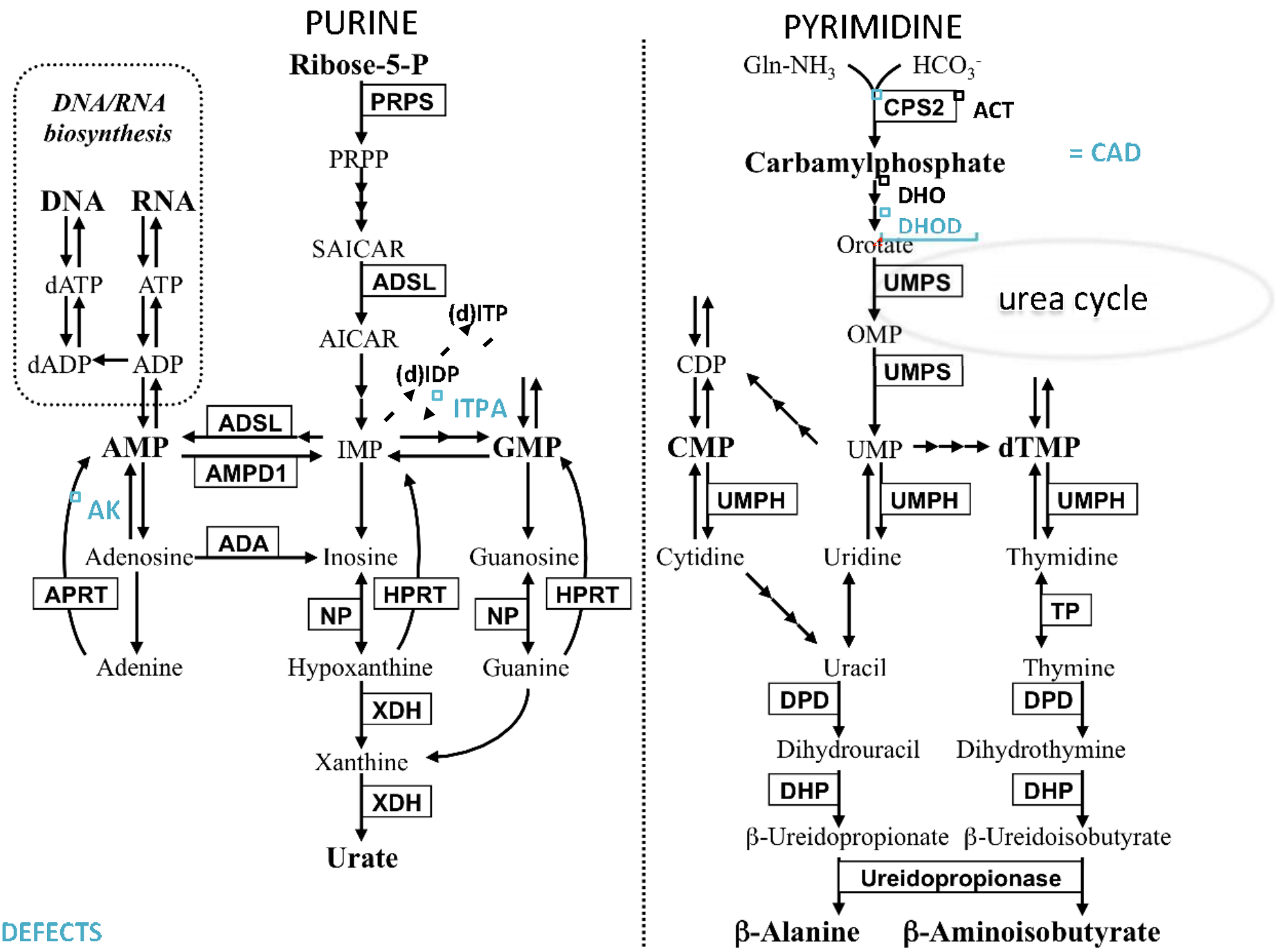
Fig. 2 Dysmorpholgical symptoms in ADK deficiency. a-d Patient 3. **a**) Age 1.2 years. Icterus is evident, frontal bossing may be noticed. Hair is sparse and of abnormal texture. **b-d**) Age 4.25 years. Facial expression of a mentally retarded girl, frontal bossing is pronounced. Severe muscular hypotonia of head, trunk and limbs. **e**) Patient 6, aged 22 years. He has short stature (151 cm / -4.5 SDS), macrocephaly (3.9 SDS) and has a long, trigonal face with frontal bossing. Teeth are distorted. Slender hands and fingers can be noted. f, g Patient 11. **f**) Age 14 months. Frontal bossing is prominent. **g**) Age 2.3 years. Relative macrocephaly, frontal bossing and muscular hypotonia



