Amino Acids

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Amino Acids: Case 1

- **Clinical Presentation**
- Female born by elective caesarean section at 39 weeks due to previous maternal caesarean section, uneventful antenatal period
- Admitted to the neonatal unit following a dusky episode after her second feed
- Metabolic acidosis (Lactate 17.7 mmol/L) (unresponsive to Bicarbonate corrections)
- Hyperammonaemia (310µmol/L)
- Transferred to PICU for possible haemofiltration (Ammonia 473 µmol/L)
- Normal neurology on admission, intubated and sedated with morphine
- Three failed attempts at haemofiltration due to blood in filter repeatedly clotting
- Commenced on sodium benzoate, sodium phenylbutyrate and carnitine
- 10% dextrose

Differential Diagnosis?

- -Organic Acid Disorders (eg. Propionic Aciduria, MMA, IVA)
- -Fatty Acid Oxidation Disorder (eg. MCADD, CPTII)
- -Mitochondrial Respiratory Chain defect
- -Pyruvate dehydrogenase deficiency
- -Congenital lactic acidosis

Metabolic Investigations (1)

- **Organic Acid Analysis**

-Massive excretion of lactate possibly secondary to clinical condition/perfusion/infection. Please check plasma lactate. Additionally marked ketonuria with appropriate dicarboxylic aciduria, and marked excretion of 4-hydroxyphenyllactate and 4-hydroxyphenylpyruvate which is suggestive of liver dysfunction. Please check liver function including clotting. Orotic acid not detected. Overall, nothing specifically diagnostic.

Metabolic Investigations (2)

Plasma Amino Acid Analysis

Taurine:	74 µmol/L	(40-420)
Threonine:	238 µmol/L	(10-400)
Serine:	57 µmol/L	(50-350)
Glutamic Acid:	34 µmol/L	(0-250)
Glutamine:	**184 µmol/L**	**(487-1031)**
Proline:	439 µmol/L	(50-450)
Glycine:	212 µmol/L	(200-600)
Alanine:	489 µmol/L	(100-800)
Ornithine:	57 µmol/L	(25-225)
Lysine:	**531 µmol/L**	**(105-315)**
Citrulline:	**121 µmol/L**	**(0-40)**
Valine:	136 µmol/L	(50-400)
Methionione:	49 µmol/L	(0-80)
Isoleucine:	46 µmol/L	(0-150)
Leucine:	85 µmol/L	(20-280)
Tyrosine:	181 µmol/L	(30-135)
Phenylalanine:	80 µmol/L	(40-110)
Histidine:	102 µmol/L	(45-150)
Arginine:	55 µmol/L	(10-70)
Aspartic Acid:	**<5 µmol/L**	**(40-420)**

Possible Diagnosis?

- **Causes of Raised Citrulline**

  -Citrullinaemia

  -Argininosuccinic Aciduria

  -Pyruvate carboxylase deficiency

  -Saccharopinuria

- **Causes of Raised Lysine**

  -Hyperlysinaemia

  -Pyruvate carboxylase deficiency

  -Saccharopinuria

Diagnosis: Pyruvate Carboxylase Deficiency

- Raised citrulline and lysine in the context of a neonate with sever lactic acidosis and hyperammonaemia are highly suggestive of pyruvate carboxylase deficiency. Recommend skin biopsy for pyrivate carboxylase enzyme assay in fibroblasts to confirm/exclude this diagnosis.

- Diagnosis confirmed by enzyme analysis in fibroblasts

- Patient became cardiovascularly unstable and died of cardiorespiratory failure 24 hours later

Pyruvate Carboxylase Deficiency

- Autosomal recessive condition
- Incidence 1:250,000 live births
- Three clinical presentations:

Type A (Infantile or North American Form)

Type B (Severe Neonatal or French Form)

Type C (Intermittent/Benign Form)

Clinical Presentations

- Type A (Infantile Form)

Mild metabolic acidosis, delayed motor development, intellectual impairment, failure to thrive, hypotonia, ataxia, convulsions. Episodes of acute vomiting, tachypnoea and acidosis precipitated by metabolic/infection stress.

Death in early infancy or early childhood, some survive to maturity

- Type B (Severe Neonatal Form)

Hypoglycaemia, hyperammonaemia, hypernatraemia, hepatomegaly, convulsions, abnormal movements, severe impaired motor development, intellectual disability.

