Amino Acids, Amino Acidopathies and the Urea Cycle Disorders

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Some slides adapted from:

Thank you, Jean Marie Saudubrey, Mark Korson,
Jerry Vockley and many others

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How to recognise different types of trees from quite a long way away.

No. 1 The Larch.

Amino acid metabolism

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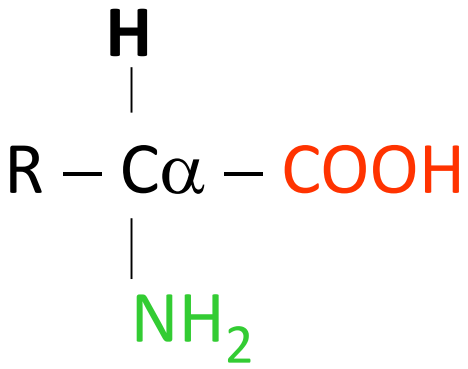
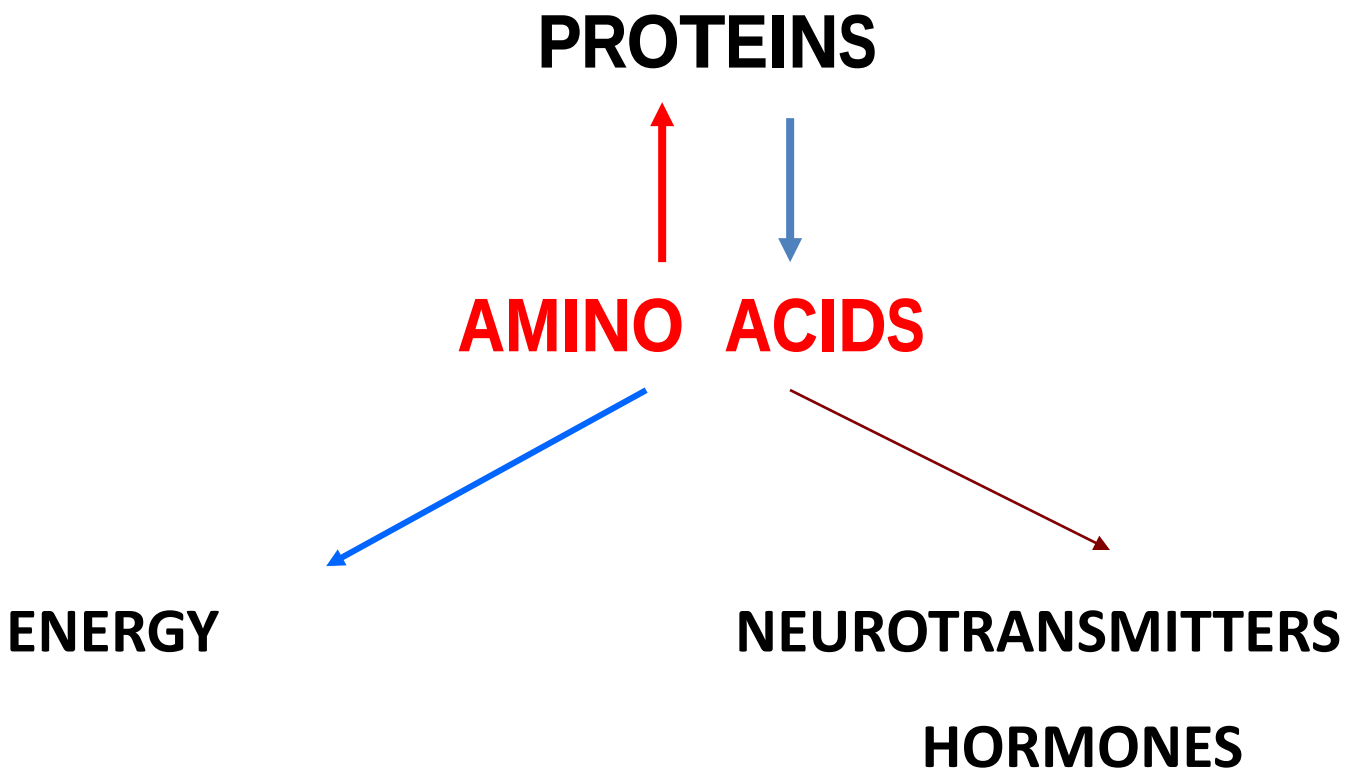


- Lateral chain R:
  - Carboxyl: Asp (β), Glu (γ)
  - Amine: Lys (ε), Orn (δ)
  - Hydroxyl: Thr, Ser, Tyr
  - Imidazole: His
  - Guanidinium: Arg
  - Thiol: Cys, Hcy



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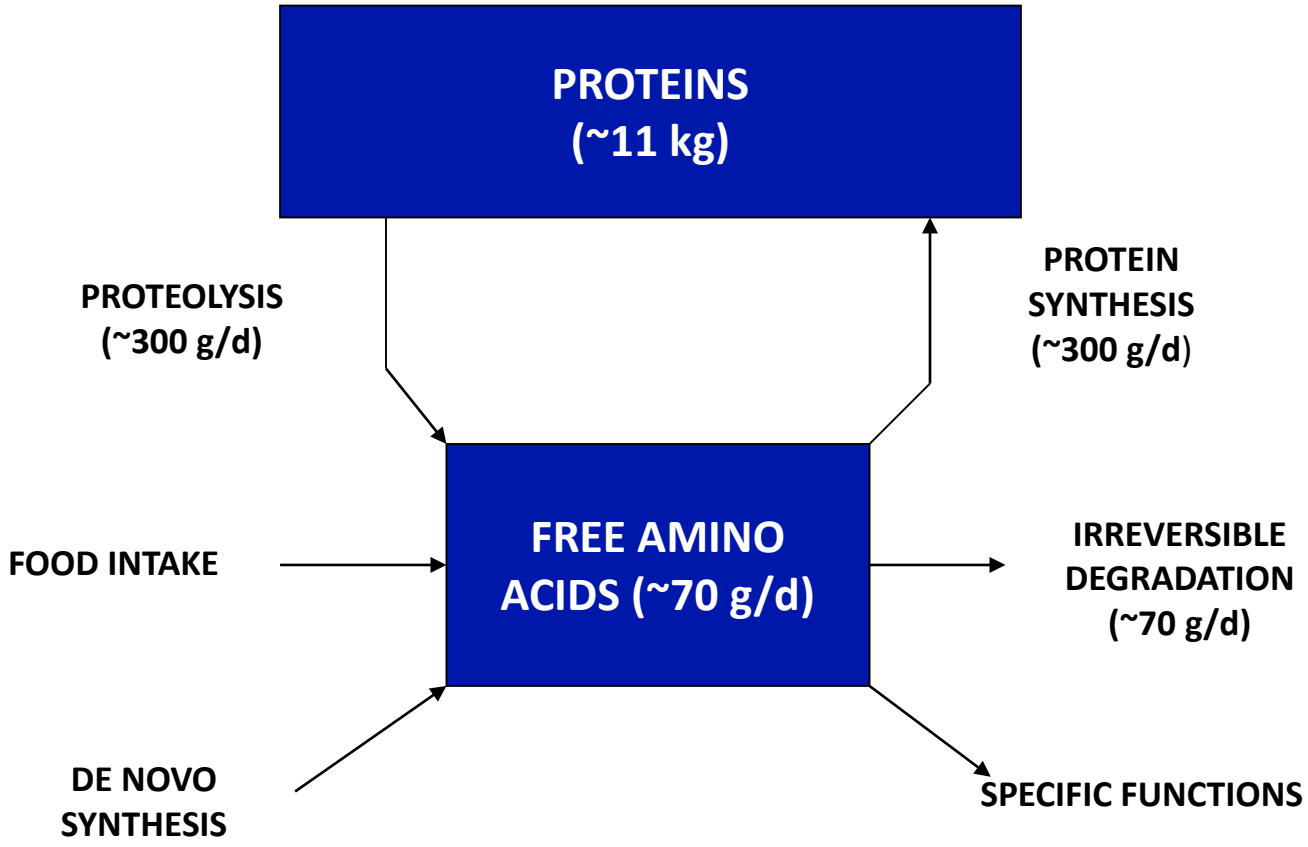
Protein metabolism (Adult 70 kg)



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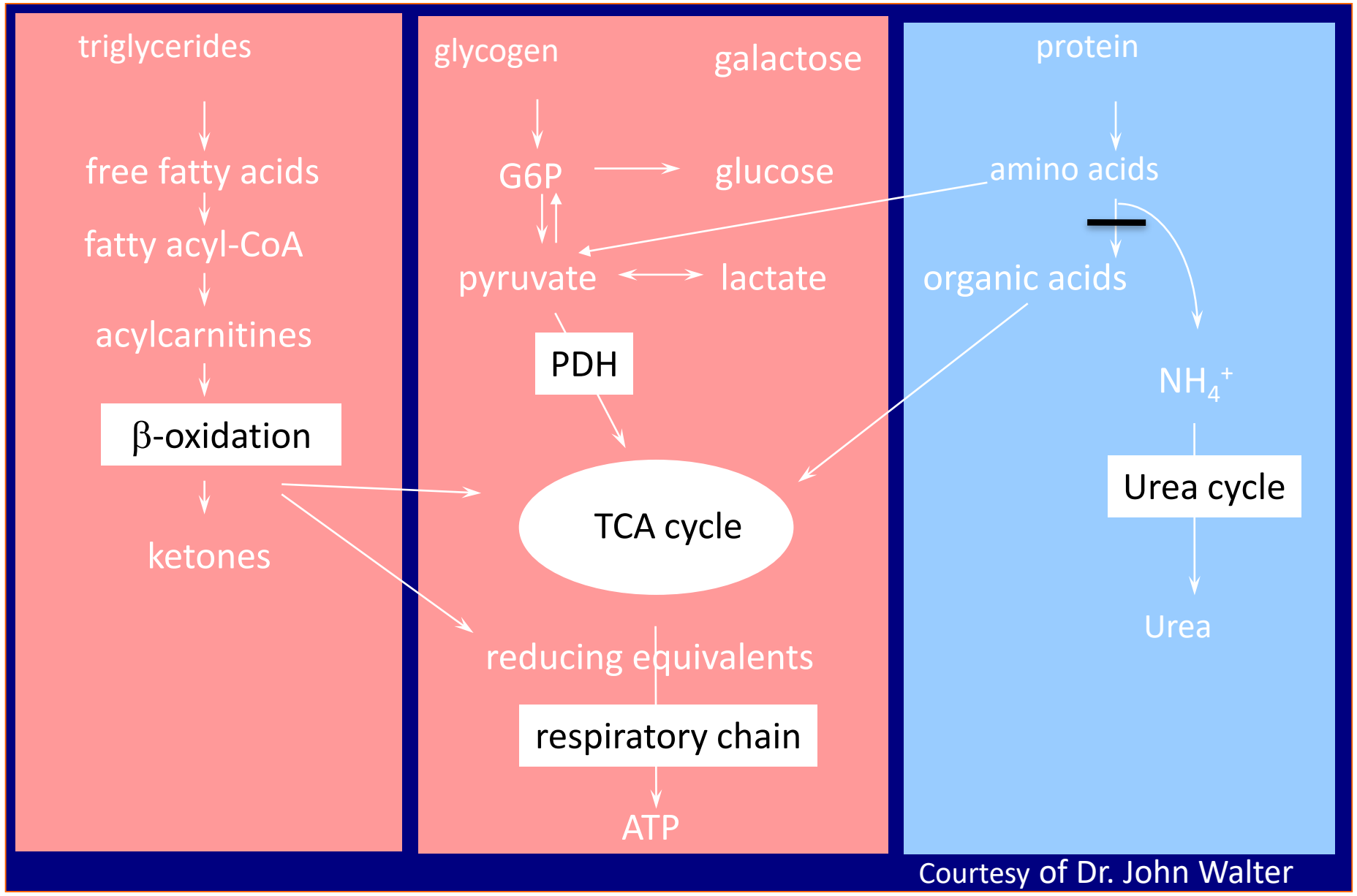

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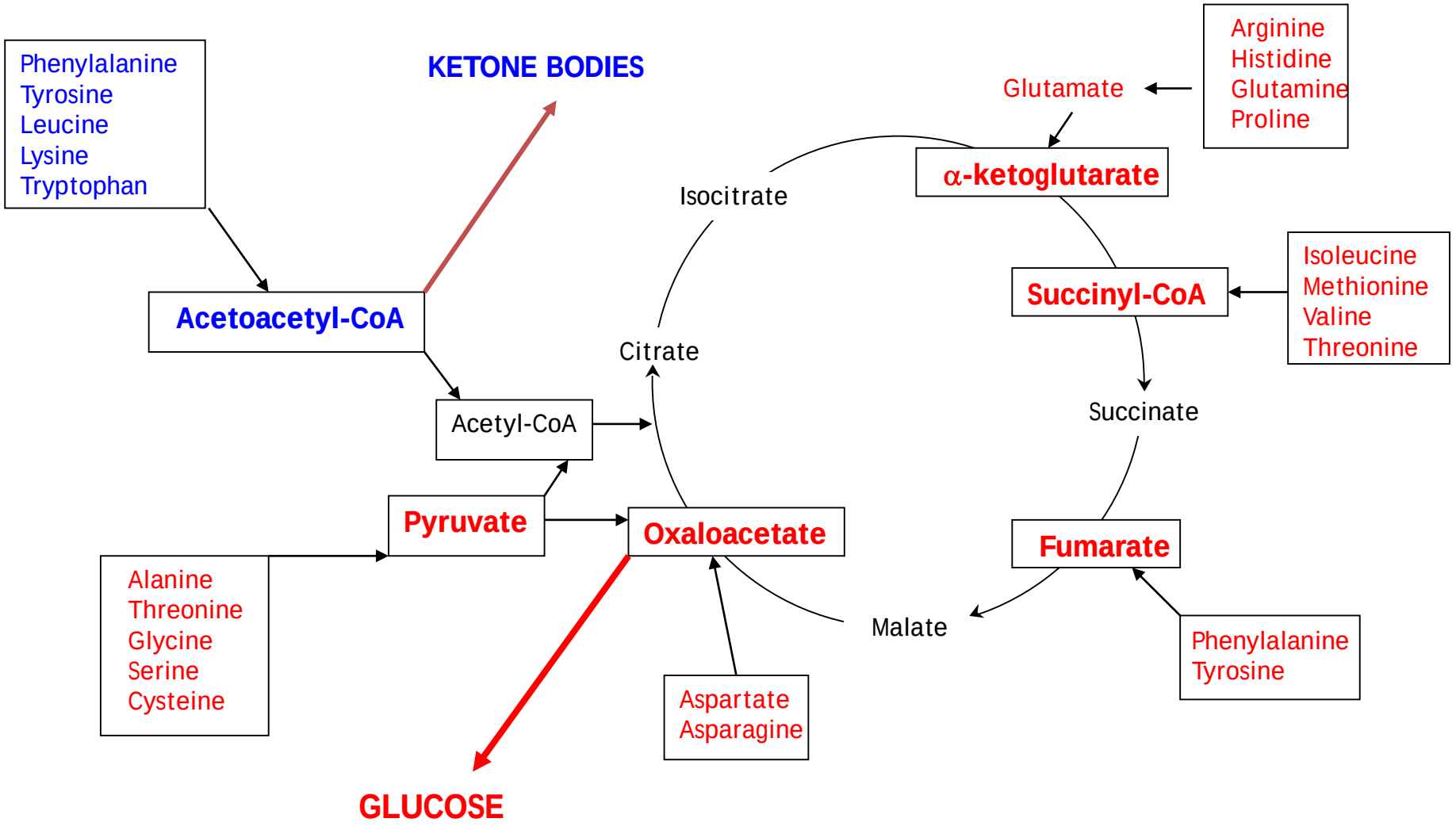
Irreversible degradation



KETOGENIC and **GLUCONEOGENIC** amino acids

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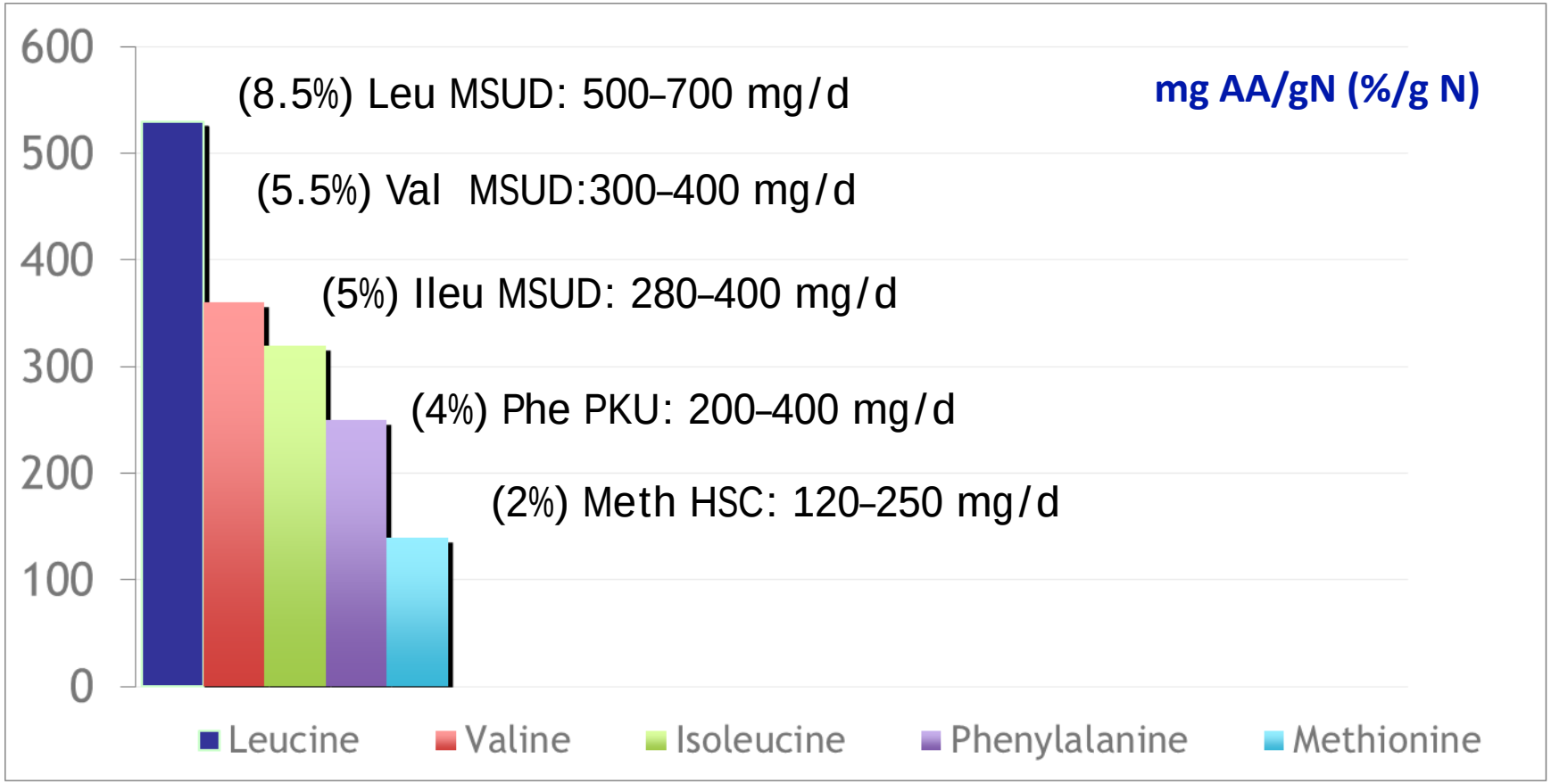
Muscle amino acids