Note: frontal bossing and muscular hypotonia

Adenosine Kinase Deficiency (Staufner et al, JIMD 2015)

- Dysmorphic features (frontal bosssing, hypertelorism)
- Developmental Delay (DD)/Intellectual Disability (ID)
- Muscular Hypotonia
- Epilepsy
- Liver dysfx: Neonatal hyerbilirubinemia, elevated transaminases, liver fibrosis
- Hyperinsulinism, Hypoglycemia
- Failure to thrive (FTT)



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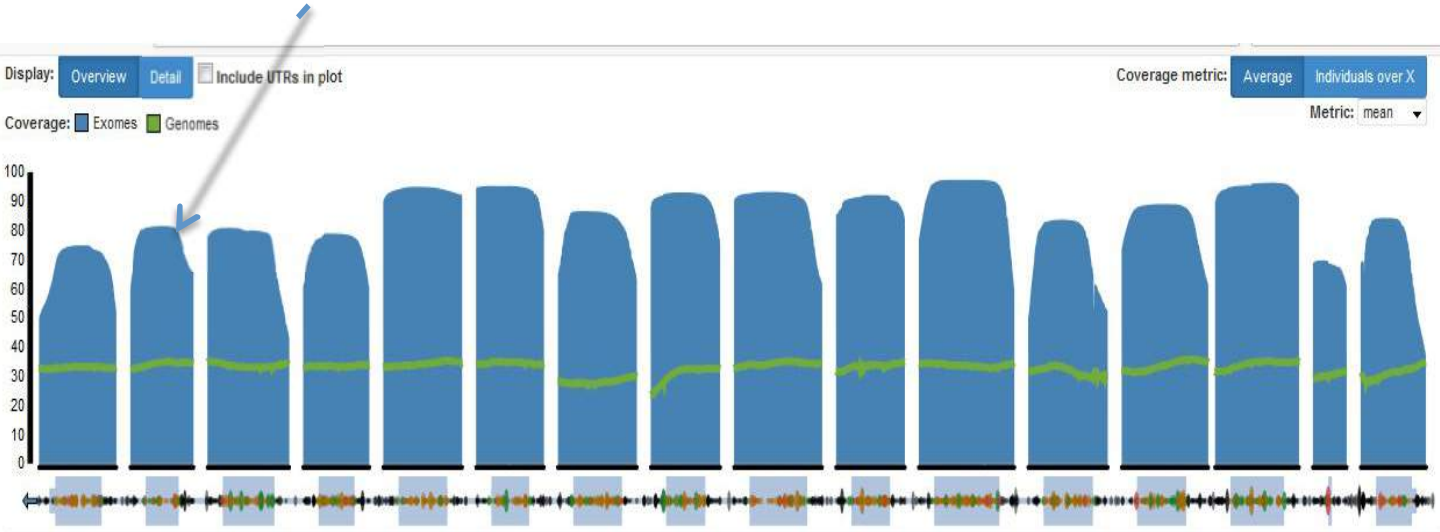
AMPD1 – non disease?

- Clin Neuropathol. 2005 Mar-Apr;24(2):77-85. Fischer S et al. Clinical significance and neuropathology of primary MADD in C34-T and G468-T mutations of the AMPD1 gene.