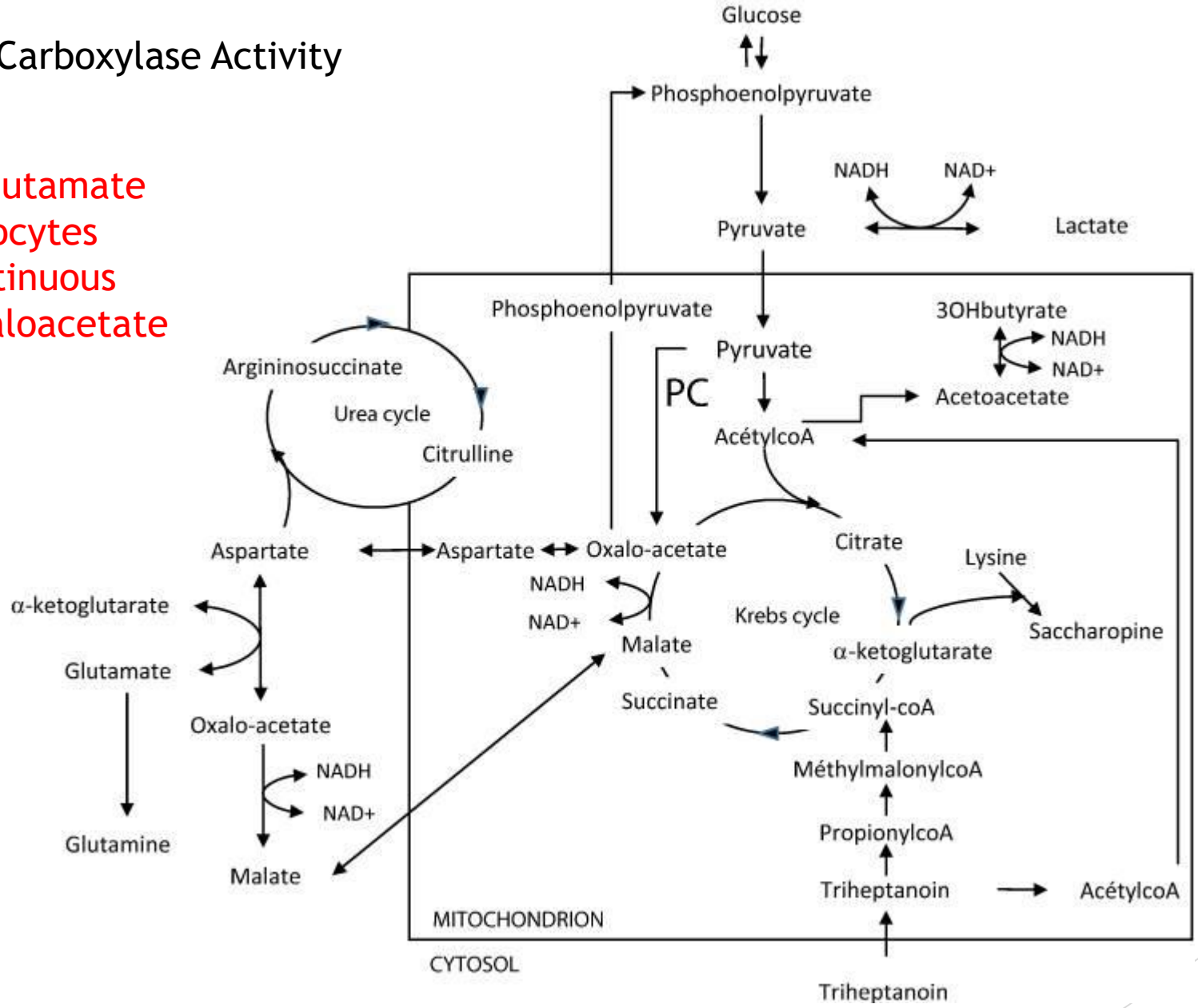
Majority of affected infants die within first three months of life

- Type C (Intermittent/Benign Form)

Mild delayed neurological development, episodic metabolic acidosis

Pyruvate Carboxylase Activity

Glutamine-glutamate cycle in astrocytes requires continuous supply of oxaloacetate