1 g N = 6.25 g protein = 30 g muscle



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De novo amino acid synthesis

- Essential and non-essential amino acids
- Essential AA: Inborn errors of AA catabolism
  - Cannot be synthesized by humans
  - Must come from food
- Non-essential AA: Inborn errors of AA synthesis
  - Can be synthesized by humans
  - Carbon skeletal comes from glucose and other amino acids
  - Nitrogen comes from other amino acids

Amino acid classification

Essential	Non-essential
Threonine	Alanine
Valine	Asparagine
Isoleucine	Aspartate
Leucine	Cysteine
Methionine	Glutamate
(Cysteine)	Glutamine
Phenylalanine	Glycine
(Tyrosine)	Hydroxyproline
Lysine	Hydroxylysine
Tryptophan	Proline
Histidine	Serine
Arginine	Tyrosine

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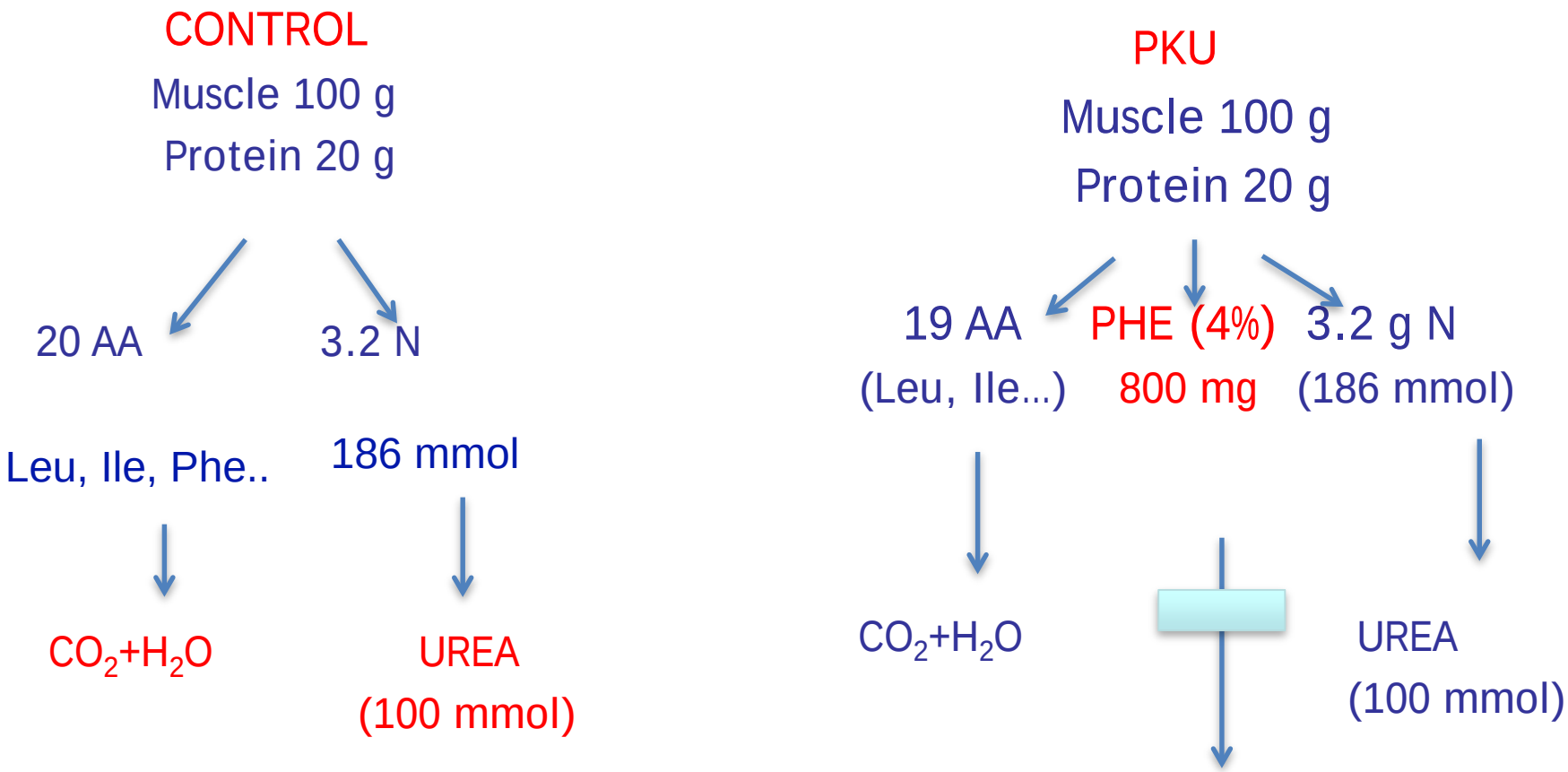
Protein catabolism

- Muscle protein content is 20 g%
- Nitrogen protein content is 16%
- 1 g nitrogen = 6.25 g protein = 30 g muscle
- Amino acid composition of proteins is genetically determined (doesn’t depend on the diet)
- In catabolic situations amino acids released from muscles are oxidized and nitrogen is converted to urea

Catabolism in control and PKU



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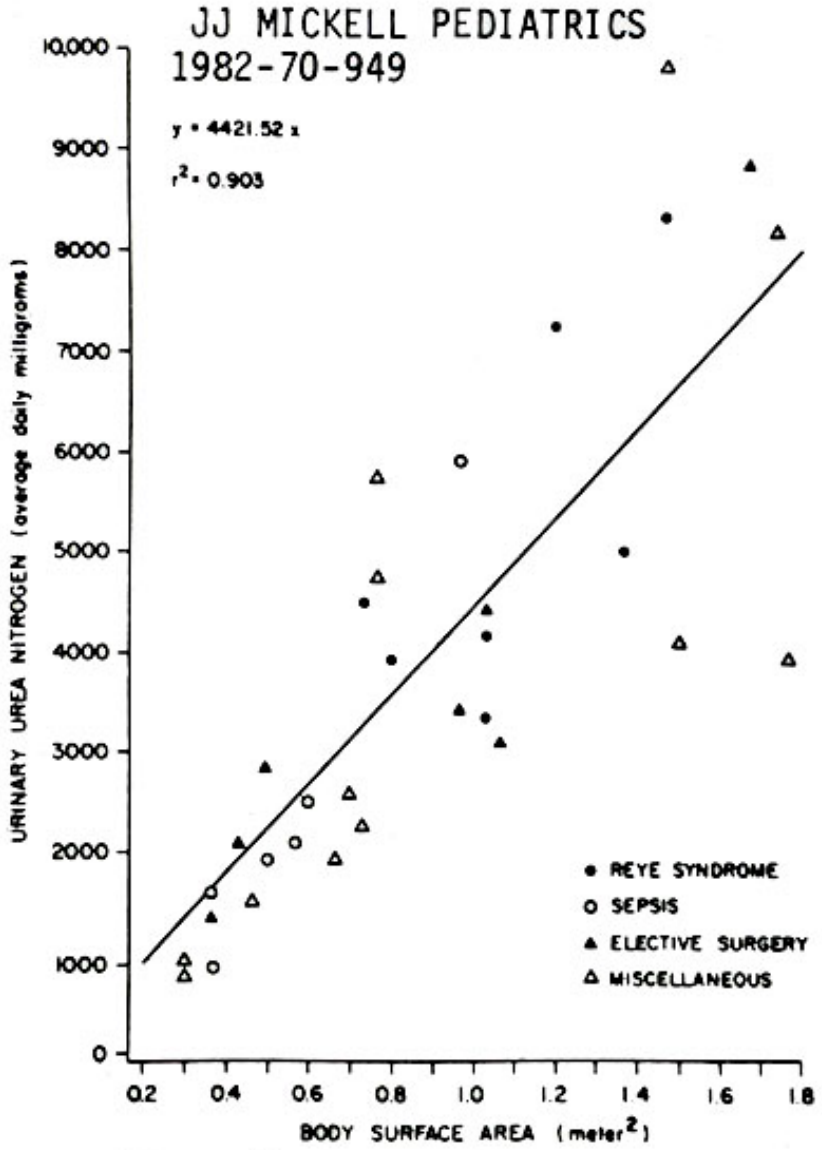
Nitrogen excretion



- Relationship between urinary urea nitrogen excretion and body surface area

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Food intake

- Feeding → exogenous proteins
  - Digestion → free amino acids and peptides (di- and tri-)
  - Essential and non-essential amino acids
  - Allows endogenous protein synthesis
- Defective intake
  - Kwashiorkor: protein-only deficit
  - Marasmus: combined deficit of protein and calories

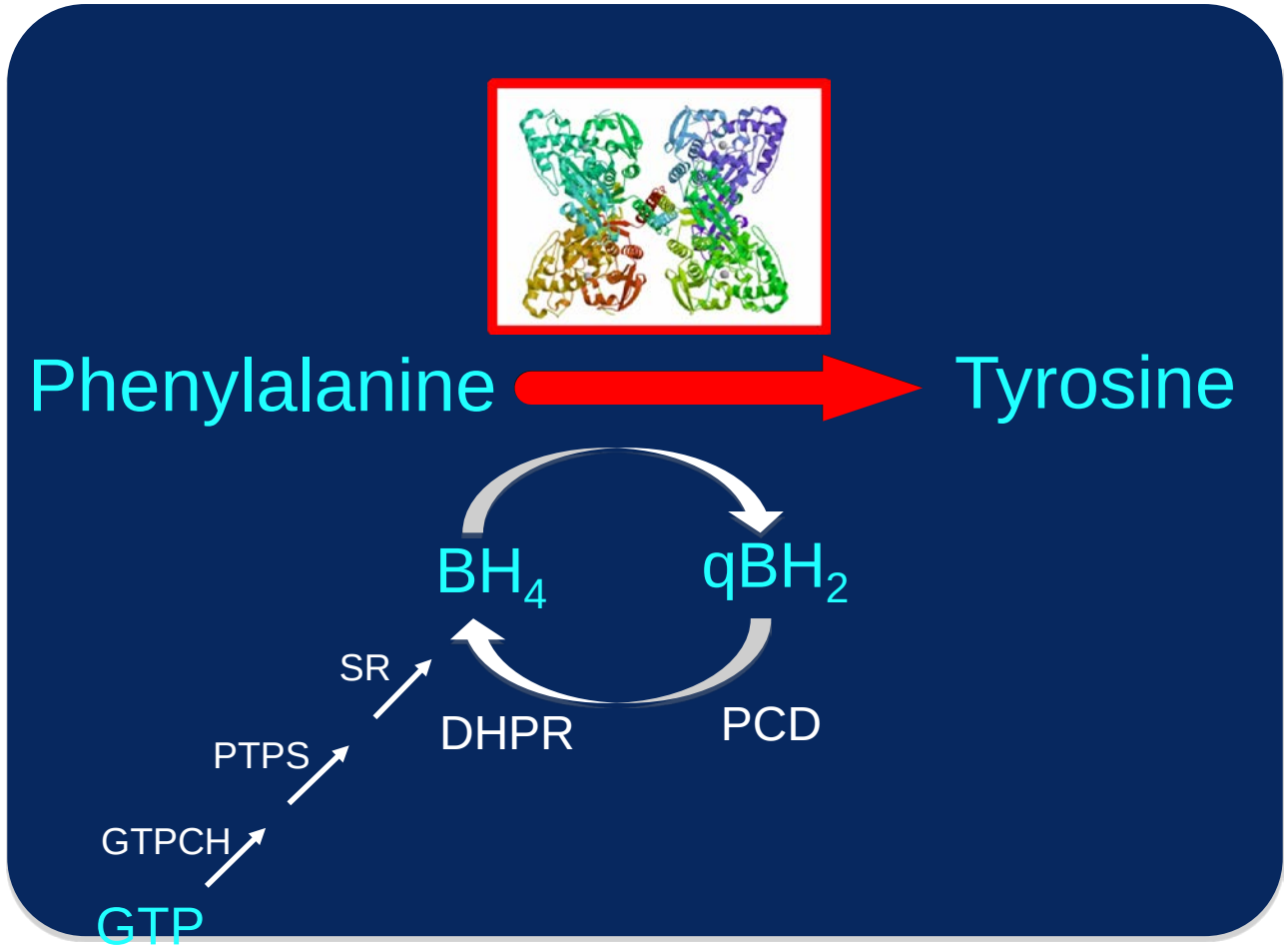
Amino acidopathies

Majority can be identified by newborn screening

Hyperphenylalaninemias

- "Classic" phenylketonuria
  - untreated phe >1200 μmol/L
- "mild PKU"
  - untreated phe 600-1200 μmol/L
- Hyperphenylalaninemia
  - untreated phe < 600 μmol/L when well

Phenylalanine hydroxylase (PAH)



BH_4 is also a cofactor for tyrosine hydroxylase (dopamine synthesis) and tryptophan hydroxylase (seratonin synthesis)

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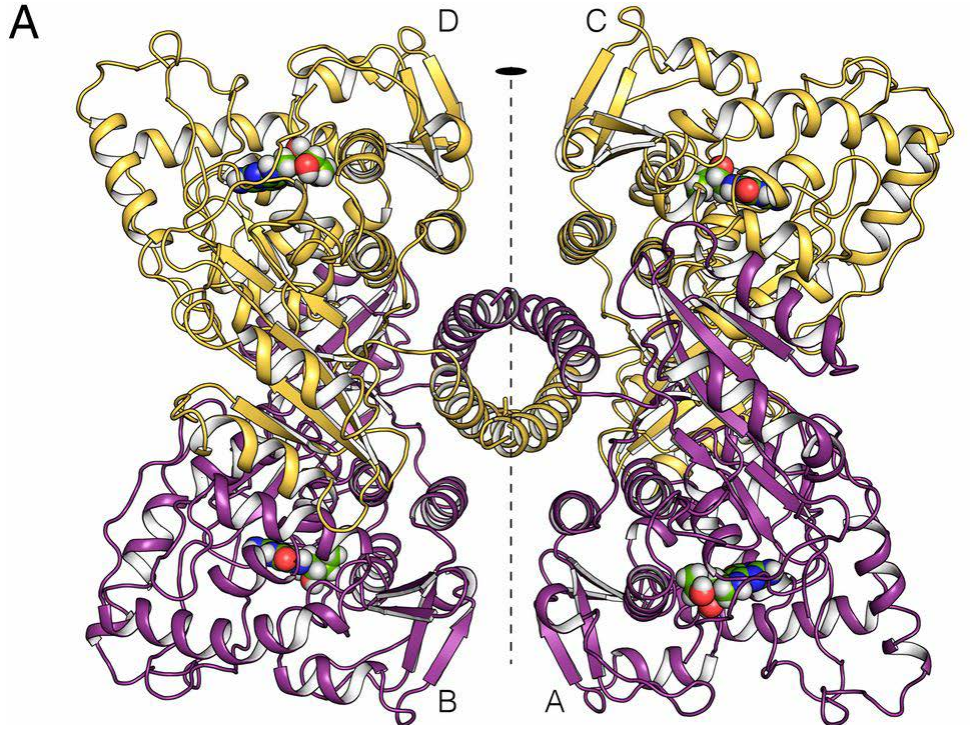
Phenylketonuria (PKU)

- Liver phenylalanine hydroxylase (PAH) deficiency
- Autosomal recessive inheritance
- Incidence ~1:16,000 live births in the US



PNAS v116 p11229 2019

- Homotetramer ("dimer of dimers")
- Allosteric activation
  - confirmation determines enzyme activity
  - Phe activates enzymatically favorable conformation
  - BH4 stablesizes tetramer, but supports lower activity confirmation

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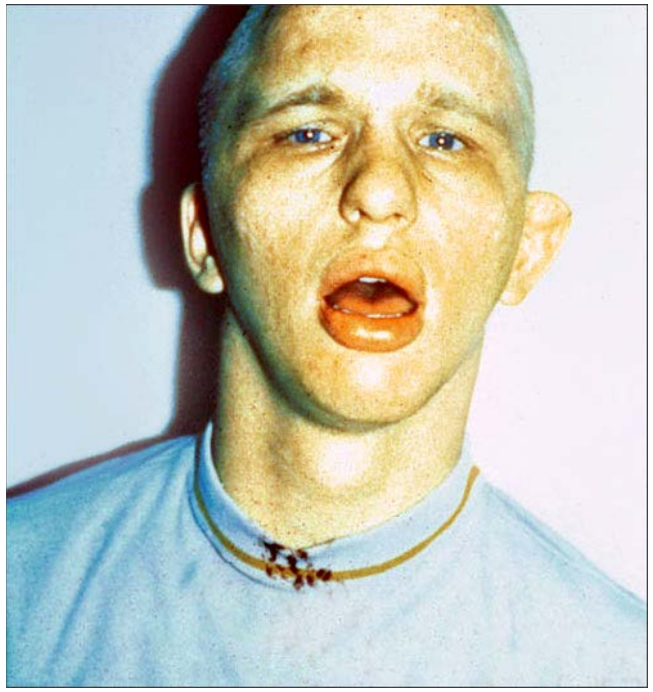
Other causes of hyperphe

- Rare variants of biopterin synthesis or recycling (about 1% of severe hyperphe)
  - GTP cyclohydrolase
  - Dihydropteridine reductase
  - 6-pyruvoyl-tetrahydropterin synthase
    - All 3 generally more difficult to treat, require BH4 and usually dopa
  - Pterin-alpha-carbinolamine dehydratase
    - Generally mild, excrete 7-biopterin
- Hyperphe, not BH4 deficient
  - *DNAJC12* – molecular chaperone for the hydroxylases – PAH, TH and TPH

Untreated PKU



- “Normal” development for 6–9 months, feeds well
- 9–12 months signs of slowing in developmental progress, head growth slows
- About 1 year clearly developmentally delayed, light hair, eczema, musty odor of “mouse urine”, may have seizures

- Severe intellectual disability with behavior problems — eventual institutionalization
- White matter hyperintensities – “pseudoleukodystrophy”

Pathophysiology

- Elevated total body phenylalanine
- Excessive phe in the brain
- Reduced large neutral amino acid transport into the brain (including tyrosine and tryptophan)
- Reduced synthesis of key neurotransmitters (e.g., dopamine, serotonin), especially during development
  - Mouse data suggests inhibition by CNS Phe of TH and TPH2 activity
- No direct pathologic effect on the liver known

Therapy

- Dietary phe reduction
- Competitive – large neutral amino acids supplements
- Chaperone therapy – sapropterin
- Enzyme substitution therapy – pegvaliase

Experimental

- Gut biome manipulation of absorption
- Gene correction or replacement therapy

Diet therapy

- Restrict dietary protein
- Phenylalanine intake: ~250-350 mg/day in classical form
  - Breastfeeding often manageable
- Supplement with phenylalanine-free medical food to guarantee the daily requirements
  - Non-offending amino acids
  - Glycomacropeptide – low phe casein product
  - Vitamins and minerals
  - Distribute through the day
- “Diet for life”

1 g dietary protein ≈ 50 mg phe

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Strategies for breastfeeding

- Alternate feedings
- Mix in a bottle (breast milk provides intact protein in a traditional formula recipe
- Bottle first with metabolic formula with each feed, followed by nursing (one breast for at least 10 min to access hindmilk)

Table 3
Guidelines for PHE, TYR, and protein intake for individuals with PKU.