- **OBJECTIVE:**
- Primary myoadenylate deaminase deficiency (MADD) is probably the most frequent inborn metabolic myopathy with a prevalence of up to 2%. It is the result of mutations in the AMPDI gene, the most common of which is a C34-T transition in exon 2. The importance of the more rare mutation G468-T in exon 5 is uncertain. Primary objective was to elucidate the clinical significance of the enzyme disorder, which remains unclear since its first description in 1978. We further examined the existence of an association of MADD with other muscle disorders, such as malignant hyperthermia and rhabdomyolysis, as was suspected in earlier studies.
- **MATERIAL AND METHODS:**
- In a large collection of 1673 muscle biopsies that had been stored deep frozen we identified 33 cases of primary MADD, 12 of which without any other coinciding muscle diseases, by histochemical, biochemical and molecular genetic examinations. Clinical and laboratory data was collected. By additional examination of randomly chosen blood samples we identified one person carrying the rare compound heterozygosity C34-T/ G468-T, who was examined in clinical respects and a muscle biopsy was taken.
- **RESULTS:**
- As underlying mutation, the most common transition C34-T/C 143-T was detected in 33 cases. One patient carried the compound heterozygosity C34-T/G468-T. The overall frequency of MADD in the contingent was 1.8%. Only three patients out of 12 with isolated primary MADD suffered from muscle complaints, one of whom did not experience the typical symptoms of exercise related myalgia, muscle cramps and weakness as described by Fishbein. The patient carrying C34-T/ G468-T was a fully healthy female. She had never experienced any muscle complaints. Any association with other neuromuscular disorders, if not completely ruled out, was found to be very unlikely.
- **CONCLUSION:**
- The results suggest that MADD itself is unlikely to be solely responsible for the manifestation of muscular symptoms. It is probable that either the loss of a compensation mechanism or coexistent disturbances in muscle metabolism which are unidentified so far are required for the emergence of complaints.



Save coverage plot | Save exon image

All | Missense + LoF | LoF

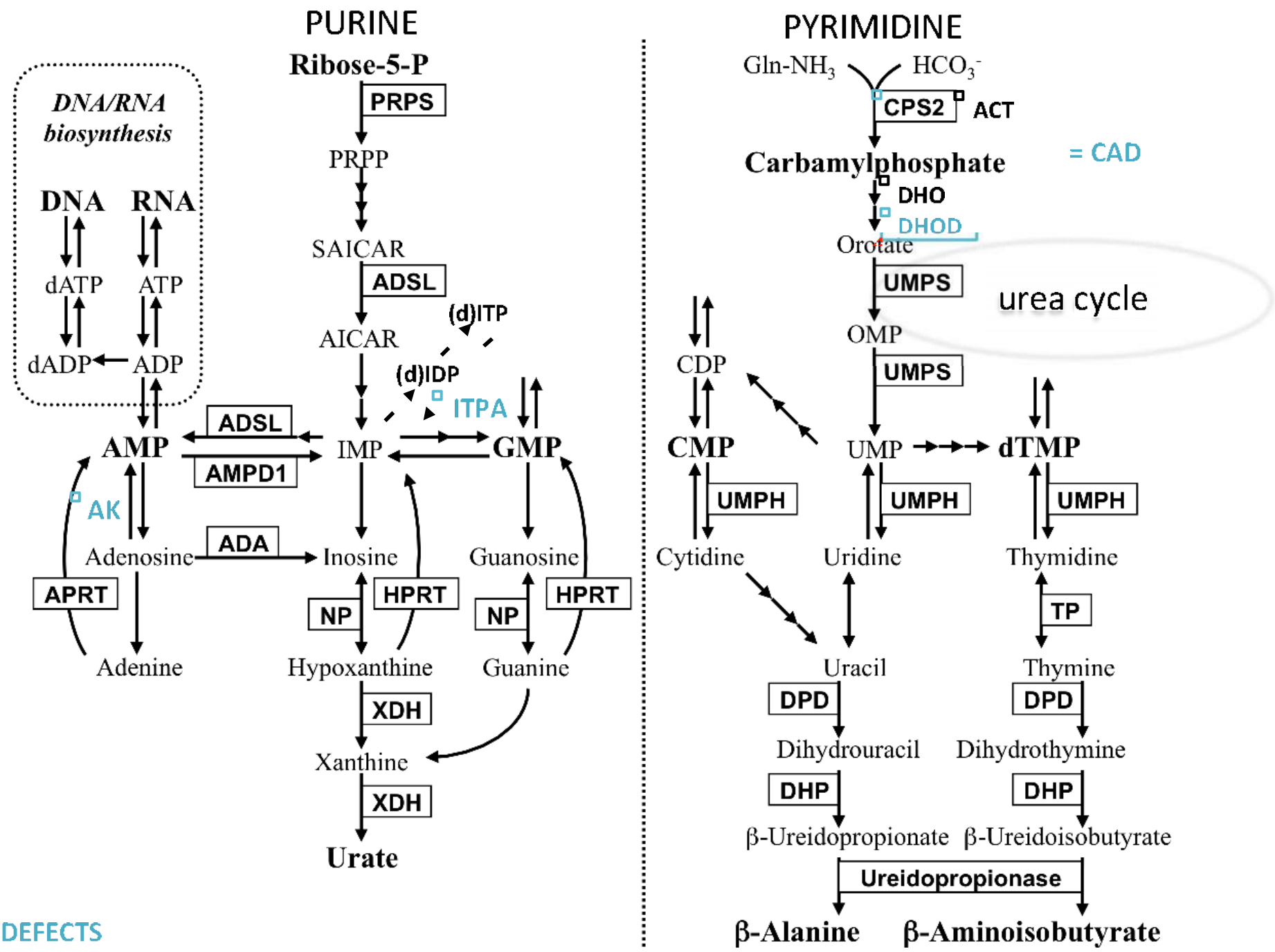
Include:

Exomes | SNPs | Filtered (non-PASS) variants
Genomes | Indels

Export table to CSV

† denotes a consequence that is for a non-canonical transcript

Variant	Source	Consequence	Annotation	Flags	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
1:115238016 CA / C (rs200909380)			intron	LCR	16528	22236	5979	0.7433
1:115231254 G / A (rs61752479)	E	p.Pro81Leu	missense		24588	277152	1514	0.08872
1:115236057 G / A (rs17602729)	E	p.Gln45Ter	stop gained		24150	276826	1458	0.08724
1:115220523 C / T (rs140176911)	E	c.1323+8G>A	splice region		5233	276980	332	0.01889
1:115222237 T / A (rs34526199)	E	p.Lys320Ile	missense		8102	277018	182	0.02925
1:115222169 G / C (rs115883987)	E		intron		8077	276898	179	0.02917
1:115238016 C / CA (rs200909380)			intron	LCR	2274	22236	147	0.1023
1:115231172 C / T (rs6683173)	E		intron		2752	277056	138	0.009933
1:115223156 A / T (rs148919817)			intron		2186	30962	90	0.07060
1:115218549 C / T (rs80266556)	E	p.Glu521Glu	synonymous		2065	277050	34	0.007454
1:115216393 A / T (rs148592763)	E		intron		2363	276520	25	0.008545
1:115229666 C / A (rs79168230)	E		intron		1522	171416	18	0.008879
1:115229523 C / T (rs61738827)	E	p.Arg108His	missense		1310	277188	12	0.004726
1:115217522 G / A (rs192311271)	E		intron		1394	276878	12	0.005035