Habarou *et al* (2015) Mol Genet Metab Rep 2, 25-31

Biochemical Abnormalities

- Lactate and Pyruvate
  - Elevated pyruvate leading to increased lactate levels
  - Increased lactate:pyruvate ratio in Type B (>20), but typically normal in types A and C
- Ketonaemia
  - Increased 3-OH butyrate and acetoacetate
- Hypoglycaemia
  - Deficiency of oxaloacetate limits gluconeogenesis
  - Not a consistent finding
- Hyperammonaemia
- CSF Measurements
  - Increased lactate and pyruvate levels
  - Reduced glutamine concentrations
  - Increased glutamic acid and proline concentrations

Pyruvate Carboxylase Deficiency and Amino Acids

- Serum and Urine Amino Acid Abnormalities

- Elevated Alanine
- Elevated Citrulline
- Elevated Lysine
- Low Aspartic Acid
- Low Glutamine

Differential Diagnosis

- Biotinidase deficiency
- Pyruvate dehydrogenase complex (PDHC) deficiency
- Respiratory chain disorder
- Krebs cycle disorder
- Gluconeogenic disorder

Ultimately diagnosis of Pyruvate Carboxylase deficiency is by enzyme analysis in cultured fibroblasts and molecular genetic analysis

Management of Pyruvate Carboxylase Deficiency

- Anaplerotic therapy
  - -Citrate supplementation
  - -Aspartic acid supplementation
  - -Biotin (little effect)
  - -Triheptanoin
- Liver transplantation
- Prevention of crisis/decompensation
  - -Emergency regimen
  - -Minimise infections, stress (fasting/ketogenesis)
  - -High-carbohydrate, high-protein diet

Amino Acid Case 2

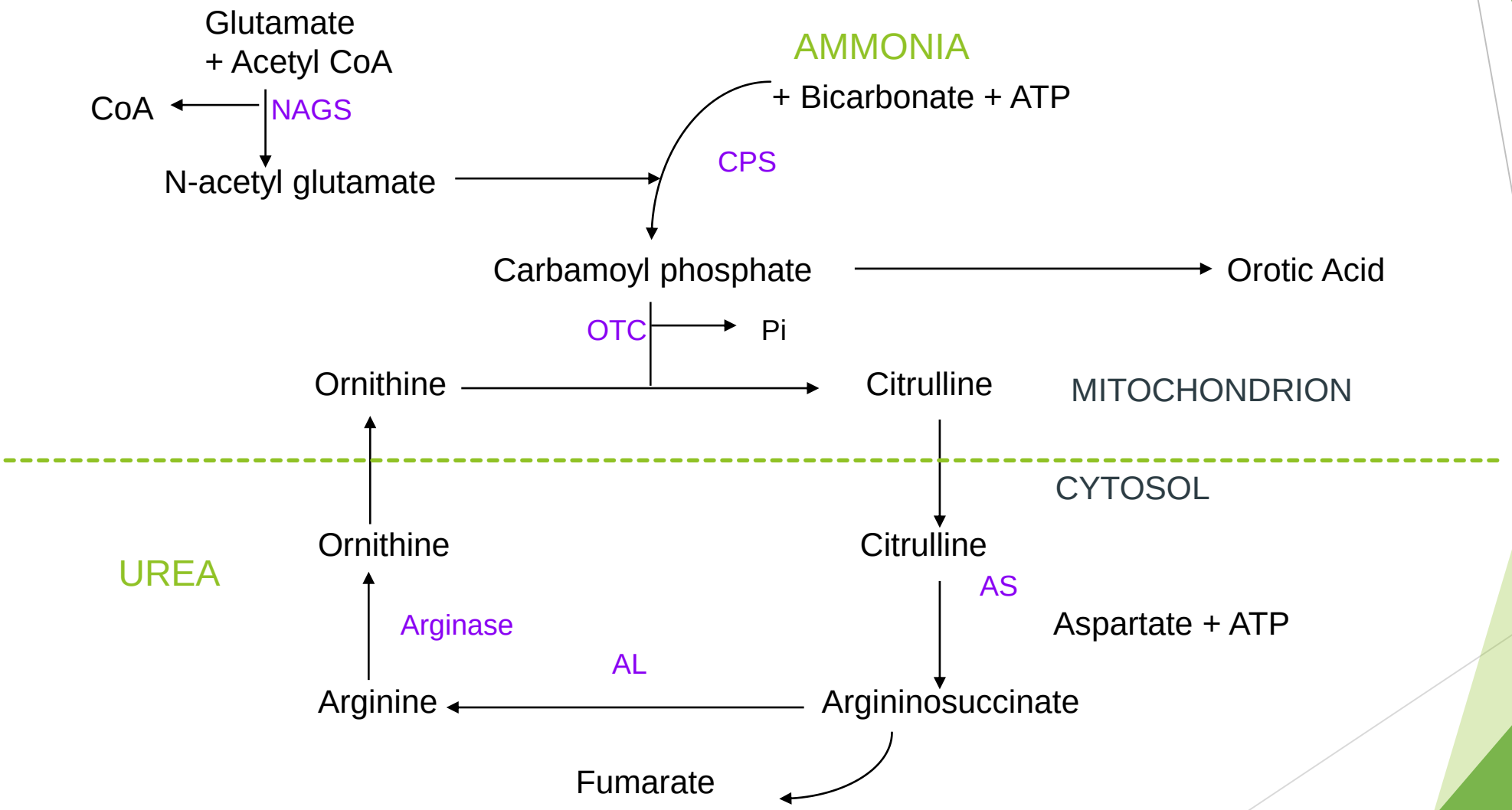
- 10 month old male patient presented with spastic diplegia
- No acute presentations and seen by community paediatrics team
- Basic metabolic investigations completed