AGE	PHE[a] (mg/day)	TYR[a] (mg/day)	Protein[b] (g/kg/day)
Infants to <4 years[a]			
0 to <3 months[c]	130–430	1100–1300	2.5–3.0
3 to <6 months	135–400	1400–2100	2.0–3.0
6 to <9 months	145–370	2500–3000	2.0–2.5
9 to <12 months	135–330	2500–3000	2.0–2.5
1 to <4 years[d]	200–320	2800–3500	1.5–2.1
After early childhood[e]			
>4 years to adult	200–1100	4000–6000	120–140% DRI for age[f]
Pregnancy and lactation[g]			
Trimester 1	265–770	6000–7600	≥70
Trimester 2	400–1650	6000–7600	≥70
Trimester 3	700–2275	6000–7600	≥70
Lactation[h]	700–2275	6000–7600	≥70

[a] Adapted from Acosta [118], recommendations for PHE and TYR intake for infants and children <4 years with more severe PKU and treated with PHE-restricted diet alone. TYR intake recommendations may require adjustment based on blood TYR monitoring.

Monitoring diet therapy

- Provide adequate calories
- Provide adequate protein, vitamins, minerals
- Maintain normal growth and development
- Monitor blood Phe and Tyr
- Monitor other parameters (development, psychological status, bone density)
  - Consider monitoring iron and Vitamin D from time to time

Other therapies

Goal to enhance phe tolerance and normalize diet

- Sapropterin
  - 20 mg/kg/day
  - Infant – 24 hour trial >30% reduction in phe (with stable or no diet treatment)
  - Older 48 hours to 30 days trial
  - May have gradual onset
  - Requires some protein to work (null alleles unaffected)

Some sapropterin responsive mutations

cDNA	Protein	Cases in PAHdb	Responsive to Sapropterin
c.1222C>T	p.Arg408Trp	6.7%	<10%
c.1066-11G>A (IVS10-11G>A)		5.3%	<10%
c.194T>C	p.Ile65Thr	4.1%	89%
c.782G>A	p.Arg261Gln	3.6%	78%
c.842C>T	p.Pro281Leu	2.9%	None [Leuders et al 2014, biopku.org]
c.1315+1G>A (IVS12+1G>A)		2.8%	12.5% [biopku.org] None [Leuders et al 2014]
c.473G>A	p.Arg158Gln	2.7%	<10%

Data obtained from: PAHdb accessed 5/8/2016 (biopku.org); and Leuders et al [2014]. All changes with >2.5% frequency in the PAHdb database were included. In database searches, homozygosity was assumed for calculations; however, this is a rare finding in consanguineous individuals. It is recommended that all affected individuals be tested for personal responsiveness. Genetic changes shown affect >2.5% of the database population. See biopku.org for the most up-to-date information and additional references.

Genereviews
https://www.ncbi.nlm.nih.gov/books/NBK1504/

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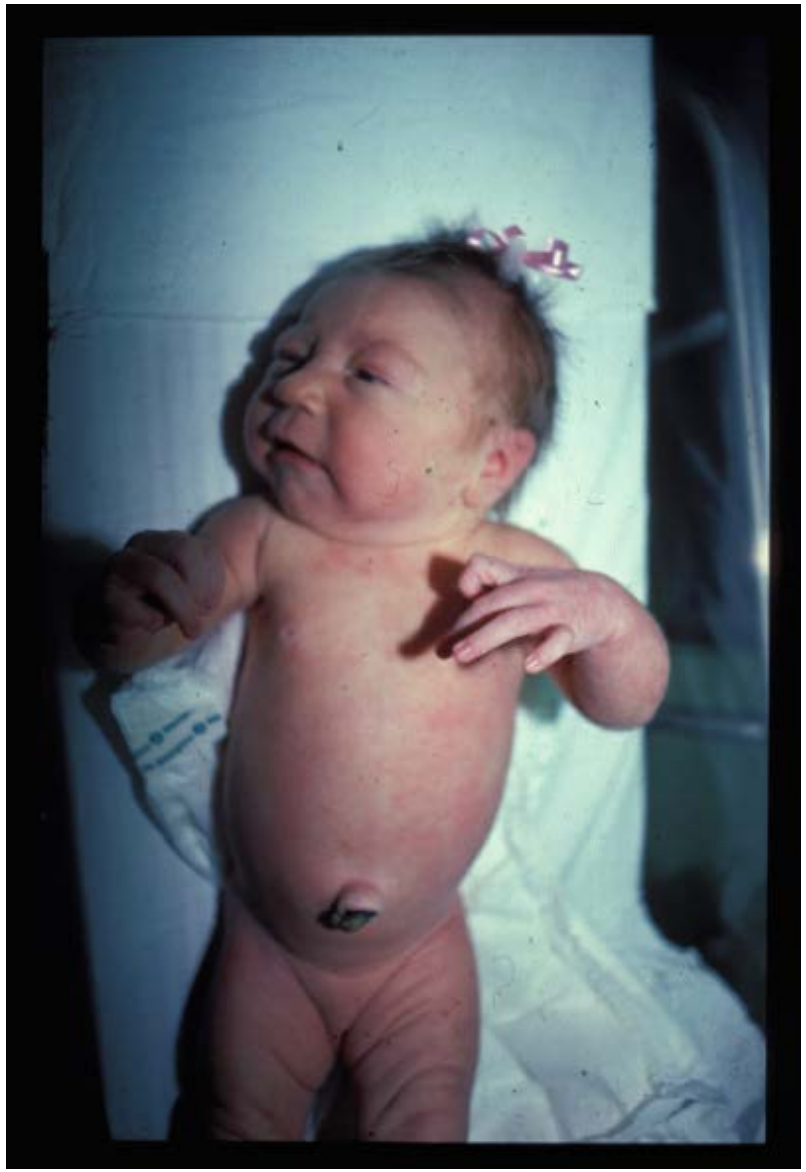
Other therapies

Goal to enhance phe tolerance and normalize diet

- Pegvaliase
  - Plant enzyme – phenylalanine ammonia lyase
  - Does not reduce need for tyrosine
  - Immunologic reactions must be managed
  - Titrate dose to keep phe in physiological range on normal diet
  - Not recommended during pregnancy
  - FDA approval for 16 years and up

“Maternal” PKU



- Phenylalanine teratogenicity
- microcephaly,
- congenital cardiac lesion
- Intellectual disability

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“Maternal” PKU Management

- Ideally start aggressive therapy before pregnancy
- Phe in target range as early as possible for unplanned pregnancy
- Often need aggressive Tyr supplements, especially 3rd trimester
- Sapropterin seems safe
- Risk of high phe likely outweighs potential risk for use of sapropterin and consideration for pegvaliase

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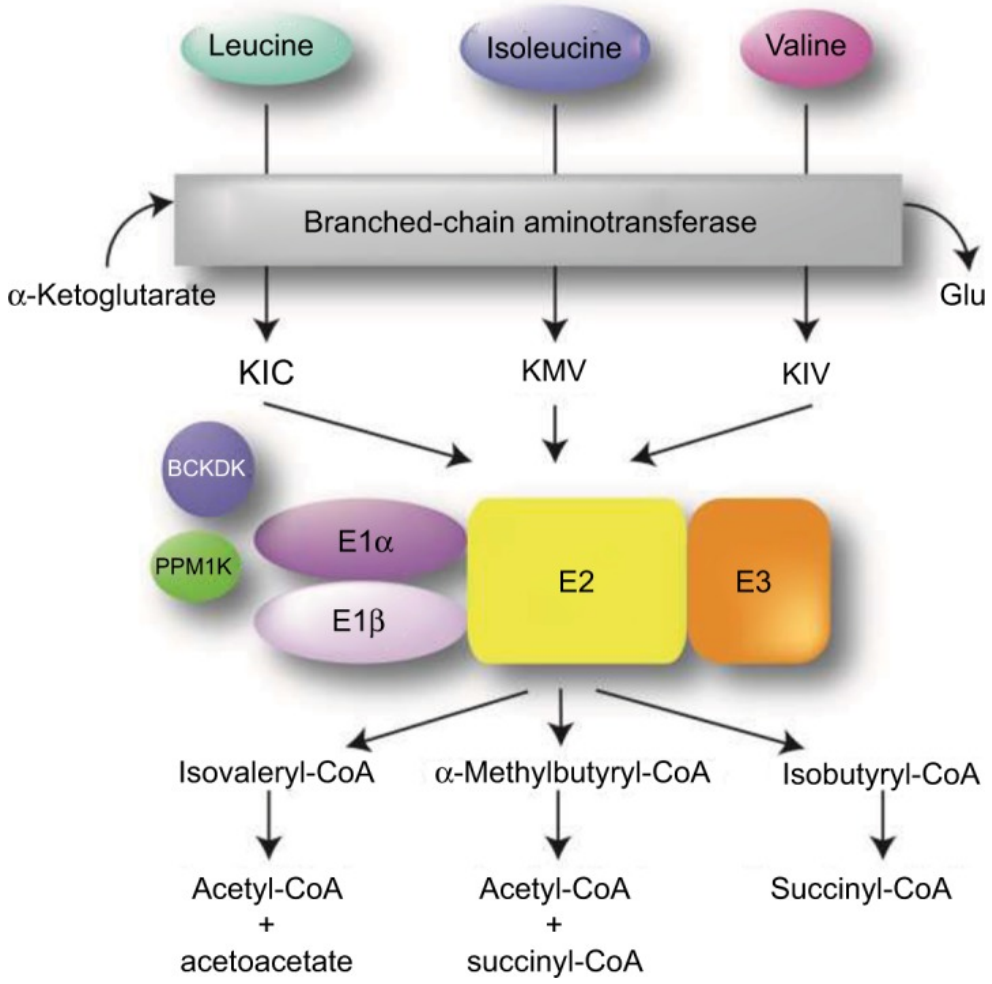
Universal lessons from PKU

- Screening and treatment can be effective
- NBS can uncover milder forms for which the need to treat may not be obvious
- Unanticipated future consequences, for example maternal PKU, may occur or be revealed
- Treatment/intervention creates a new “natural history”
- The pathogenesis is more complicated than you think
- Alternative therapies may be developed over time

Branched chain AA pathway



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Maple syrup urine disease

Branched chain ketoacid dehydrogenase deficiency

- Autosomal recessive inheritance
- Incidence = ~1/185,000 births
- 4 subunits – E1α, E1β, E2, and E3
- Mutations known in all four genes
  - Tyr391Asn substitution in E1a protein is a common founder mutation in the Mennonite population
  - Mutations in E2 subunit are sometimes thiamine responsive

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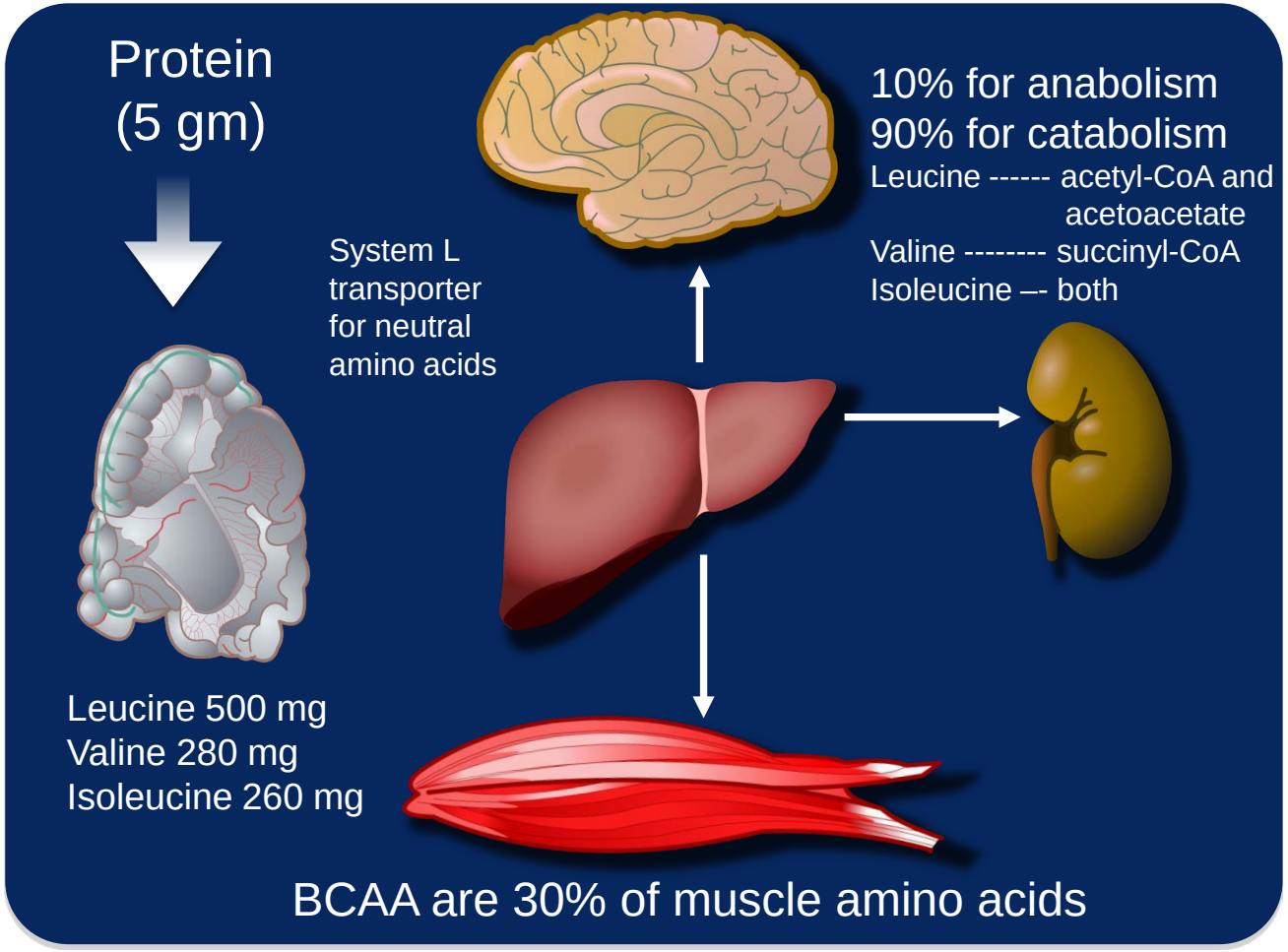
3 presentations

- Severe neonatal form (<1% residual activity)
  - Few abnormalities on routine lab tests
  - Maple syrup odor in urine (2-hydroxyisoleucine)
  - May have hyperammonemia, hypoglycemia
- Acute intermittent form (with residual activity)
  - Late onset
  - Ataxia
  - Ketoacidotic coma sometimes with hypoglycemia
  - Amino acid and keto acids can be normal between attacks

3 presentations

- Subacute chronic form (with residual activity)
  - Hypotonia and developmental delay
  - Failure to thrive
  - Spastic paraplegia

- Acute intoxication looks like intoxication
  - Ataxia prominent
  - Slurred speech
  - Disordered thinking
  - Lethargy progressing to coma



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Acute treatment

- Eliminate dietary protein intake
- Supplement valine and isoleucine
  - Isoleucine, in particular, can become limiting so that leucine remains increased because of protein catabolism
- Provide adequate non-protein energy source
- Avoid hypotonic fluids
- Treat cerebral edema if symptoms develop
- Dialysis

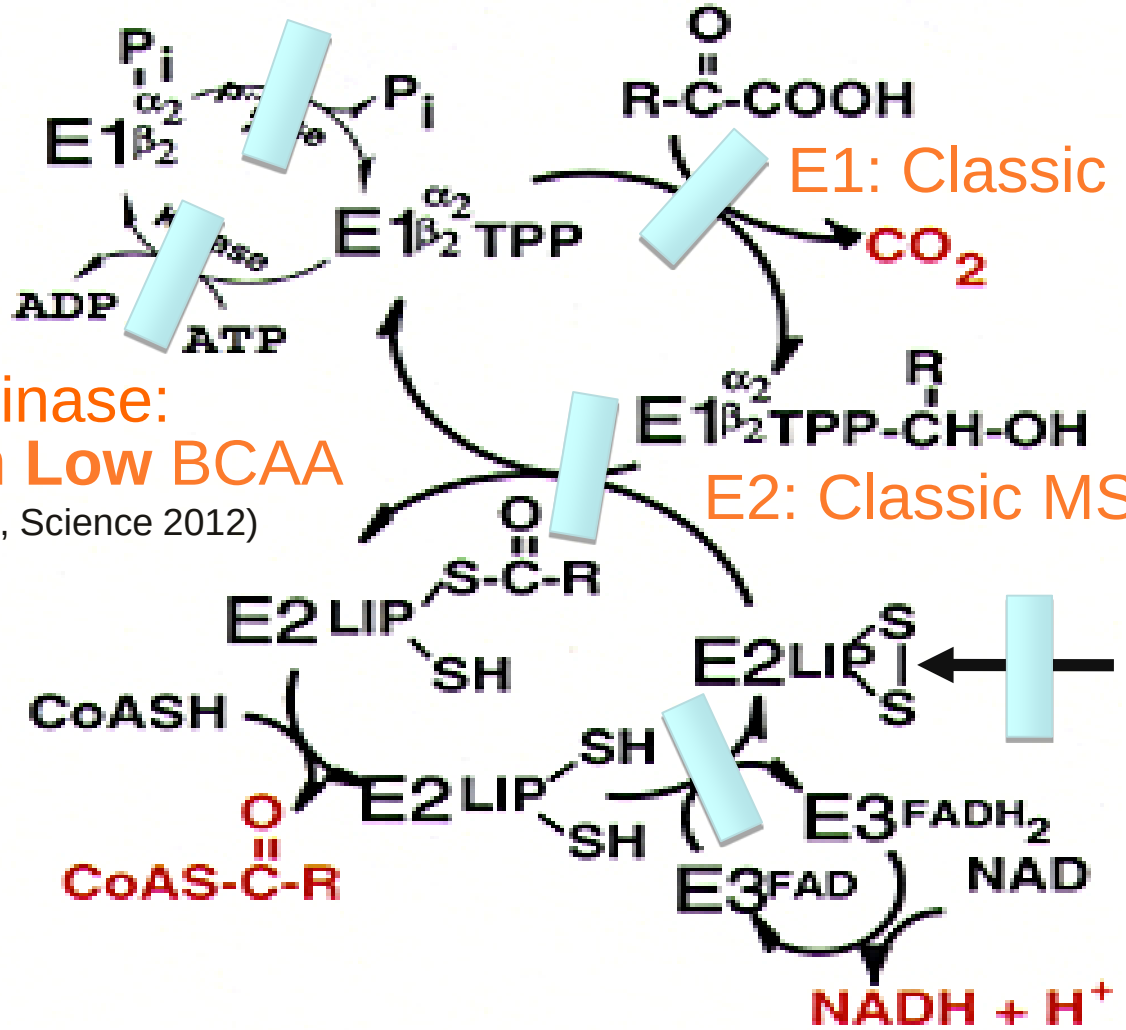
- Be careful with “sick day” formulas
  - Data regarding utility are limited to non-existent
  - they can be overused by families leading to chronic protein deficiency
  - Isoleucine, in particular, can become limiting so that leucine remains increased because of protein catabolism
  - Family should not start without contacting metabolic provider
  - Rarely use for more than 24 hours without adding back protein
- Role of glucose/carbohydrate
  - Prevent gluconeogenesis that drives protein breakdown for substrate (same mechanism as steroids)
  - Provides calories and drives insulin (anabolic driver)
  - Frequent small doses best