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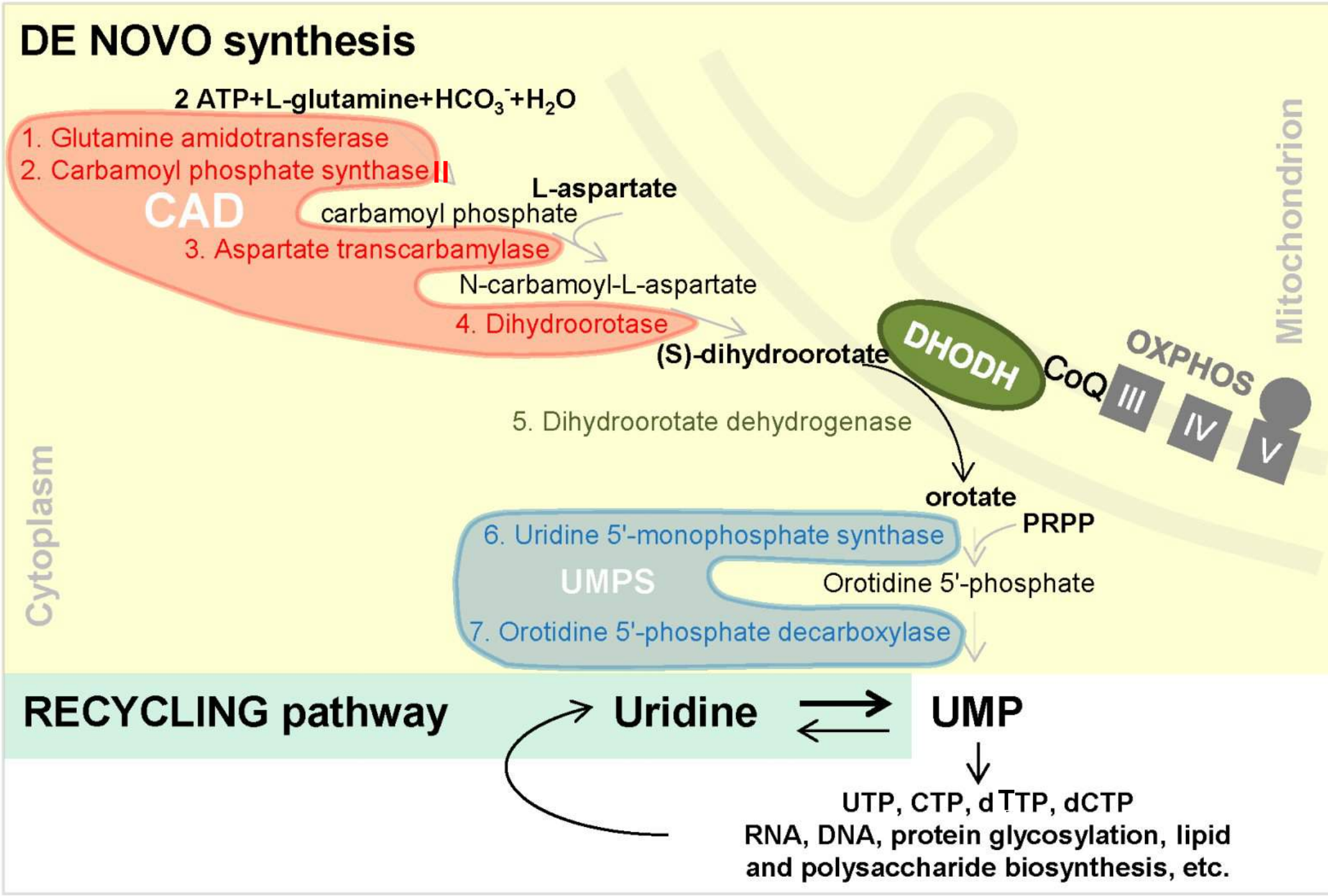
Dihydropyrimidine Dehydrogenase Deficiency: Metabolic Disease or Biochemical Phenotype?

Fleger et al, JIMD reports 2017

- Dihydropyrimidine dehydrogenase (DPD) deficiency is an autosomal recessive disorder of pyrimidine metabolism that impairs the first step of uracil und thymine degradation. The spectrum of clinical presentations in subjects with the full biochemical phenotype of DPD deficiency ranges from asymptomatic individuals to severely affected patients suffering from seizures, microcephaly, muscular hypotonia, developmental delay and eye abnormalities.
- We report on a boy with intellectual disability, significant impairment of speech development, highly active epileptiform discharges on EEG, microcephaly and impaired gross-motor development. This clinical presentation triggered metabolic workup that demonstrated the biochemical phenotype of DPD deficiency, which was confirmed by enzymatic and molecular genetic studies. The patient proved to be homozygous for a novel c.2059-22T>G mutation which resulted in an in-frame insertion of 21 base pairs (c.2059-21_c.2059-1) of intron 16 of DPYD. Family investigation showed that the asymptomatic father was also homozygous for the same mutation and enzymatic and biochemical findings were similar to his severely affected son. When the child deteriorated clinically, exome sequencing was initiated under the hypothesis that DPD deficiency did not explain the phenotype completely. A deletion of the maternal allele on chromosome 15q11.2-13-1 was identified allowing the diagnosis of Angelman syndrome (AS). This diagnosis explains the patient's clinical presentation sufficiently; the influence of DPD deficiency on the phenotype, however, remains uncertain.