Plasma Amino Acid Analysis

Taurine:	83 µmol/L	(0-250)
Threonine:	46 µmol/L	(20-280)
Serine:	80 µmol/L	(40-280)
Glutamic Acid:	64 µmol/L	(0-300)
Glutamine:	**381 µmol/L**	**(550-830)**
Proline:	121 µmol/L	(75-450)
Glycine:	135 µmol/L	(100-425)
Alanine:	349 µmol/L	(100-800)
Ornithine:	42 µmol/L	(20-200)
Lysine:	50 µmol/L	(40-280)

Citrulline:	**9 µmol/L**	**(10-60)**
Valine:	64 µmol/L	(75-387)
Methionine:	17 µmol/L	(0-50)
Isoleucine:	18 µmol/L	(0-150)
Leucine:	85 µmol/L	(20-150)
Tyrosine:	28 µmol/L	(20-160)
Phenylalanine:	32 µmol/L	(40-140)
Histidine:	59 µmol/L	(35-130)
Arginine:	**503 µmol/L**	**(0-120)**
Aspartic Acid:	**<5 µmol/L**	**(0-110)**

Urea Cycle


Glutamate
+ Acetyl CoA
CoA
NAGS
N-acetyl glutamate
AMMONIA
+ Bicarbonate + ATP
CPS
Carbamoyl phosphate
Orotic Acid
OTC
Pi
Ornithine
Citrulline
MITOCHONDRION
CYTOSOL
Ornithine
Citrulline
UREA
AS
Aspartate + ATP
Arginase
AL
Arginine
Argininosuccinate
Fumarate

Arginase Deficiency

- Rare inborn error of the urea cycle 1:950,000 live births (Summar *et al*, 2013)
- Presents with progressive spastic paraplegia, typically in first decade of life

- Clinical Symptoms: (Freua *et al* (2022) Cold Spring Harb Mol Case Stud **8** a006232)

  -Progressive spasticity (predominantly in lower limbs)