Chronic therapy

- Protein-restricted diet supplemented with branched chain amino acid-free medical foods
  - Recall that muscle (especially red meat) is enriched in leucine
- Leucine intake about 400–600 mg per day (childhood) in severe neonatal forms. Then 600–800 after adolescence
- Supplement valine and isoleucine
- Some forms are responsive to thiamine supplementation
- Liver transplantation is very successful

Branched chain ketodehydrogenase defects: New disorders

Phosphatase:Variant MSUD (Oyarzabal A, Hum Mut 2012)



Kinase:
Autism **Low** BCAA
Novarina G, Science 2012)

E1: Classic MSUD

E2: Classic MSUD

Lipoic acid Synthetase
(Mayr J, Am J Hum Genet 2011)

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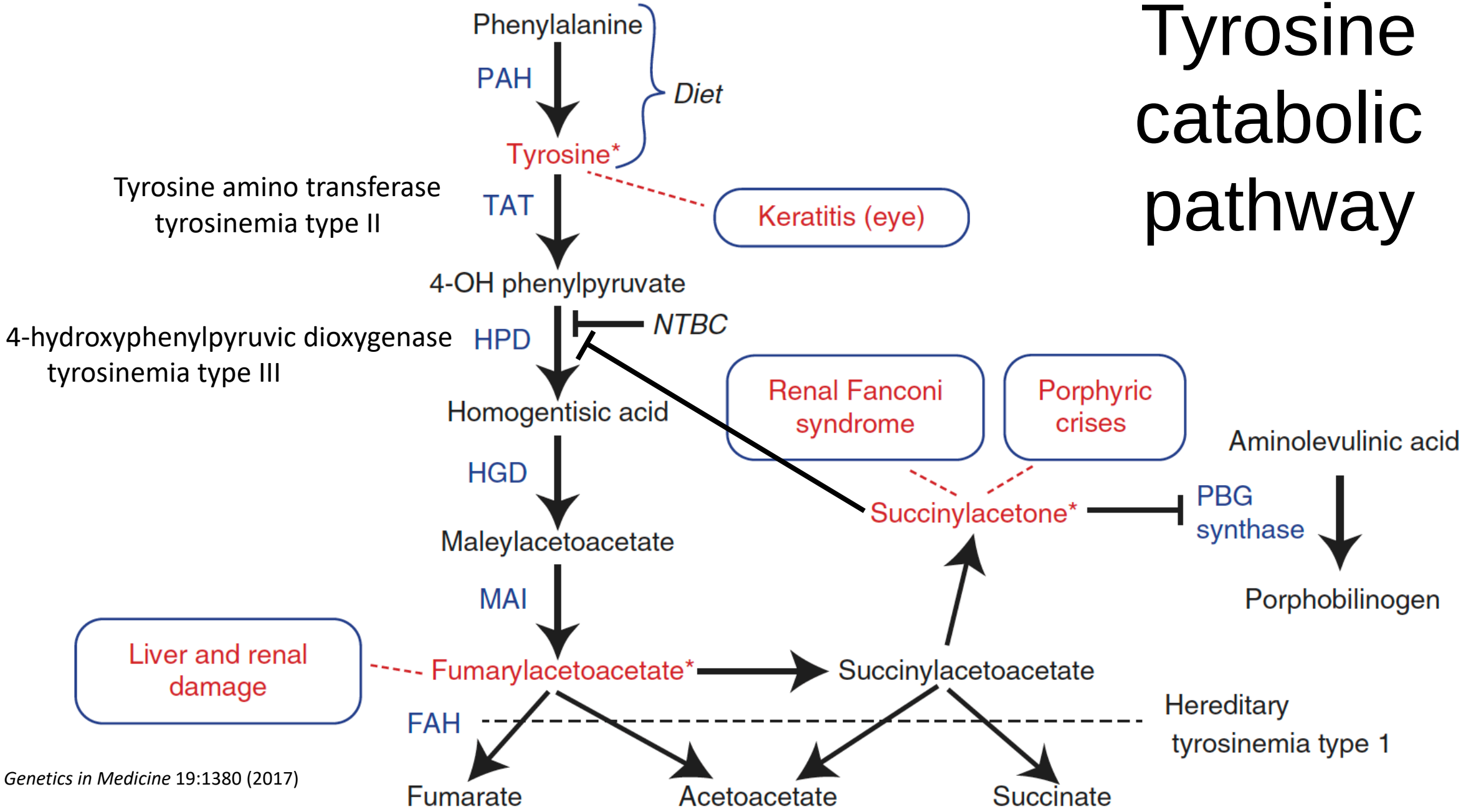
Tyrosine catabolic pathway



Genetics in Medicine 19:1380 (2017)

Tyrosinemia type 1

- Fumarylacetoacetate hydrolase deficiency
- Autosomal recessive inheritance
- Founder effect
  - Quebec, Canada
  - Finland
- 3 presenting forms:
  - Early in infancy (1 to 6 months): Liver disease (hepatic failure or cholestatic jaundice or cirrhosis with renal tubulopathy)
  - Late infancy: Rickets due to renal tubulopathy (Fanconi syndrome) with no obvious liver failure
  - Porphyria-like attack at any age (can be presenting sign)

Cellular effects tyrosinemia 1

- Toxic compounds (don’t cause ”intoxication” symptoms)
  - Fumarylacetoacetate, maleylacetoacetate
  - Succinylacetone
- Hepatocellular damage
  - Cirrhosis
  - Hepatocellular carcinoma
  - High alpha fetoprotein
- Renal tubule damage
  - Renal Fanconi syndrome
  - Hypophosphatemic rickets

Succinylacetone

- Succinylacetone inhibits
  - –Δ-aminolevulinic acid dehydratase activity
    - Porphyria-like abdominal pain crises
    - Peripheral neuropathy
  - –4-hydroxyphenylpyruvic dioxygenase
    - Tyr II enzyme defect, target of NTBC

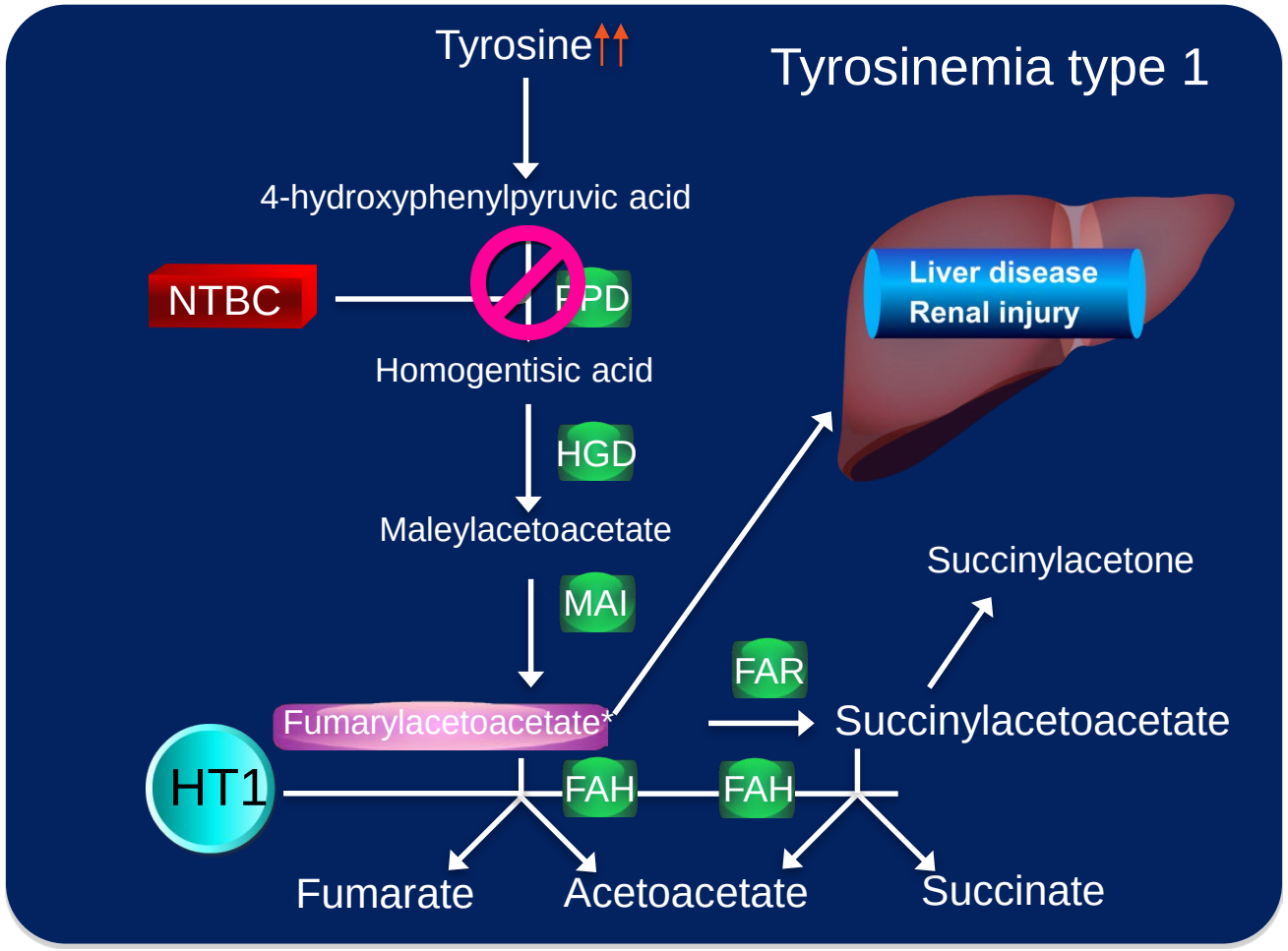
Treatment

- 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexane-dione (NTBC)
  - Inhibits 4-hydroxyphenylpyruvic acid dioxygenase
  - Further increases plasma tyrosine
  - Decreased production of FAA and succinylacetone
  - Markedly reduces, but may not eliminate, hepatocellular carcinoma
- Phenylalanine and tyrosine restriction to avoid excessive hypertyrosinemia (risk of keratitis as in TYR II)
- Liver transplant if hepatocellular carcinoma develops

Monitoring

- Therapeutic response to nitisinone
  - Plasma drug concentrations >35 μmol/L inhibit enzyme 99.9%
  - Some also monitor plasma succinylacetone to see complete suppression (plasma SA normal)
  - Start nitisinone at 1 mg/kg/day (usually divided BID for first year)
  - Titrate dose to desired plasma concentration and/or suppression of SA
- Dietary restriction of Phe and Tyr to keep plasma tyr <600 μmol/L
- Dried blood spot testing including SA, nitisone concentration, tyr and phe is available but drug concentrations may not correlate well with plasma

Tyrosine catabolic pathway



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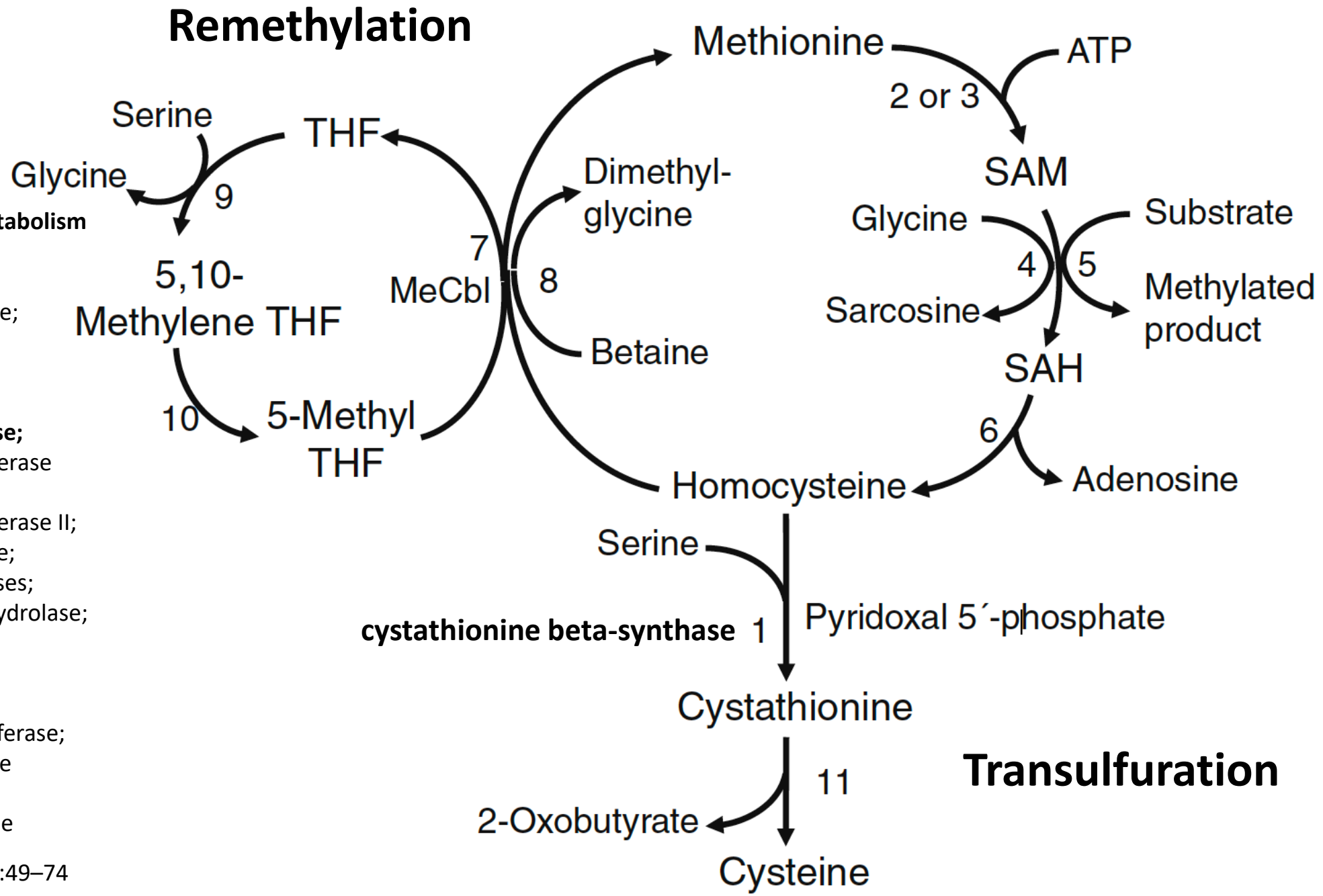
Other tyrosinemias

- Type II – tyrosine transaminase
  - Corneal crystals (painful)
  - Plantarpalmar hyperkeratosis with pits (painful)
  - Intellectual disability in some
- Type III – 4-hydroxyphenylpyruvic acid dioxygenase deficiency (enzyme inhibited by NTBC)
  - Intellectual disability
  - Seizures
- Transient tyrosinemia of the newborn
  - Cause unknown
  - Self limiting over 1 to 2 months
  - Apparently benign
  - More common in premature infants



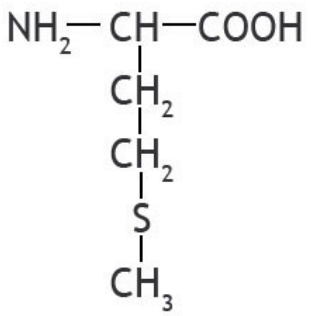
Pathways of methionine metabolism

SAM, S-adenosylmethionine;
SAH, S-adenosylhomocysteine;
THF, tetrahydrofolate;
MeCbl, methylcobalamin.