DE NOVO synthesis
2 ATP+L-glutamine+HCO3-+H2O
1. Glutamine amidotransferase
2. Carbamoyl phosphate synthase II
CAD
L-aspartate
carbamoyl phosphate
3. Aspartate transcarbamylase
N-carbamoyl-L-aspartate
4. Dihydroorotase
(S)-dihydroorotate
DHODH
CoQ
OXPHOS
III
IV
V
Mitochondrion
5. Dihydroorotate dehydrogenase
orotate
PRPP
6. Uridine 5'-monophosphate synthase
UMPS
Orotidine 5'-phosphate
7. Orotidine 5'-phosphate decarboxylase
Cytoplasm
RECYCLING pathway
Uridine
UMP
UTP, CTP, dTTP, dCTP
RNA, DNA, protein glycosylation, lipid
and polysaccharide biosynthesis, etc.

DD of orotic aciduria

- urea cycle (esp. ornithine transcarbamylase deficiency)
- pyrimidine metabolism
  - uridine monophosphate synthase (UMPS) deficiency
  - phosphoribsyl pyrophosphatase synthetase (PRPS) superactivity
- mitochondrial disorders
- lysinuric protein intolerance (LPI)
- liver disease
- Rett syndrome, malignancies
- side effect of certain medications

Three patients with orotic aciduria I

- DK:
  - 17 y, male, autism spectrum disorder, diabetes mellitus I (DMI), acute liverfailure > PICU
  - highly elevated transaminases, initially high then normal NH3, lactate 7-9 mmol/l
  - hepatomegaly, increased echogenicity on US abdomen

  = Mauriac disease (secondary glycogenosis in poorly controlled DMI)
  - urinary orotic acid 11 (RR <4) umol/mmol creat.
  - 2nd disorder? OTC-deficiency? mitochondrial disease?

Three patients with orotic aciduria II/III

- LV
  - one year, female, developmental delay, hyperlaxicity;
  - catch up development, near normal at five years
  - urinary orotic acid 16, 7, 8, 15, 16, 15, 13,
    after allopourinol loading 44, 134, 182, 143 (RR <4) umol/mmol creat.
  - Deletion chr2p, also in mother with no relevant medical complaints

- MvH
  - one year, female, developmental delay
  - generalized muscular hypotonia, joint hypermobility, and weakness of the ocular, oral and limb girdle musculature
  - Muscle biopsy: fibre type dysproportion
  - DD congenital myopathy
  - urinary orotic acid 16,14,23 (RR <4) umol/mmol creat.

NGS

- DK
  - No disesae causing variants in genes known to be related to disease, no candidate genes.
  - *UMPS* heterozygous c.889G>T, (p.Glu297*) > premature stop

- LV
  - Known pathogenic mutation associated with late onset limb girdle myopathy
  - *UMPS* heterozygous c.688C>T, (p.Arg230Cys), not listed in ExaC, PP2: Probably damaging, SIFT: deleterious, mutation taster: Disease causing, conserved „down“ to C. elegans

- MvH
  - No disesae causing variants in genes known to be related to disease, no candidate genes.
  - *UMPS* heterozygous c.889G>T, (p.Glu297*) > premature stop

Table 1 Clinical and metabolic findings and *UMPS* carrier status of all individuals