  -Progressive mental impairment

  -Growth retardation

  -Epilepsy/Seizures

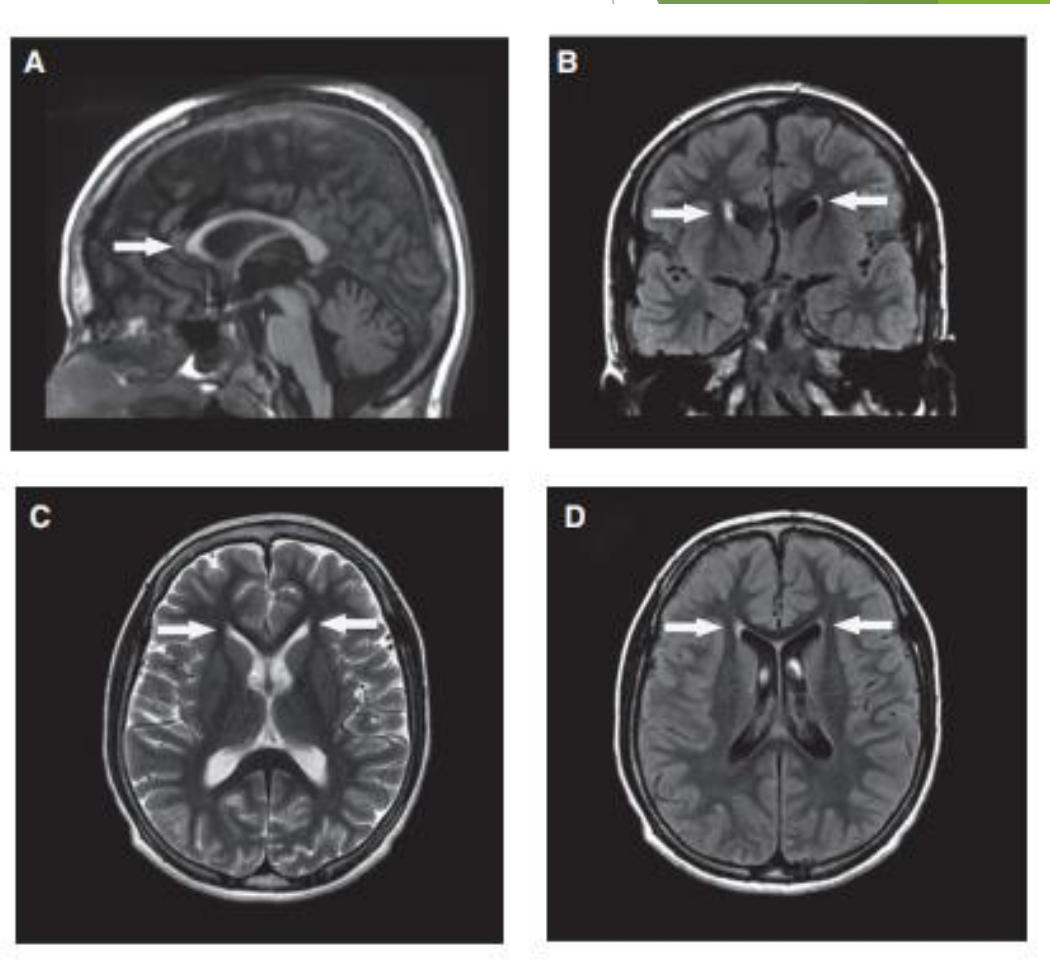
  -Periodic episodes of hyperammonaemia

  (May not present with hyperammonaemic encephalopathy in neonatal period and ammonia levels not as high as other urea cycle disorders)

  -Slow disease progression

Neurological Abnormalities with Arginase Deficiency

- Variable reports of abnormal neuroradiological findings
- Mild cerebellar atrophy frequent finding
- Signal changes in the posterior putamen
- Global brain oedema (more common in neonatal-onset patients)
- Basal ganglia involvement
- Cystic lesions