1 **cystathionine beta-synthase;**
2 methionine adenosyltransferase I/III;
3 methionine adenosyltransferase II;
4 glycine N-methyltransferase;
5 numerous methyltransferases;
6 S-adenosylhomocysteine hydrolase;
7 methionine synthase;
8 betainehomocysteine methyltransferase;
9 Serine hydroxymethyltransferase;
10 methylenetetrahydrofolate reductase;
11 cystathionine gamma-lyase

J Inherit Metab Dis (2017) 40:49–74

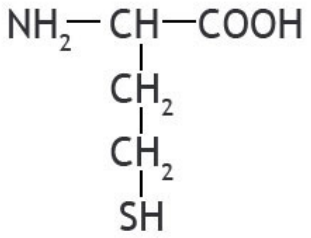
Disulfide bonds



Methionine

Normal Plasma Concentration

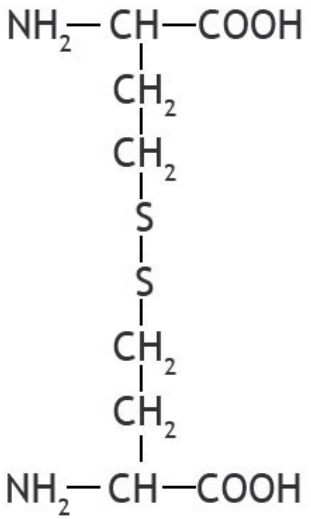
10-35μM



Homocysteine

Undetectable in normal plasma

5-15μM in plasma treated with a reducing agent



Homocystine

Undetectable

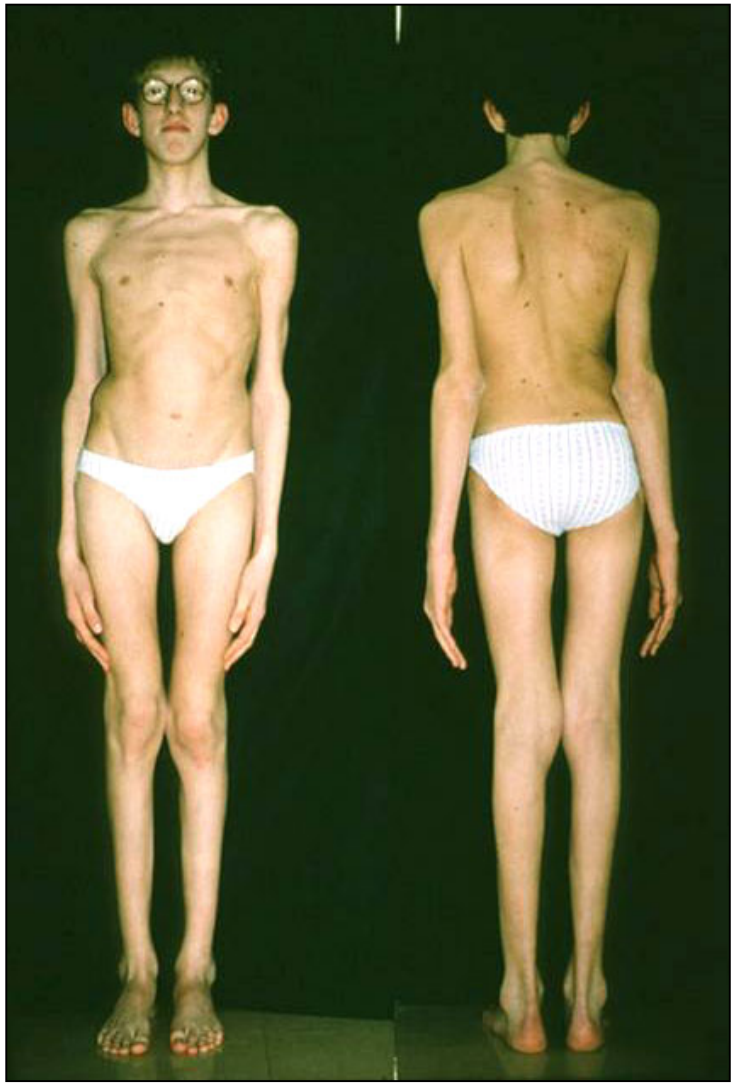
Elevated MET: caveats

- When accurate homocysteine measurements are important, measure “total homocysteine” and don’t rely on amino acid analysis, unless:
  - You can make sure the specimen gets to the lab quickly, and…
  - The specimen will be deproteinized soon after arrival in the laboratory
- Total homocysteine in this case measured 150 µM

Classical homocystinuria

- Cystathionine β-synthase deficiency
- Autosomal recessive inheritance
- Incidence = 1/200,000 to 1/400,000 births
  - Incomplete ascertainment
  - Cases often missed on newborn screens obtained during the first week of life
- 50% of CBS mutations are pyridoxine (vitamin B_6) responsive

Classical untreated homocystinuria



Courtesy JM Saudubray

- Skeletal malformations
  - Marfanoid habitus
  - Osteoporosis
  - Scoliosis
  - Most common in B_6 non-responsive forms

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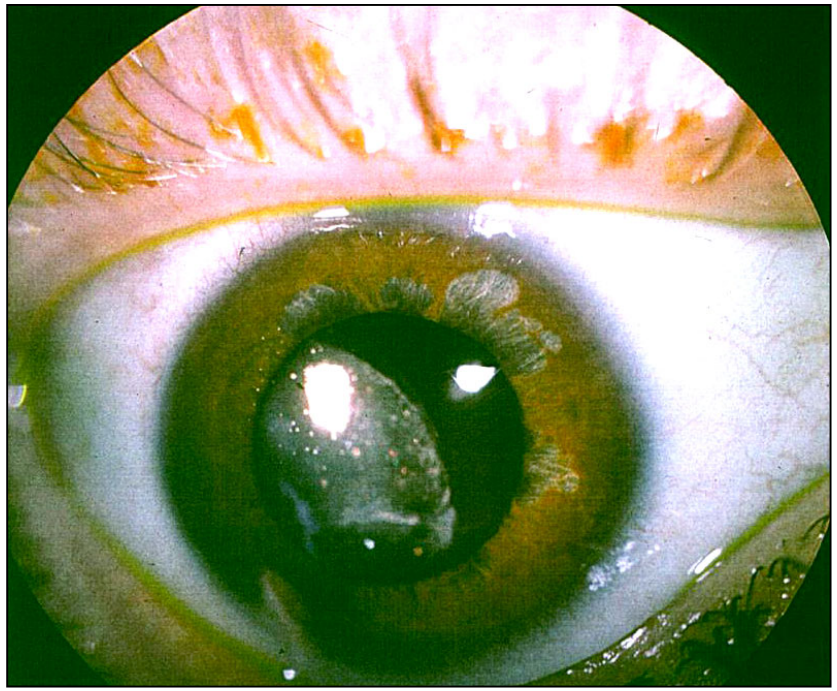
Other clinical findings

- Eye abnormalities
  - Ectopia lentis
    - 90% of affected individuals
    - Often bilateral
    - Typically down and toward nose (opposite of Marfan)
  - Myopia
  - May be an isolated presenting sign in children or adults
- Developmental disability and neuropsychiatric symptoms in many, but not all



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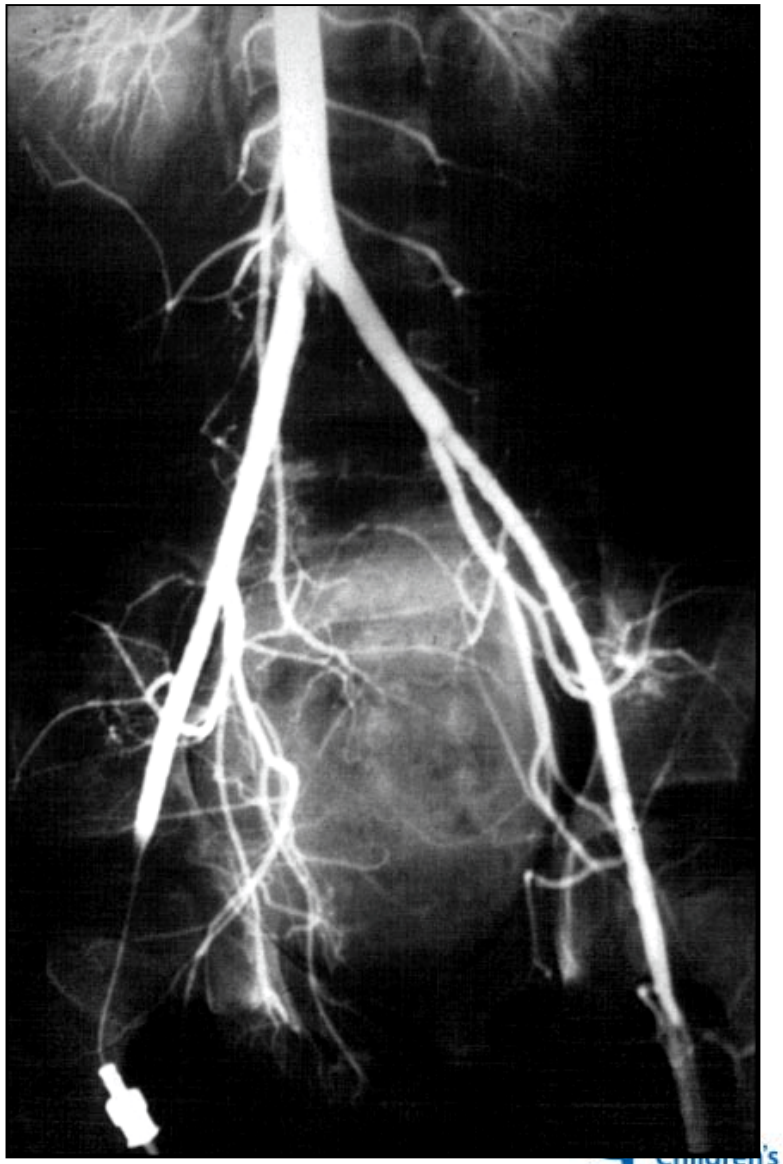
Recurrent thromboembolism

- May be a isolated presenting sign in late-onset B_6 responsive forms
- Thromboembolism can be a presenting sign
  - Phlebitis
  - Pulmonary embolism
  - Cerebrovascular accident
- Environmental triggers
  - Anesthesia
  - Catabolism
  - Smoking
  - Oral contraceptives

Atherosclerotic disease

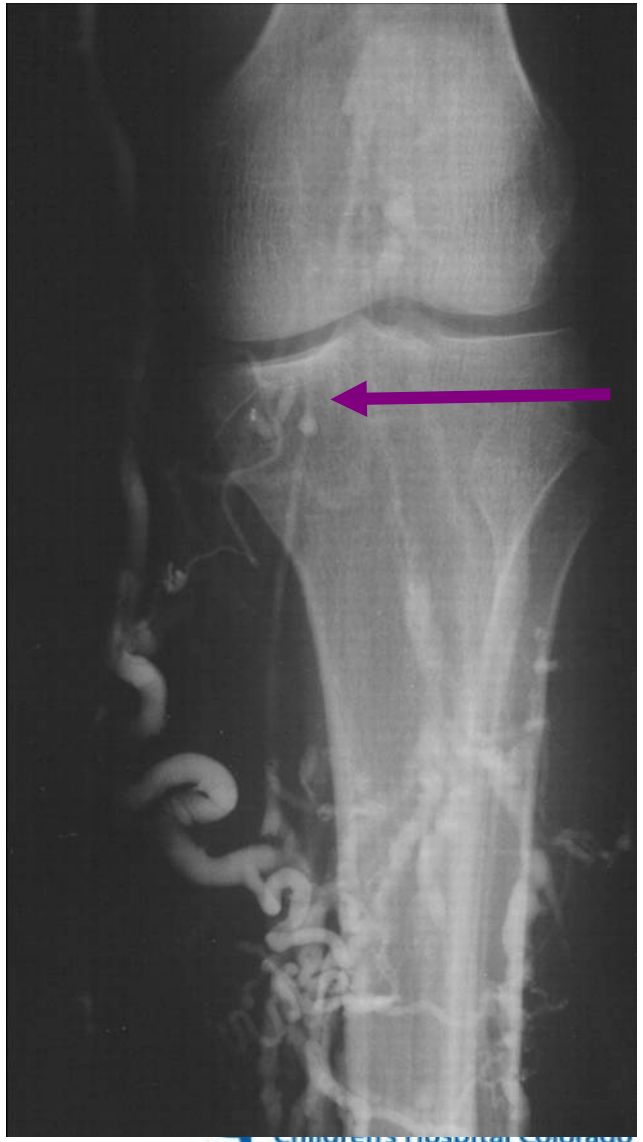


Courtesy of JM Saudubray

Thrombosis

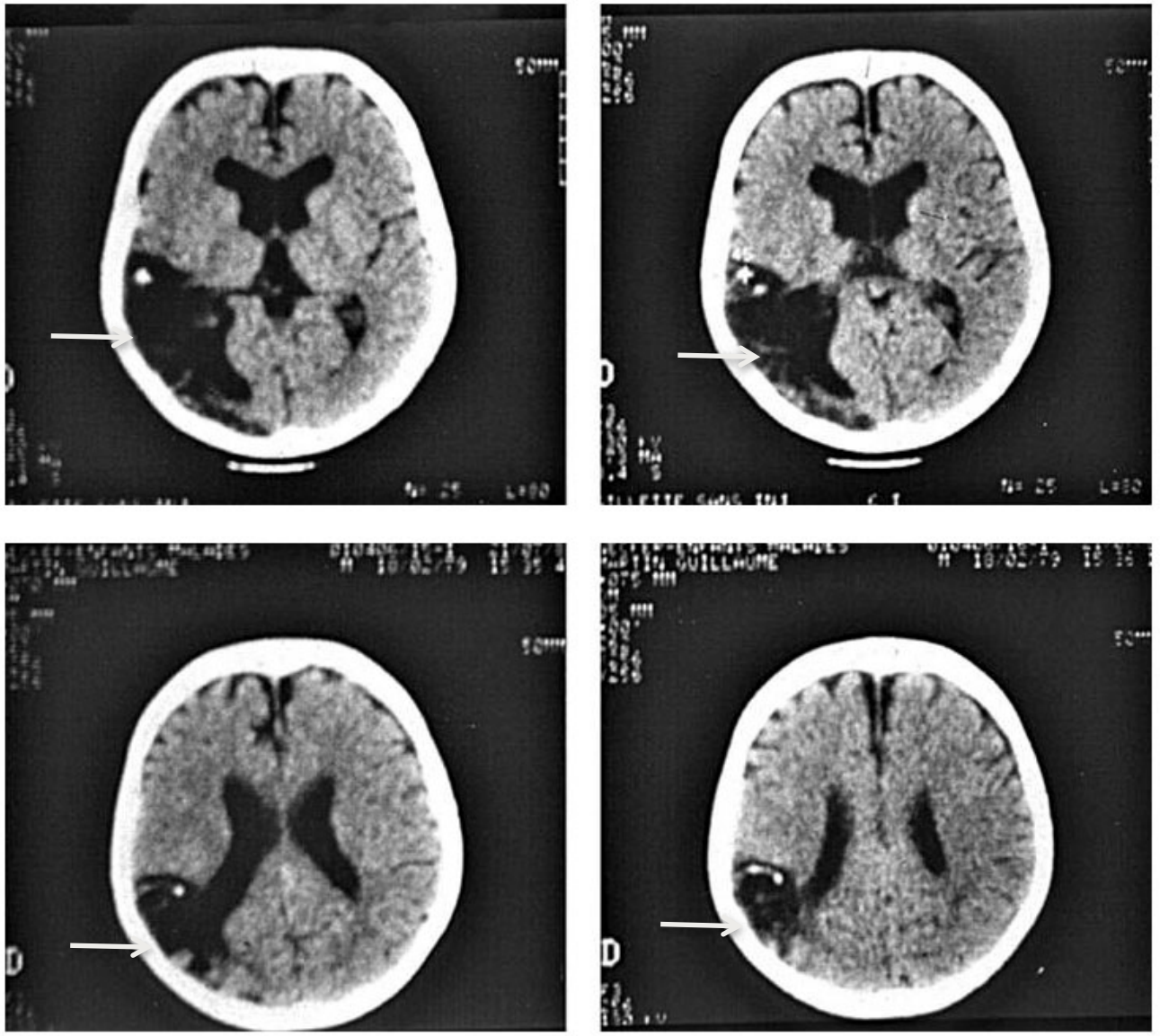
Homocystinuria

Thrombus in popliteal vein. Note the collateral circulation.



Sueyoshi E et al. Am J Roentgenol 2004;182:830

Thromboembolic stroke



Courtesy JM Saudubray

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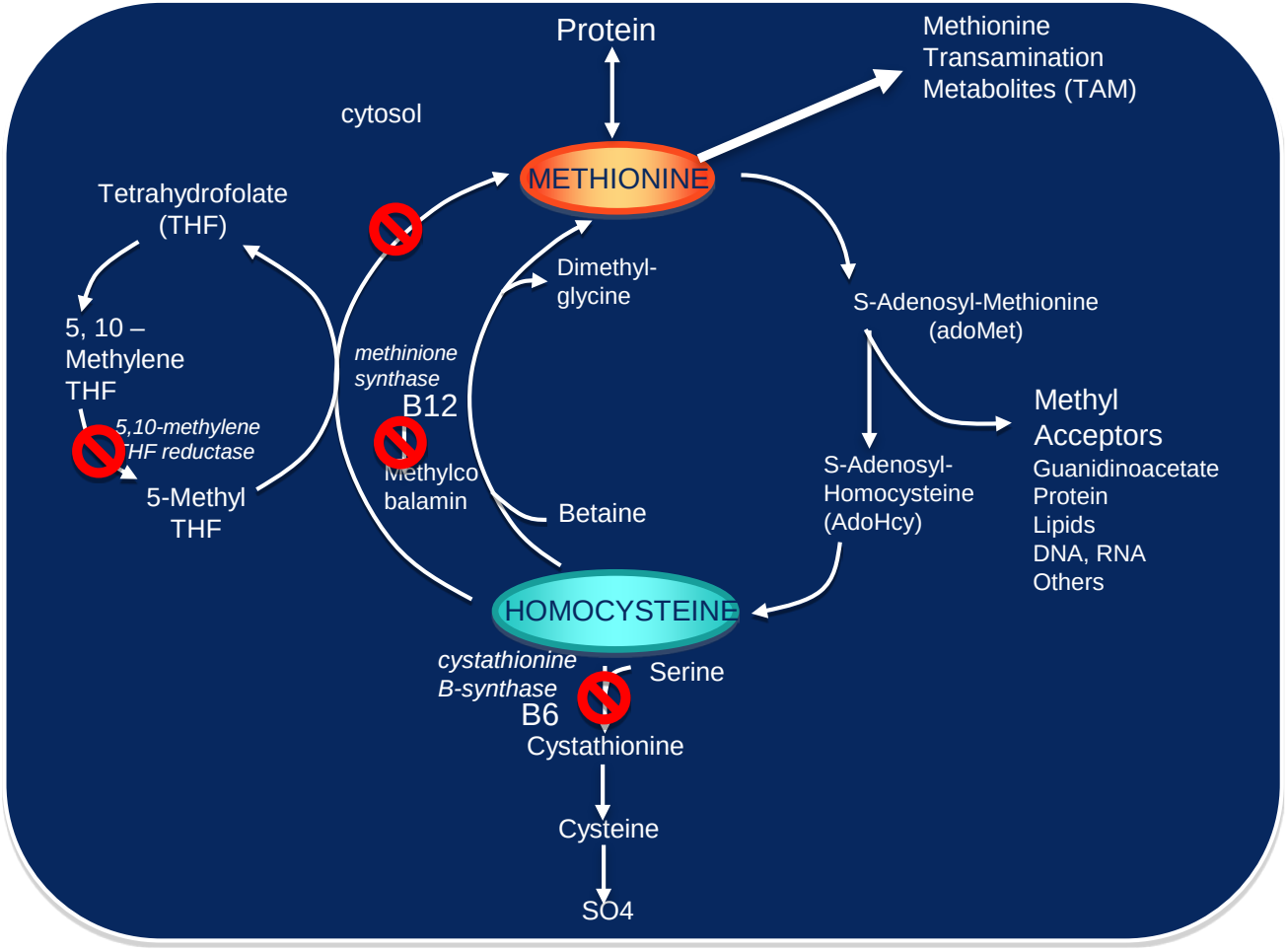
Other causes of homocystinuria