	UMPS mutation	Clinical signs and symptoms	Urinary orotic acid (umol/mmol creatinine)	Max. factor of urinary orotic acid elevation	Orotate/ orotidine ratio
I1	+	Neonatal encephalitis, feeding difficulties, mild LD	30, 31 (RR<4.9)	6.3	n/a
M1, F1	–	NRMC	n/a		n/a
Ped2	+	12 x NRMC	22–43.1 (RR 0.05–3)	14.3	n/a
Ped2	–	13 x NRMC, 1x ID/DD, infantile seizures	<0.2–3 (RR 0.05–3)		
I3	+	ID	10.5 (RR <1.5 +/−0.4)	5.3	n/a
M3	+	n/a	38 (RR 4.9 +/−1.8)	5.7	n/a
I4	+	DD, myoclonic seizures	17.3 (RR 1–3.2)	5.4	13.5
I5	+	Mild LD	15.5–49 (RR <1.5)	6 (after allopurinol 32.3)	5–15.2
M5	+	NRMC	7.3 (RR <1.5)	5	n/a
F5	–	NRMC	Unmeasurably low		n/a
I6	+	FTT, speech delay	34–53 (RR <4.9)	10.8	6.4
M6	+	mild ID	13.8 (RR <4.9)	2.8	n/a
S6	–	NRMC	<4.9 (RR <4.9)		
I7	+	DD, dysmorphic features, hypotonia	40 (RR <8)	5	3.36
F7	–	NRMC	n/a		n/a
M7	+	NRMC	n/a		n/a
S7	+	NRMC	7.8 (RR <3.4)	2.32	n/a
I8	+	DD, joint hypermobility	7–16, after allopurinol 44–182 (RR <4)	4 (after allopurinol 45)	1.6–7.3
M8	+	NRMC	4 (RR <4)	–	5
I9	+	DD	18–35 (RR <7)	5	n/a
I10	+	DD, hypotonia, joint hypermobility, exercise intolerance	14–23 (RR <4)	5.75	5.4–8.5
M10	+	NRMC	19 (RR <4)	4.75	n/a
F10	–	NRMC	<4 (RR <4)	–	n/a
I11	+	diabetes mellitus I, autism, Mauriac syndrome	11 (RR <4)	2.75	8.5
M11	–	NR			
F11	+	NR			
I12	+	NR			
M12	n/a	NR			
F12	n/a	NRMC	<5 (RR <5)		n/a

30 individuals with heterozygous mutations in *UMPS*

11 individuals with heterogenous signs and symptoms

19 healthy family members with no relevant medical complaints

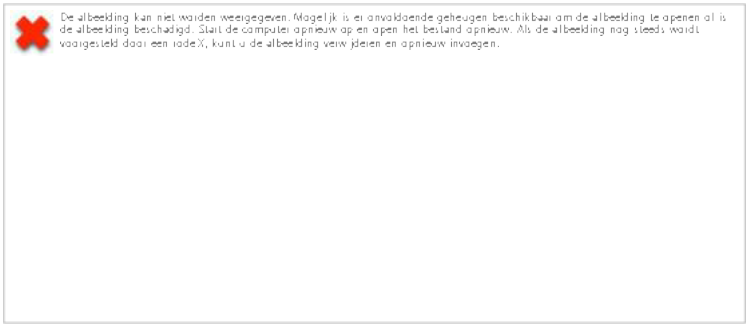
DD developmental delay, *F* father of, *FTT* failure to thrive, *I* individual, *ID* intellectual disability, *LD* learning disability, *M* mother of, *n/a* not available, *NRMC* no relevant medical complaints, *Ped* pedigree, *RR* reference range, *S* sibling of