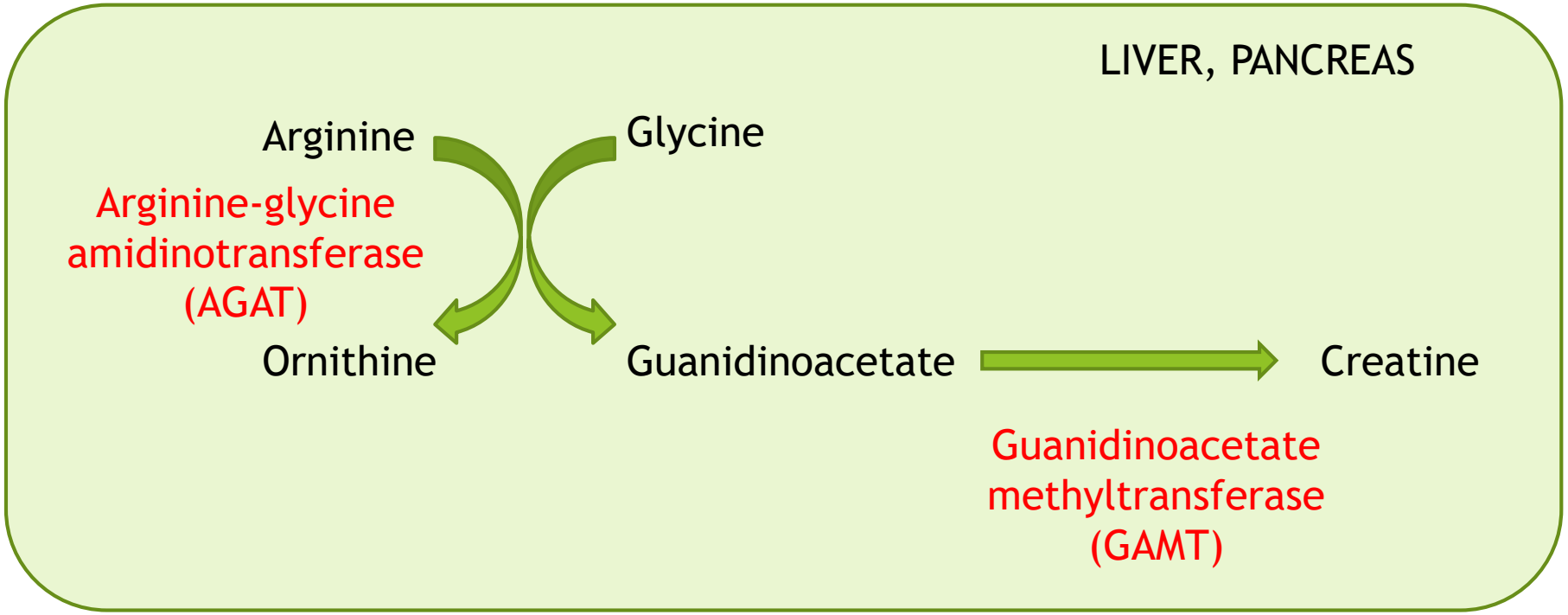


(Freua *et al* (2022) Cold Spring Harb Mol Case Stud **8** a006232)

Treatment of Arginase Deficiency

- Arginine-restricted diet
  - -Early treatment can stabilise condition and lead to clinical improvement
  - -Not all patients respond
  - -Late adolesence/Adulthood treatment showed no clinical response
- Ammonia scavenging drugs
  - -Benzoate/Phenylbutyrate
- Role for measurement of guanidinoacetate?

Arginase Deficiency and Guanidoacetate



Creatine Biosynthesis Pathway

Guanidinoacetate is an epileptogenic compound and may be the cause of neurological impairment and epilepsy (Amayreh *et al* (2014)). Guanidino compounds result in oxidative stress *in vitro* which may also cause neurological damage.

Dietary arginine restriction, creatine and ornithine supplementation and benzoate treatment (as utilised in GAMT deficiency) could potentially "treat" the disorder.

Arginase Deficiency and Guanidoacetate

Urine Guanidinoacetate: 352 µmol/mmol creatinine (4-220)

Urine Creatine: 2584 µmol/mmol creatinine (6-1200)

- Guanidinoacetate synthesised from accumulation of arginine
- Treatment with ornithine, creatine and benzoate decreased levels of guanidinoacetate and has been shown in some patients to reduce number and duration of seizures

Amino Acid Case 3

- 15 month old female patient presented with “early developmental impairment
- No previous history and standard metabolic investigations completed

Amino Acid	Initial Results (µmol/L)	One Month Later (µmol/L)	Two Months Later (µmol/L)	Reference Range
Taurine	98	97	61	29-211
Aspartate	10	6	5	5-52
Threonine	100	82	77	48-195
Serine	140	116	116	66-231
Glutamic Acid	109	96	56	20-180
Glutamine	533	428	442	279-695
Proline	203	172	156	95-429
Glycine	129	134	96	133-455
Alanine	202	190	174	145-563
Citrulline	9	19	11	10-51
Valine	673	585	479	115-339
Methionine	18	20	22	11-40
Isoleucine	209	200	148	29-102
Leucine	337	361	273	62-209
Tyrosine	73	87	83	34-127
Phenylalanine	57	71	111	35-105
Histidine	77	76	68	47-108
Ornithine	66	82	62	24-139
Lysine	170	168	156	73-250
Arginine	31	32	68	17-149