Yaghmai R et al. Am J Med Genet 2001;108:57

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Therapy (CBS deficiency)

- Pyridoxine responsiveness – 10 mg/kg/day (max. 500 mg)
  - Test total Hcy 2-3 X before Rx and 2-3 X on Rx after 4-6 weeks
  - >20% decrease is considered responsive (starting above 50 μmol/L)
  - High dose pyridoxine (>900 mg) can cause peripheral neuropathy
- Folate for all, B12 if deficient
  - HCU formulas usually have plenty of both
- Diet therapy – low protein, low-met formula
- Betaine – start at 50-100 mg/kg/day divided BID
  - Can increase up to 200 mg/kg/day, rarely benefit to higher dose
  - BHMT is satuable enzyme, so demonstrating additional benefit on plasma Hcy is helpful for higher doses

Urea Cycle Disorders

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Neonatal UCD presentation

- 40% of urea cycle disorder cases
- Neurological
  - Lethargy/poor feeding
  - Decreased mental status/apnea
  - Hypotonia
  - Seizures
- GI
  - Poor feeding/vomiting (neurological symptoms)
  - Liver dysfunction with increase in ALT, AST and PT, INR
- Pulmonary
  - Tachypnea

Initial UCD lab findings

- Initial respiratory alkalosis in neonates (7.56/22)
- Hyperammonemia (>150 umol/L, up to thousands)
- Low BUN
- Mildly elevated liver enzymes, coagulopathy
- Glucose is usually normal; no acidosis unless there is shock/circulatory collapse (metabolic acidosis) or apnea (respiratory acidosis)
- Amino acid alterations specific to disease, see table

Later-onset UCD presentations

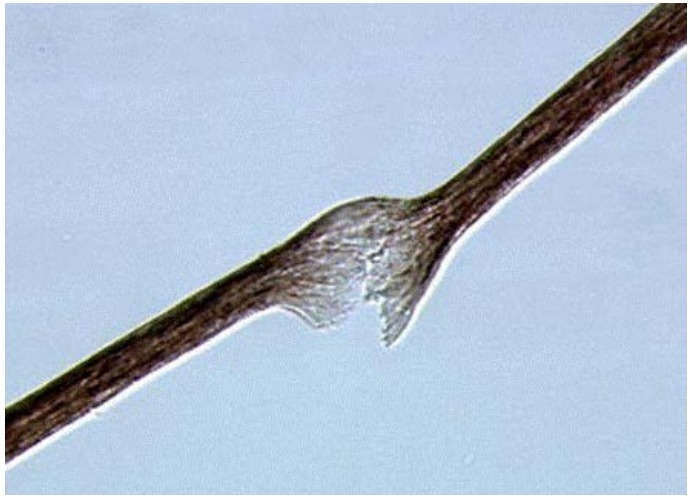
CNS

- Seizures
- Stroke
- Ataxia
- Coma mimicking encephalitis
- Visual loss
- Intellectual disability
- Neuropsychiatric symptoms (e.g., hallucinations)
- Migraine headaches
- Spastic diplegia/quadriplegia

Later-onset UCD presentations

GI

- Failure to thrive/protein intolerance
- Recurrent vomiting
- Hepatomegaly/hepatic fibrosis



Trichorrhexis nodosa (ASA)

Other

- Reye-like episodes
- Hair fragility and skin rash

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Proximal urea cycle blocks

Intramitochondrial, expressed only in liver and intestine

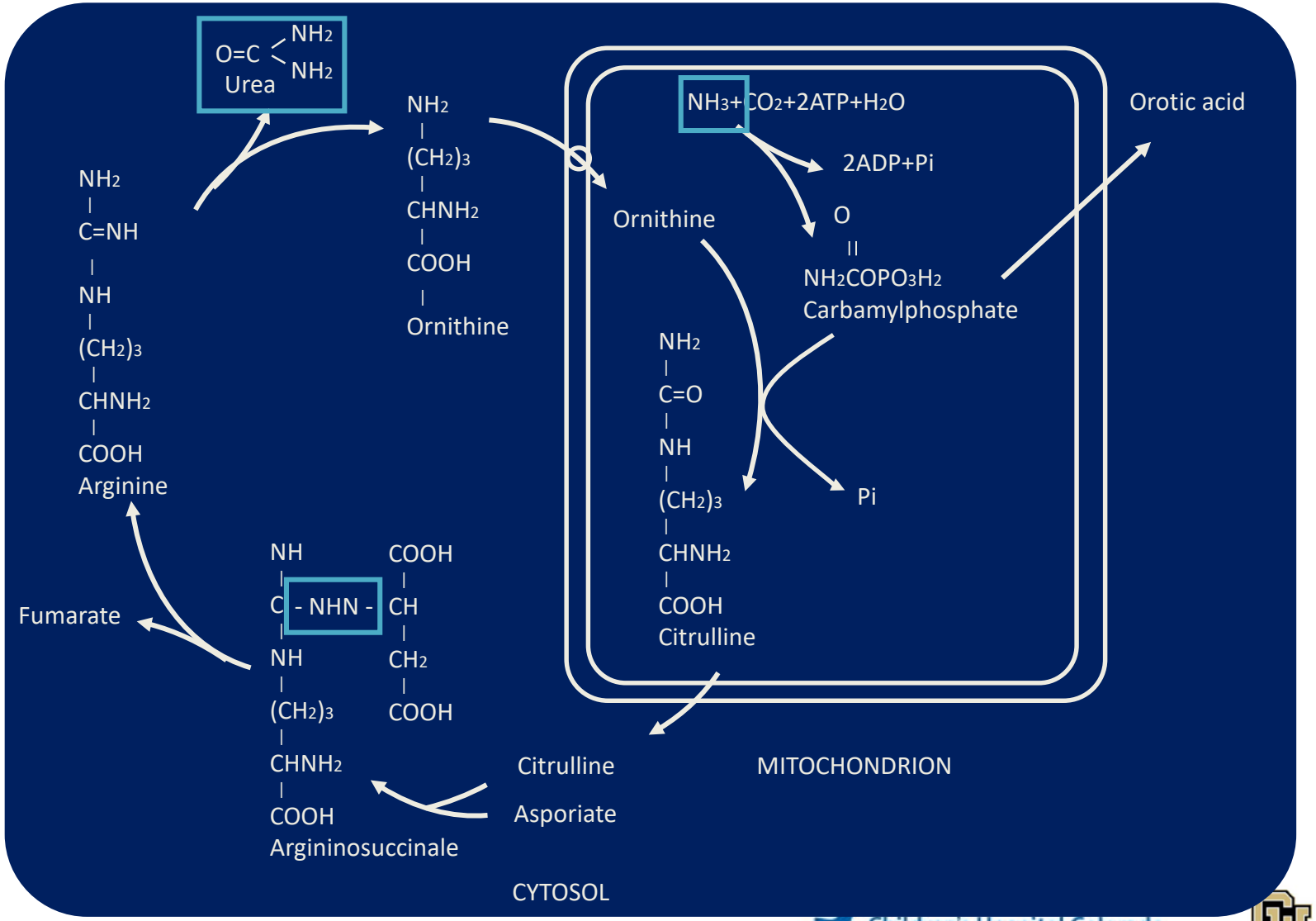
- N-acetylglutamate synthetase (NAGS)
  - Primary defect
  - Secondary inhibition in OAs/FAOs from depletion of acetyl-CoA and interference with synthesis of N-acetylglutamate, which activates CPS1
- Carbamyl phosphate synthetase 1 (CPS1)
- Carbonic anhydrase VA (CAVA) with CPS and mitochondrial carboxylases deficiency
- Ornithine transcarbamylase (OTC)

Distal urea cycle blocks

Extramitochondrial, ubiquitous expression

- Argininosuccinic acid synthetase (ASS, citrullinemia type I)
- Argininosuccinic acid lyase (ASL, argininosuccinic aciduria)
- Arginase I (ARG, arginase deficiency)

Urea cycle pathway



Nyhan and Ozand. Atlas of Metabolic Diseases.

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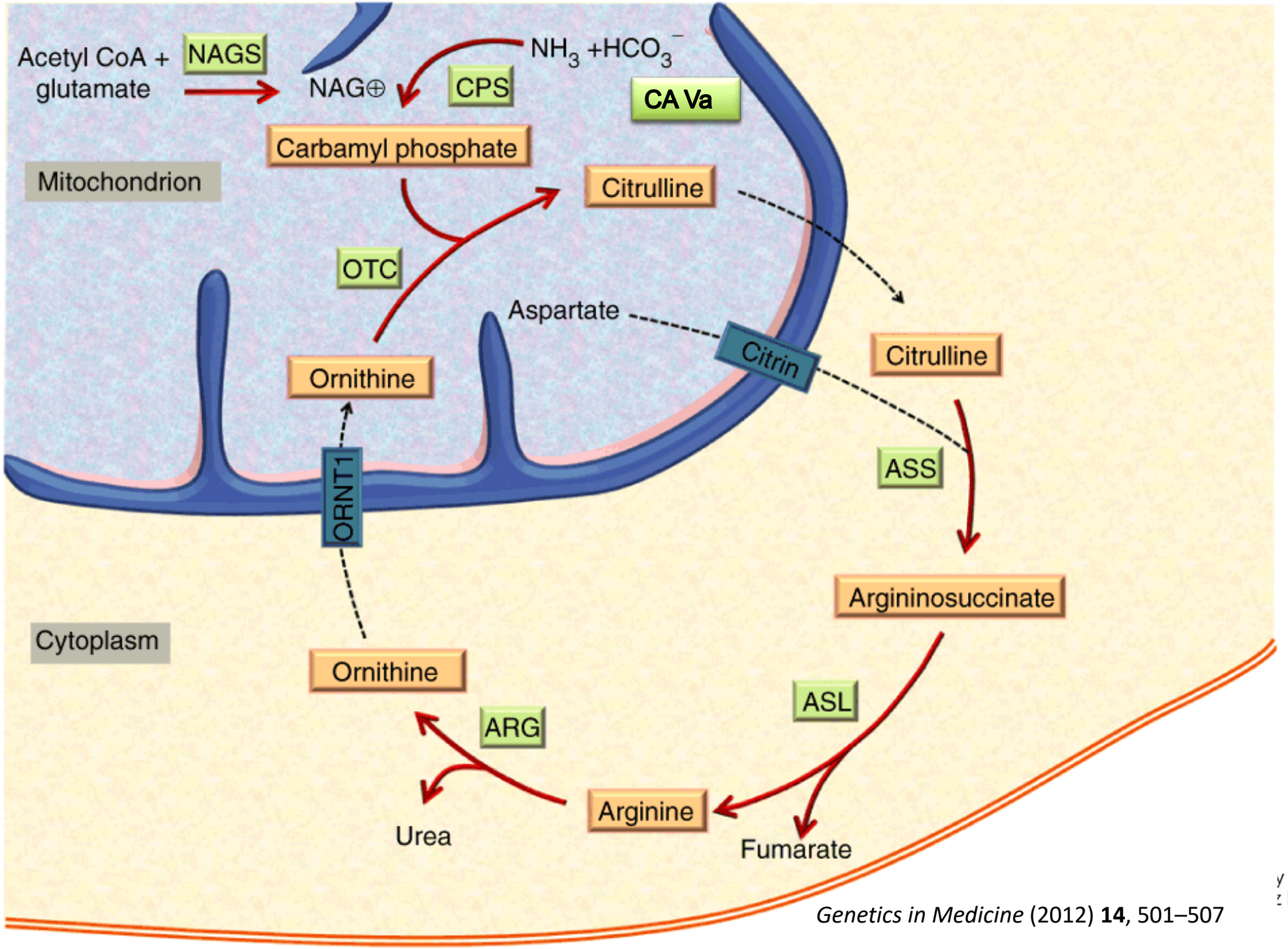

Genetics in Medicine (2012) **14**, 501–507

Relative incidence of UCDs

Molecular diagnosis frequency

Dx	US FDA	US NIH-UCDC	France	Japan
OTC	55%	55%	70%	68%
	F (51%)	F (66%)	F (25%)	F (35%)
	M (49%)	M (34%)	M (75%)	M (65%)
ASS	27%	14%	15%	11%
CPSI	14%	6%	6%	12%
ASL	3%*	16%	9%	6%
Arg	<1%	5%	0%	3%
Undx	18%	0%		
N for group	**316**	**480**	**217**	**216**

Estimated incidence of UCDs

Urea Cycle Disorder	Estimated Incidence
NAGS deficiency	<1:2,000,000
CPS1 deficiency	1:1,300,000
OTC deficiency	1:56,500
ASS1 deficiency	1:250,000
ASL deficiency	1:218,750
ARG deficiency	1:950,000

Overall 1:36,000

Mol Genet Metab. 2013;110:179–80

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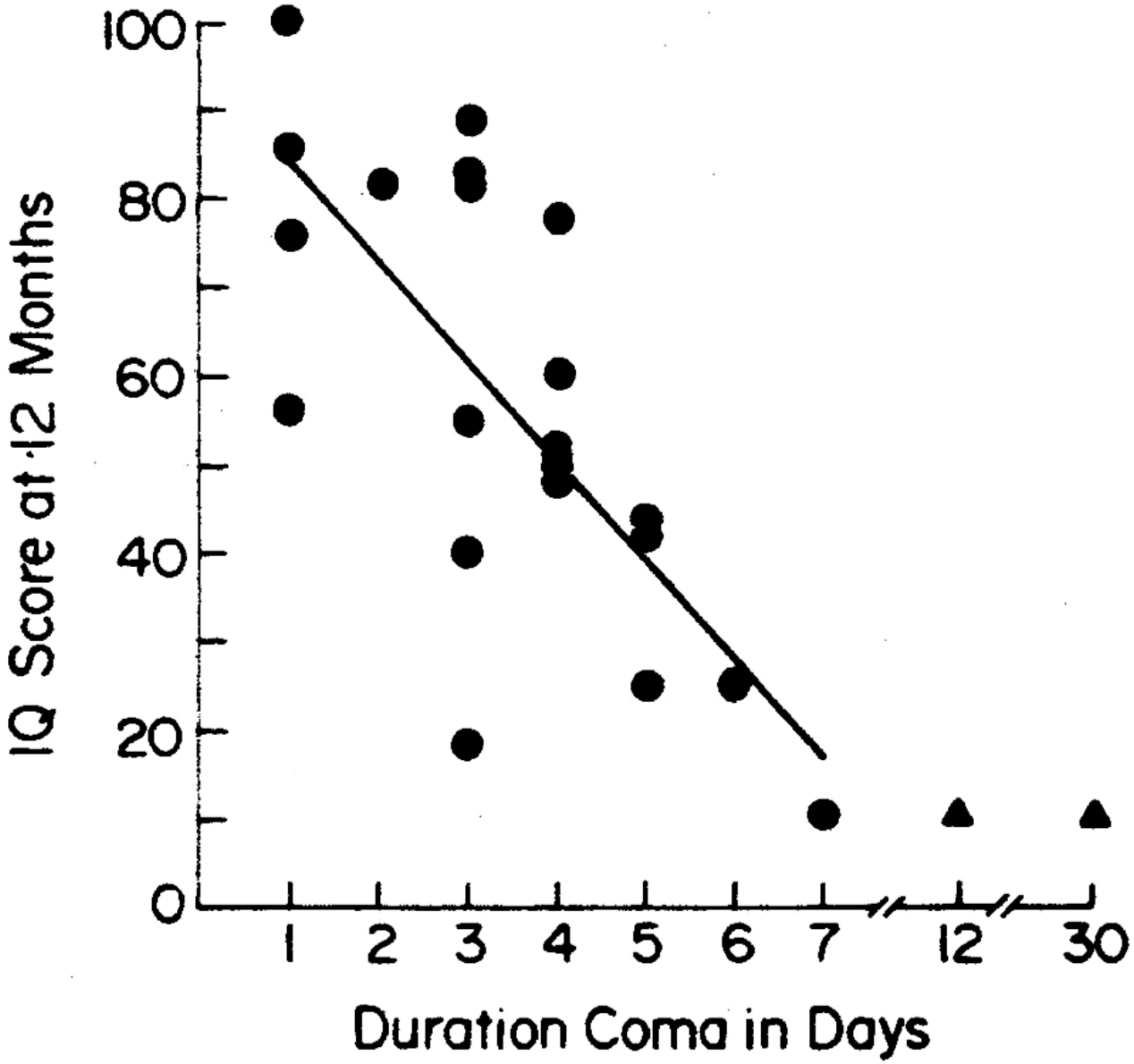
Blood tests in acute encephalopathy

- Ammonia
- Blood gas
- Electrolytes, bicarbonate, glucose
- Liver function tests and coagulation factors
- Lactate (± pyruvate)
- Carnitine and acylcarnitine profile
- Plasma amino acids
- Plasma total homocysteine
- Creatine kinase
- Check newborn screen results!