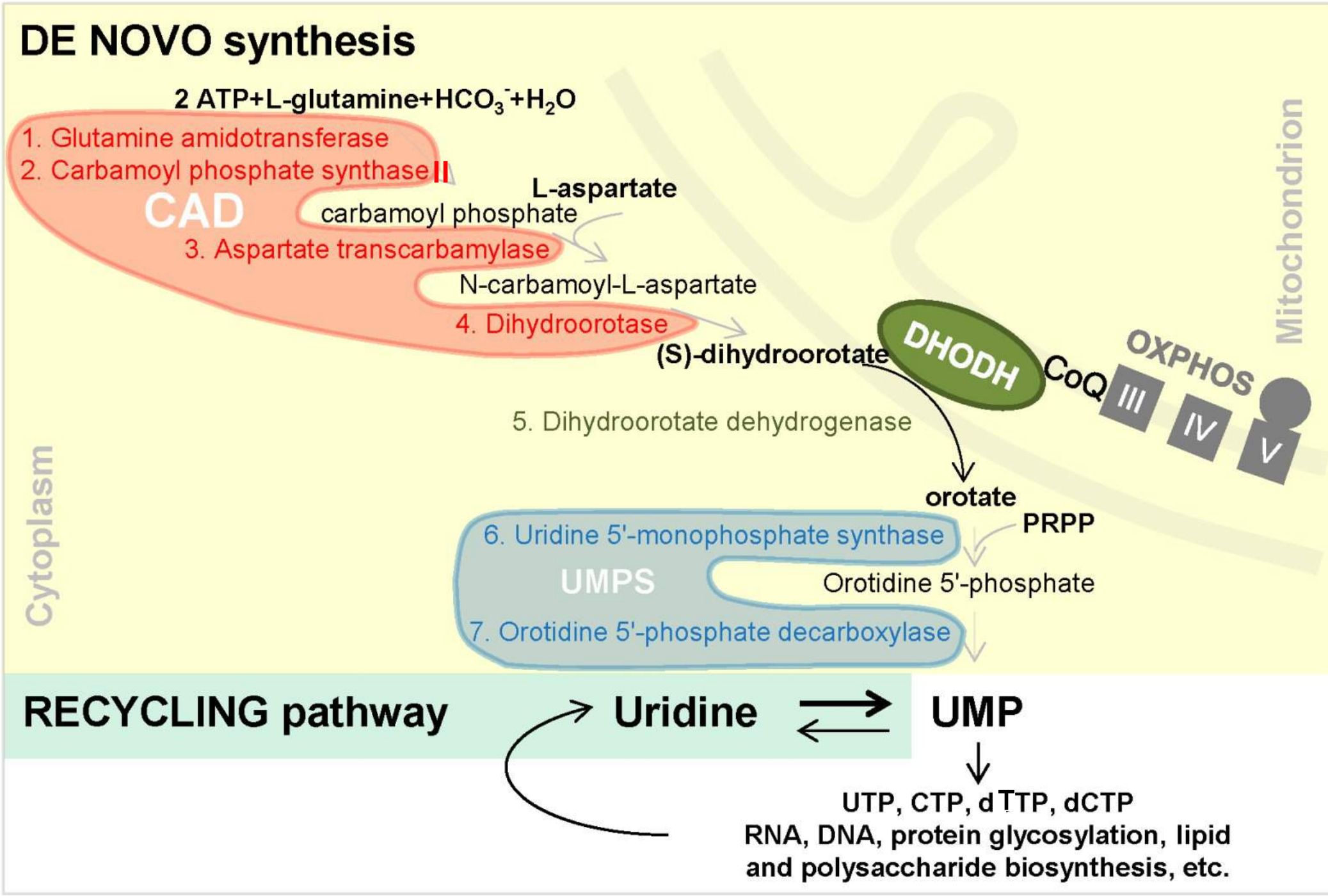
*in pedigree 2 26 family members were tested (see Fig. 1 for individual values)

Summary and conclusions

- 30 individuals with heterozygous mutations in *UMPS*
  - *11* individuals with heterogenous signs and symptoms
  - 19 healthy family members with no relevant medical complaints
  - mild orotic aciduria (max. 2.5 -11 fold)
  - no hyper NH3, no blood count abnormalities, no growth delay

- Benign metabolic disturbance
- Important DD for mild orotic aciduria




DE NOVO synthesis
2 ATP+L-glutamine+HCO3-+H2O
1. Glutamine amidotransferase
2. Carbamoyl phosphate synthase II
CAD
L-aspartate
carbamoyl phosphate
3. Aspartate transcarbamylase
N-carbamoyl-L-aspartate
4. Dihydroorotase
(S)-dihydroorotate
DHODH
CoQ
OXPHOS
III
IV
V
Mitochondrion
5. Dihydroorotate dehydrogenase
orotate
PRPP
6. Uridine 5'-monophosphate synthase
UMPS
Orotidine 5'-phosphate
7. Orotidine 5'-phosphate decarboxylase
Cytoplasm
RECYCLING pathway
Uridine
UMP
UTP, CTP, dTTP, dCTP
RNA, DNA, protein glycosylation, lipid
and polysaccharide biosynthesis, etc.