Patient not hypoglycaemic/catabolic at any of the times of sampling

Causes of Raised Branched Chain Amino Acids

- Catabolism/Hypoglycaemia
- Obesity
- Diabetes

- E3 Lipoamide dehydrogenase deficiency
- Maple Syrup Urine Disease (MSUD)
- Hypervalinemia (Valine only raised)
- Hyperleucine-isoleucinemia and hypervalinemia (BCAT2)

Post Pyridoxine Treatment

Amino Acid	Post Treatment Results (µmol/L)	Reference Range
Taurine	89	29-211
Aspartate	44	5-52
Threonine	104	48-195
Serine	268	66-231
Glutamic Acid	103	20-180
Glutamine	369	279-695
Proline	97	95-429
Glycine	353	133-455
Alanine	384	145-563
Citrulline	19	10-51
Valine	107	115-339
Methionine	16	11-40
Isoleucine	33	29-102
Leucine	71	62-209
Tyrosine	56	34-127
Phenylalanine	47	35-105
Histidine	71	47-108
Ornithine	109	24-139
Lysine	123	73-250
Arginine	63	17-149

Amino Acid results normalised following pyridoxine treatment.
?BCAT2 deficiency

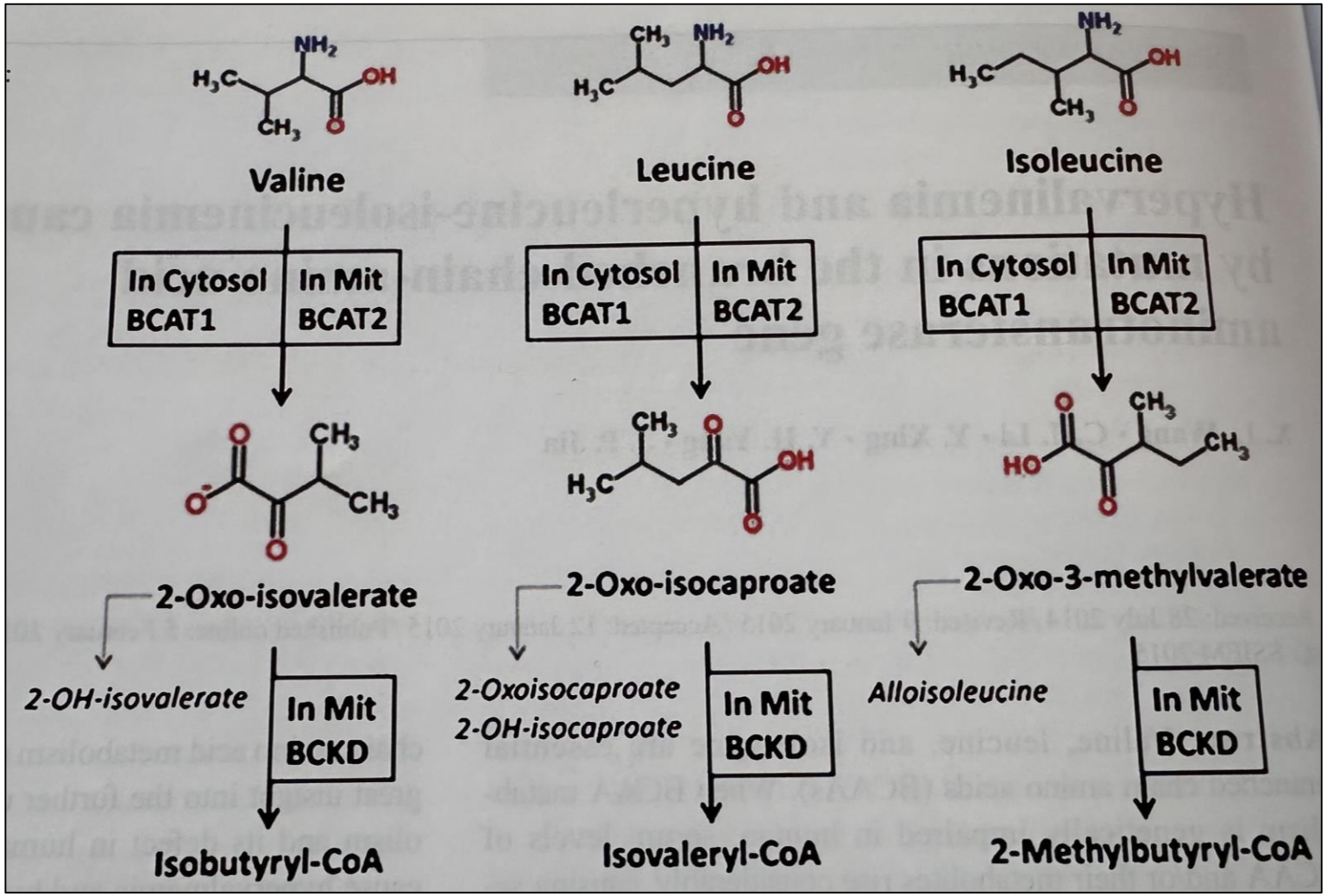
BCAT2 Deficiency

- The branched chain amino acids (Valine, Leucine and Isoleucine) are essential amino acids as they cannot be synthesised *de novo* in mammals (Tom and Nair, 2006).

- Branched chain amino acids account for 35% of all essential amino acids in muscle proteins and 40% of preformed amino acids required by mammals (Lu *et al*, 2013).

- Dysregulation of the branched chain amino acid catabolic pathways results in accumulation of excess branched chain amino acids and this is toxic to the central nervous system (Hutson et al,2005).

Branched Chain Metabolism (1)



J. Inherit Metab Dis (2015) 38: 855-861

Branched Chain Amino Acid Metabolism (2)

- The first two steps in BCAA catabolism are common to all three amino acids
- BCAA amino transferase (BCAT) catalyses the transamination of the BCAA to their α-ketoacids
- Two isoforms of BCAT: BCAT1 (cytoplasm) and BCAT 2 (mitochondria)
- BCAT2 is the predominant human form and is expressed in most tissues

- First case of BCAT2 deficiency was published in 2015 (*J. Inherit Metab Dis* 38: 855-861)

Clinical details:	25 year old male presented with headache (lasted 6 yrs), mildly impaired memory, normal muscular power and co-ordination