N Engl J Med 1984; 310:1500-1505

Natural history

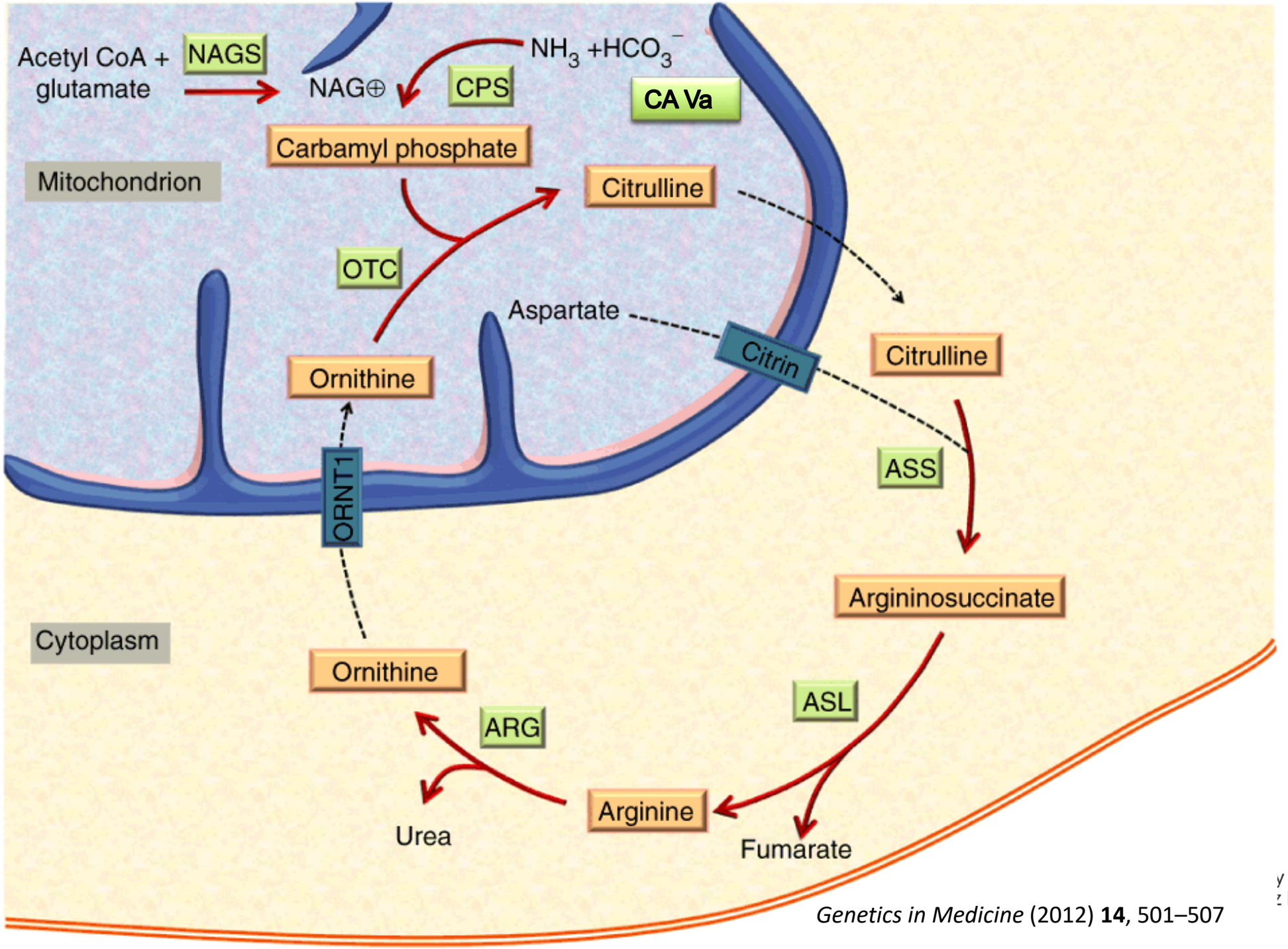
- Early-onset patient may have an apparent “honeymoon” period in early infancy (before 9 months)
- Difficulties start from end of year 1 to ~3
- Difficulties sometimes occur around puberty and/or with menstrual periods
- The disease course is more brittle with the severe, early-onset cases

“Honeymoon period” — why?

- Rapid neonatal growth and protein utilization
- Restricted environmental exposure to infection
- Simpler diet
- Later complications
  - Switch from breast to cow’s milk
  - Diet more complex and chances to cheat
  - Exposure to infections
  - Decrease in growth velocity and nitrogen building

Urine tests in acute encephalopathy

- Urinalysis with metered pH, check ketones
- Organic acids
- Orotic acid — quantitative
- Amino acids



Genetics in Medicine (2012) **14**, 501–507

OTC deficiency (males)

- Most common urea cycle defect
- X-linked inheritance
- Little or no enzyme activity in early-onset males
- Laboratory
  - Respiratory alkalosis — an important clue
  - Hyperammonemia (can rise to the 1000s µmol/L, normal <100 in infant)
  - Low BUN
  - Elevated AST and ALT, increased PT and INR
  - No or low ketosis (in contrast to organic acidemias), order urinalysis

Biochemical lab findings

- Plasma amino acids
  - Marked elevation in plasma glutamine
  - Glutamine to ammonia ratio >1.6 (µmol/L for both)
  - Very low citrulline
  - Arginine normal to low, lysine high
- Urine organic acids
  - Urine orotic acid elevated (order quantitative value)

OTC deficiency (males) — late onset

- Residual enzyme activity present
- Presentations
  - May present at any age
  - Wide range of neurologic/psychiatric phenotypes
  - Can be unmasked by steroids, valproate, anesthesia
  - Order an ammonia level for unexplained altered mental status
  - Brain damage as a result of marked hyperammonemia may be irreversible
  - Early recognition of hyperammonemia can prevent death and disability

OTC deficiency (females)

- If symptomatic, generally have null mutations on one X
  - Symptoms depend on lyonization pattern in liver
- Onset at any age
- Can present with neonatal symptoms as with males
- Recurrent emesis, ± elevated AST, ALT, PT, INR
- Protein avoidance may be present, ± failure to thrive

OTC deficiency (females)

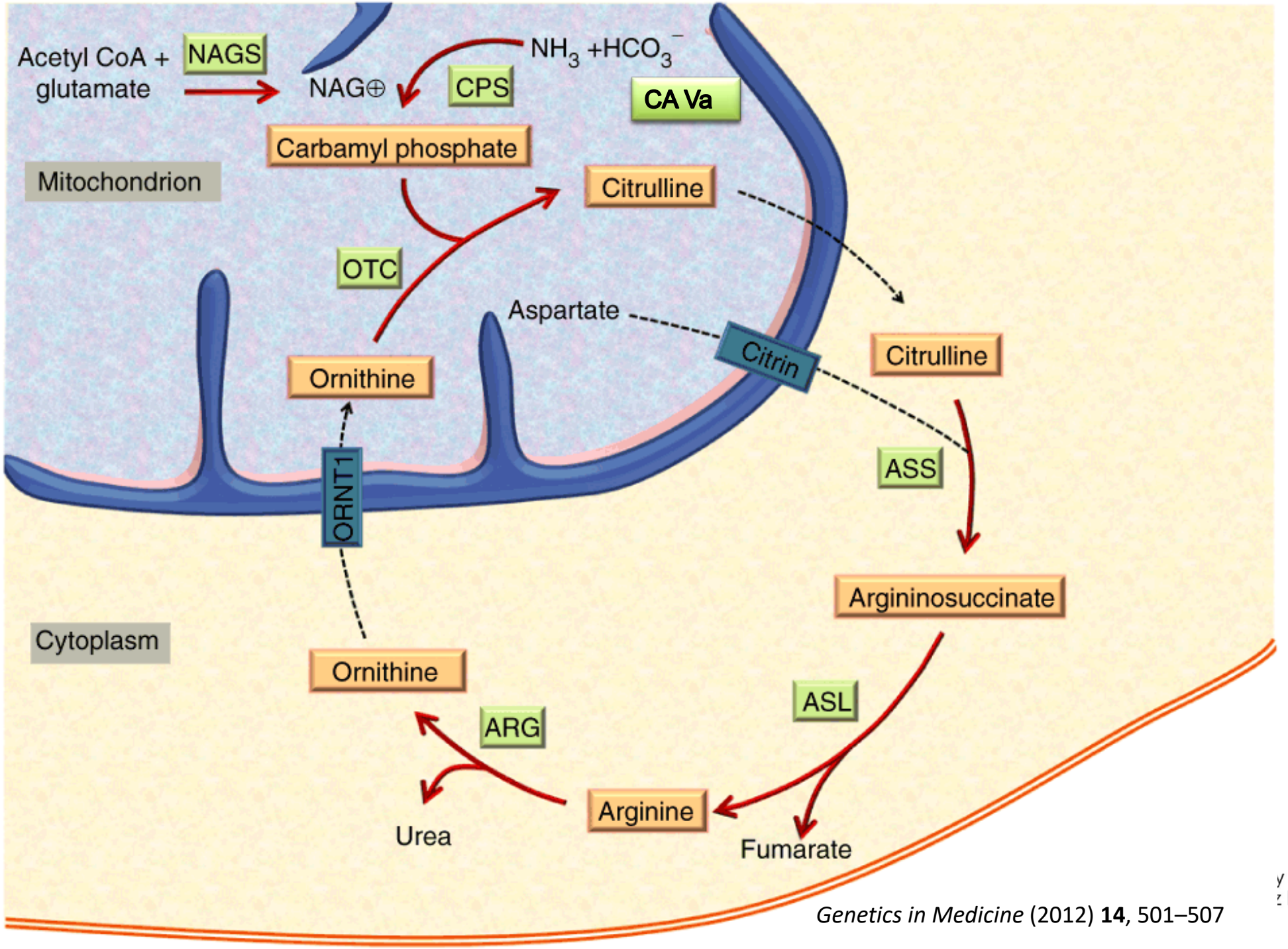
- Recurrent hyperammonemia ± coma
- Neuropsychiatric symptoms: confusion, ataxia, seizures, hallucinations, vision loss, developmental delays
- At risk during the post-partum period
- Other triggers: infection, diet change, change in growth, steroids, stress, chemotherapy, anesthesia (as for males)

Lab testing in OTC females

- Urine orotic acid and plasma glutamine may be elevated, but may be normal when well (like late-onset males)
- Diagnostic testing
  - Allopurinol challenge, allows detection of increase in orotic acid production (not sensitive or specific)
  - OTC gene sequencing and deletion/duplication analysis is easiest (80–90% sensitive)
  - Enzymatic testing
    - Requires liver tissue (sampling error possible in females)
    - Secondary partial CPS1 decrease due to negative feedback, CPS1 is also assayed
    - If withdrawing care, GET LIVER TISSUE for post-mortem enzyme testing to confirm diagnosis, as DNA may be negative

Other Proximal UCDs: CPS1, NAGS and CAVA deficiency

- Clinical
  - Both are similar to OTC, see above
  - Severe and mild forms described (early- and late-onset)
  - Equal number of males and females (recessive)
- Laboratory
  - Plasma AAs similar to OTC: high glutamine, low citrulline
  - BUT no elevation in urine orotic acid
  - Lactic acidemia and organic aciduria in CAVA
- Diagnosis
  - CPS1 enzyme assay in liver, can also sequence
  - Must sequence NAGS, no enzymatic assay

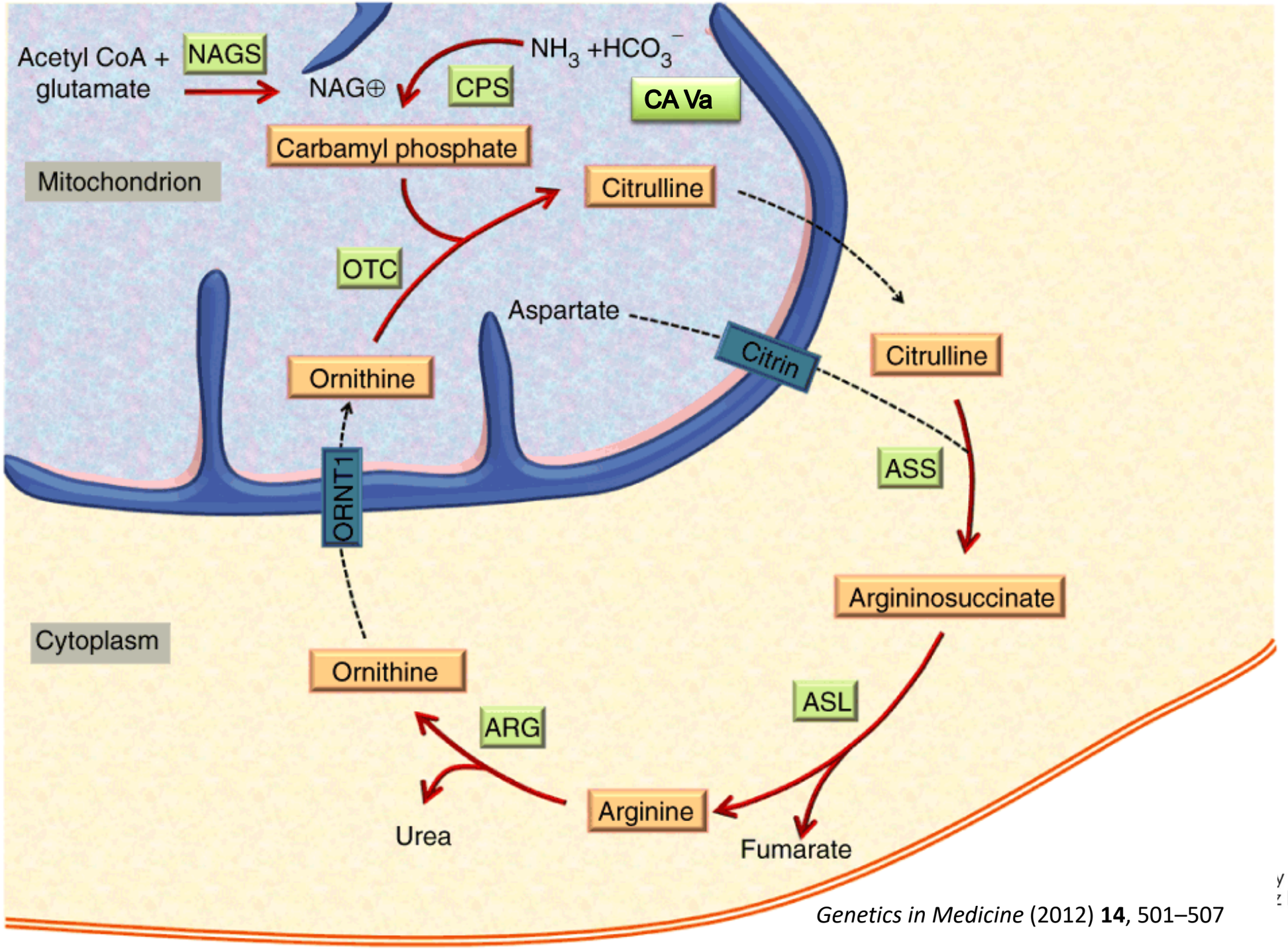


Genetics in Medicine (2012) **14**, 501–507

NAG analog is a therapy!

Cofactor therapy in NAGS deficiency — carbamylglutamate

- Stable analog of N-acetylglutamate, therefore is a cure for NAGS deficiency
- May be effective in some individuals with CPS1 (e.g., with N-acetylglutamate binding site mutations) and CAVA
- Approved in US for NAGS deficiency, current US multi-center trial in organic acidemias, OTC and CPS1 disorders



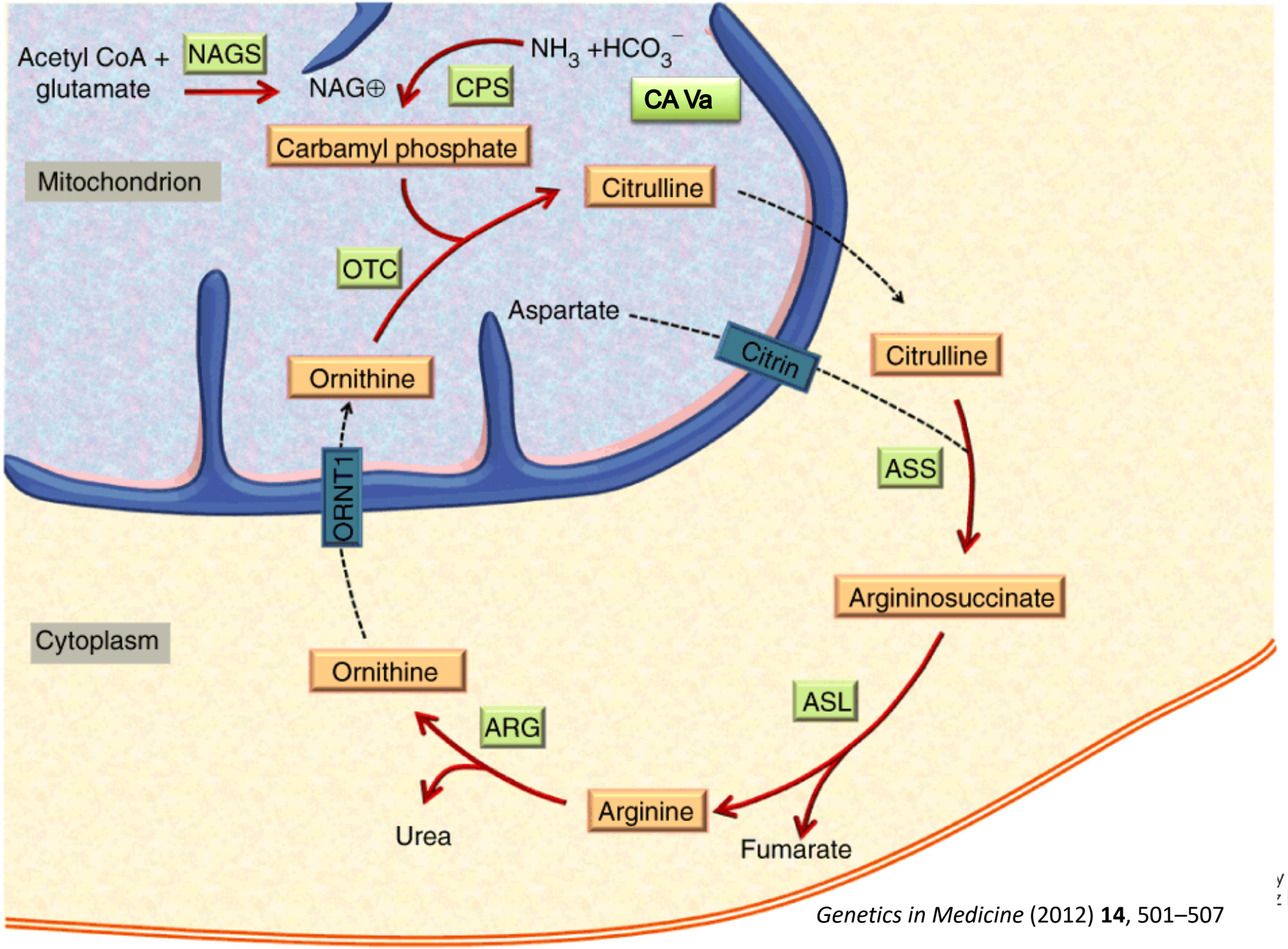
Genetics in Medicine (2012) **14**, 501–507

ASS deficiency (citrullinemia 1)

- Clinical
  - Like OTC, CPS1, NAGS deficiency
  - Early- and late-onset forms, recessive condition
  - Mild forms identified by newborn screening
- Diagnosis
  - DNA sequencing
  - Enzyme testing in fibroblasts
- Laboratory
  - Plasma citrulline high (>500 µmol/L)
  - May be lower in individuals identified by newborn screening
  - Heterozygotes may have mild elevations of plasma citrulline
  - Orotic acid high to normal

Citrin deficiency (citrullinemia 2)

- Clinical
  - Neonatal intrahepatic cholestasis due to citrin deficiency (NICCD)
    - Transient jaundice
    - No hyperammonemia
    - Can resolve spontaneously
  - Late onset
    - Neuropsychiatric disorders, sleep disturbances, developmental delay
    - Sugar aversion due to carbohydrate-induced hyperammonemia
- Laboratory
  - Moderate hyperammonemia
  - Citrulline — 30–300 µmol/L, can see elevated threonine, methionine
- Diagnosis
  - DNA sequencing of SLC25A13
  - Common mutation in Asian populations



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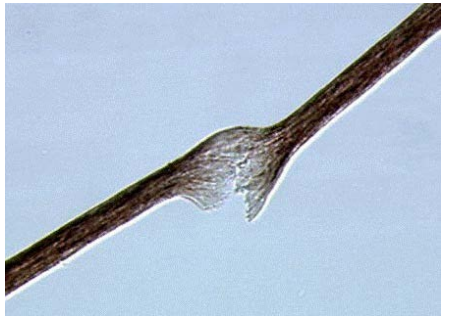
ASL deficiency

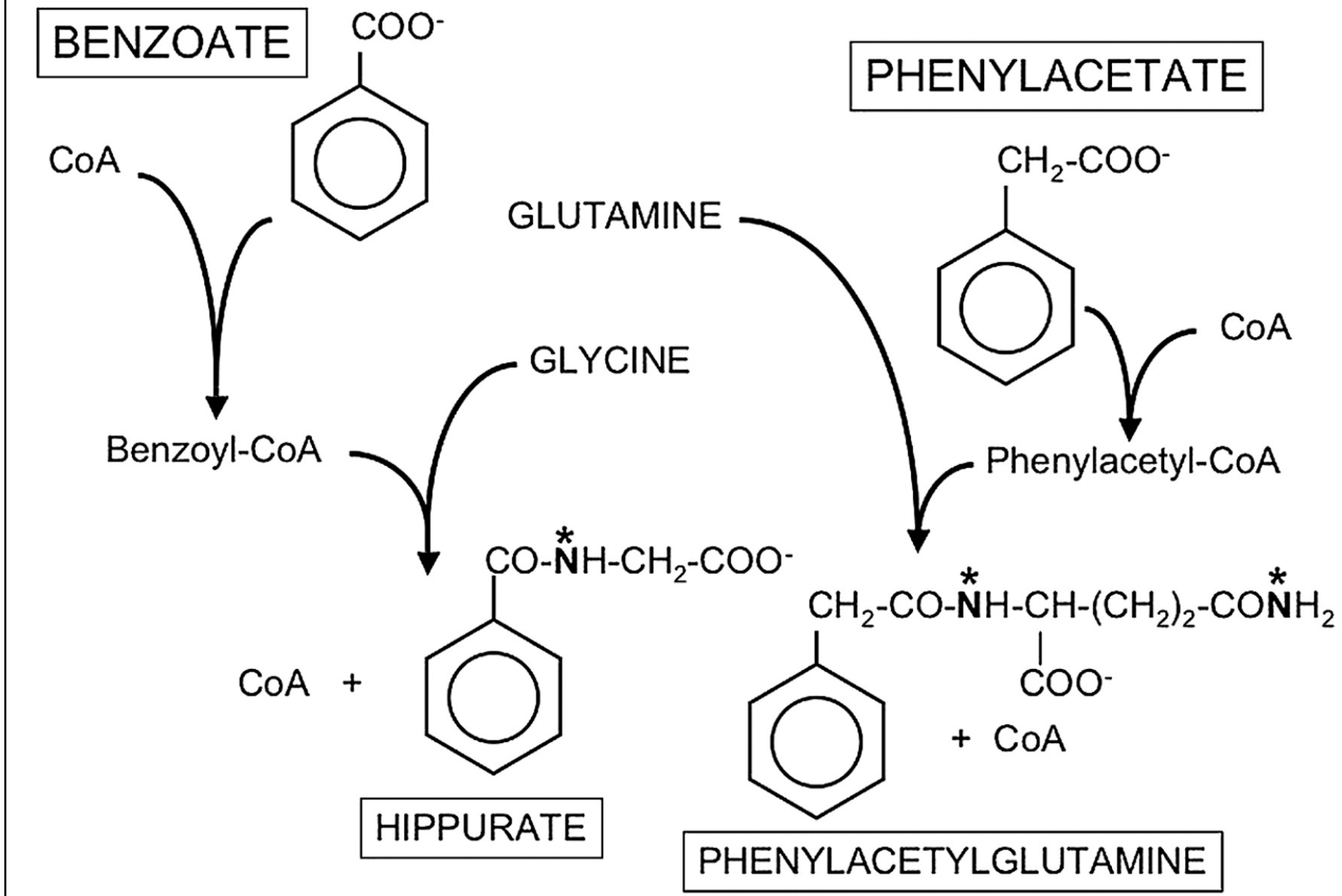


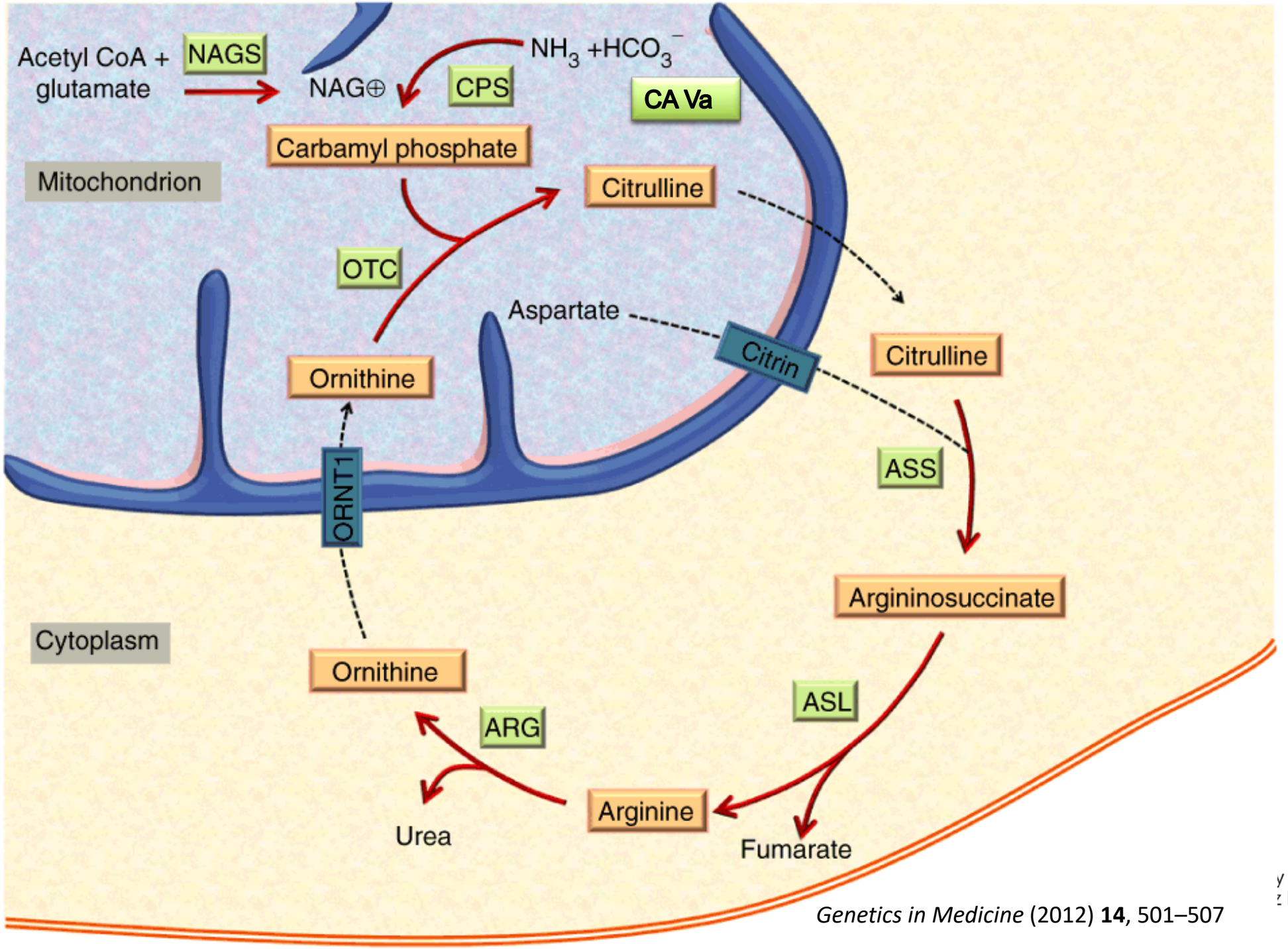
- AKA argininosuccinic aciduria
- Clinical
  - Neonatal hyperammonemic coma
  - Fragile hair (trichorrhexis nodosa)
  - Liver disease: hepatomegaly, dysfunction, even chronic cirrhosis
  - Late-onset hyperammonemia ± neuropsychiatric disease
- Laboratory
  - Plasma and urine ASA (only disorder where it's detected)
  - Plasma citrulline and glutamine elevated
  - Plasma arginine low or normal
- Diagnosis
  - DNA sequencing
  - Enzyme activity measured in fibroblasts

Alternative Treatment Strategies for Acute Hyperammonemia

1. Reduce ammonia synthesis (adequate energy, low protein, essential amino acids)
2. Remove ammonia (hemodialysis)
3. Provide a detour (benzoate, phenylacetate)
4. Reprime open urea cycle (arginine)
5. Remove inhibitor (carnitine)
6. Replace activator (carbamylglutamate)

"AMMONIA-SCAVENGING" MEDICATIONS





Genetics in Medicine (2012) **14**, 501–507

Arginase deficiency

Clinical

- Unique clinical presentation
- Rarely neonatal jaundice
- Spastic diplegia, progressive intellectual disability with deterioration
- Growth arrest
- Asymptomatic or mild forms in case of residual enzyme activity

Arginase deficiency

- Laboratory
  - Elevated plasma arginine, glutamine
  - Hyperammonemia may be intermittent
  - Elevated to normal urine orotic acid
- Diagnosis
  - DNA sequencing
  - Enzyme assay in red blood cells

Hyperammonemia in IEMs

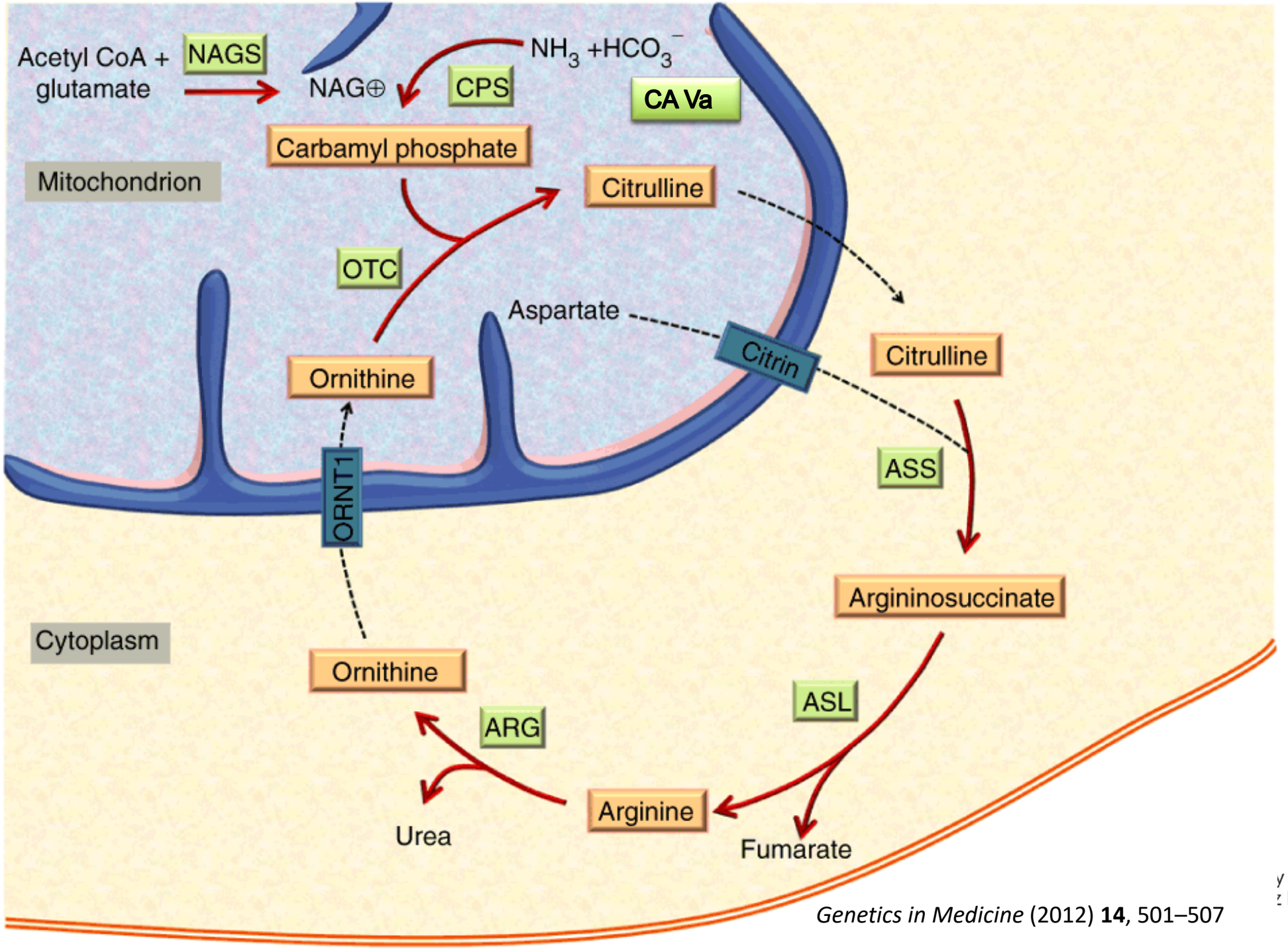
Primary

- Urea cycle enzymes (6 of these)
- Related disorders
  - Transporter defects
    - Lysinuric protein intolerance (renal, intestinal SLC7A7)
    - Hyperammonemia, hyperornithinemia, homocitrullinuria (mitochondrial, SLC25A15 (ORNT1))
    - Citrullinemia type II due to Citrin deficiency (SLC25A13)
  - Ornithine aminotransferase (OAT) deficiency, in infancy
  - Pyrolline-5-carboxylate synthetase
  - Carbonic anhydrase VA (CAVA, bicarbonate donor to CPS)

Hyperammonemia in IEMs

Secondary

- Organic acidemias
- Fatty acid β-oxidation defects
- Mitochondrial respiratory chain defects
- Primary lactic acidoses (pyruvate carboxylase deficiency, pyruvate dehydrogenase deficiency)
- Carbonic anhydrase VA (CAVA) (bicarbonate donor to CPS and to mitochondrial carboxylases)



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Non-IEM causes

- Drug-related (e.g., valproate — but valproate can unmask a UCD)
- Acute liver failure (e.g., hepatotoxins, infections such as herpes in newborns — but UCDs can mimic liver failure)
- Reye syndrome (a clinical diagnosis — rule out IEMs including UCDs)

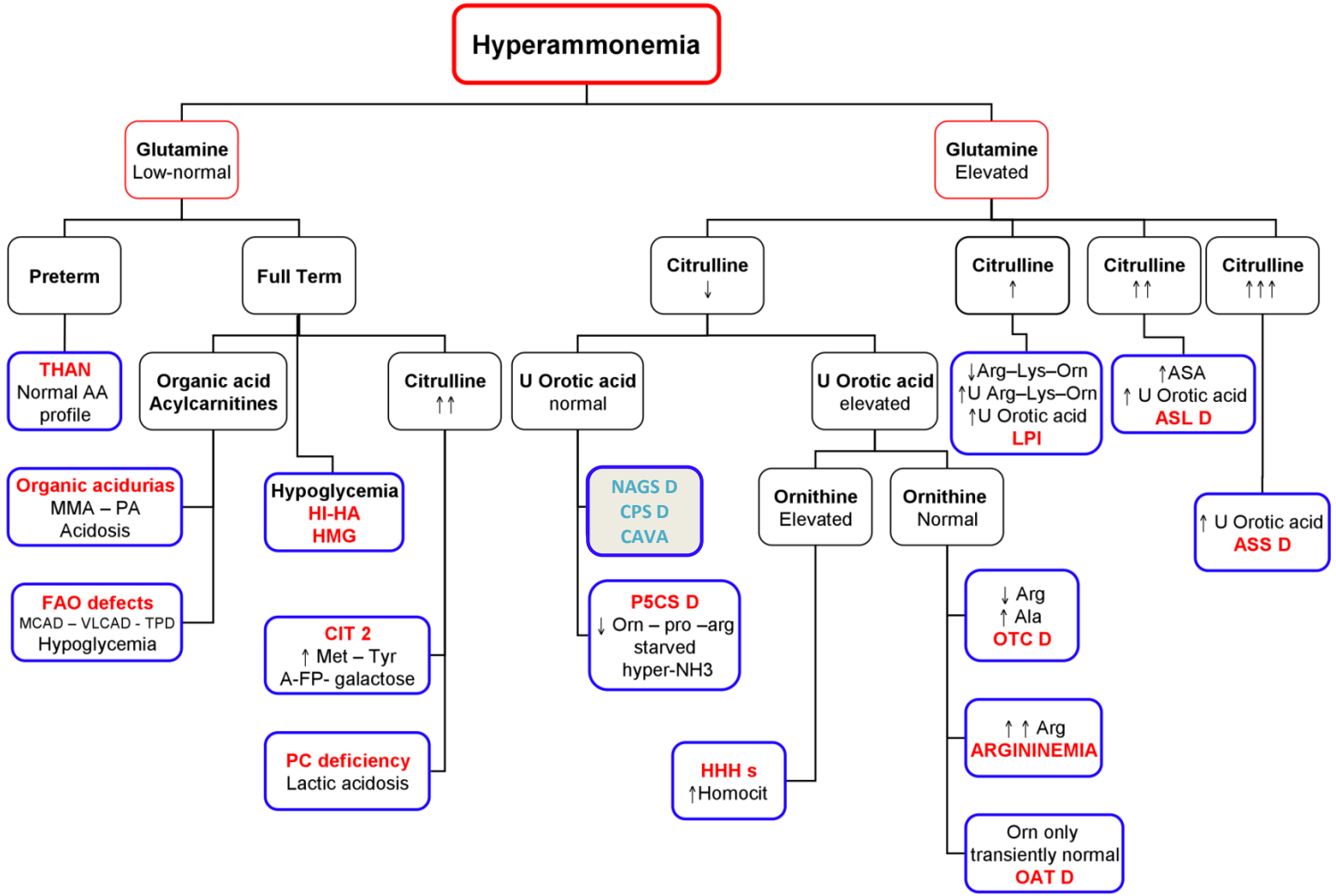
Non-IEM causes

- Transient hyperammonemia in the newborn (high ammonia to glutamine ratio, >1.6)
  - Cause remains unknown
  - Transient portocaval shunt?
- Chronic urinary tract infection with urinary retention, due to urease-producing bacteria, esp. with bladder reconstructive surgery, stasis
- Overgrowth of bowel flora

Non-IEM causes

- Chronic degenerative liver disease (cirrhosis)
- Portocaval shunts (get liver ultrasound with Doppler)
- Massive tissue necrosis (e.g. leukemia treatment)
- Bacterial overgrowth with urea-splitting organisms
  - Short gut; severe constipation in children with developmental disabilities
  - Worsened by renal insufficiency – increased recycling of urea

Neonatal hyperammonemia



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- Pete Baker – for running with the idea of this series
- Johan Van Hove – originally suggested the idea after the 6th zoom call in one day in March
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