Brain MRI and MRS:	Symmetric abnormal signals in bilateral frontal lobes, occipital lobes, periventricular white matter and callosum. Low T1 signals and high T2 signals. Decreased N-acetyl aspartate (NAA) peak by MRS.
Laboratory data:	Valine – 1754 µmol/L, Leu/Ile – 646 µmol/L
BCAT genetics:	Two heterozygous mutations in BCAT2.
	Expression of mutant forms as His-tagged recombinant proteins showed reduced BCAT2 enzyme activity

BCAT2 Deficiency

- Consider BCAT2 deficiency in cases of hypervalinaemia and hyperleucine-isoleucinaemia without elevated branched chain α-keto acids (BCKAs)

- Clinical symptoms may be mild but brain MRI show serious white matter lesions. Decreased NAA signal by MRS indicates neuronal damage due to prolonged exposure to high brain BCAA concentrations

- BCKAs and leucine may cause oxidative stress and/or energy deficits leading to neurological damage.

  BCAT catalyses the transfer of an amino group from a BCAA to α-ketoglutarate, forming glutamate. As glutamate is the major excitatory neurotransmitter and precursor of the major inhibitory neurotransmitter (GABA), BCAT mutations could lead to abnormal glutamate metabolism resulting in neurological symptoms.

- Treatment with Vitamin B6 (coenzyme of BCAT2) reduces BCAA levels and has shown MRI improvements (decreased size distribution of white matter lesions and reduced T2 high signals). Some improvement in memory impairment.

?BCAT2 Deficiency

- Genetic analysis didn't identify any mutations within the BCAT2 gene
- The metabolic team determined that the patient had poor nutritional status and instigated NG feeding. Following supported feeding the branched chain amino acids have remained within the normal range.
- Likely that patient was previously catabolic resulting in increased branched chain amino acids
- Consider other causes for raised branched chain amino acids if they are persistent or not associated with catabolism

Amino Acid Case 4

- Full-term baby boy born by CS due to fetal distress and poor growth
- Birth weight (10^{th}-25^{th} centile), Head circumference (<5^{th} percentile)
- Intractable seizures within hours after birth
- Myoclonic seizures
- Discharged once seizures controlled with antiepileptic medication but continued to have intermittent myoclonic seizures

- Follow-up

  -Severe microcephaly

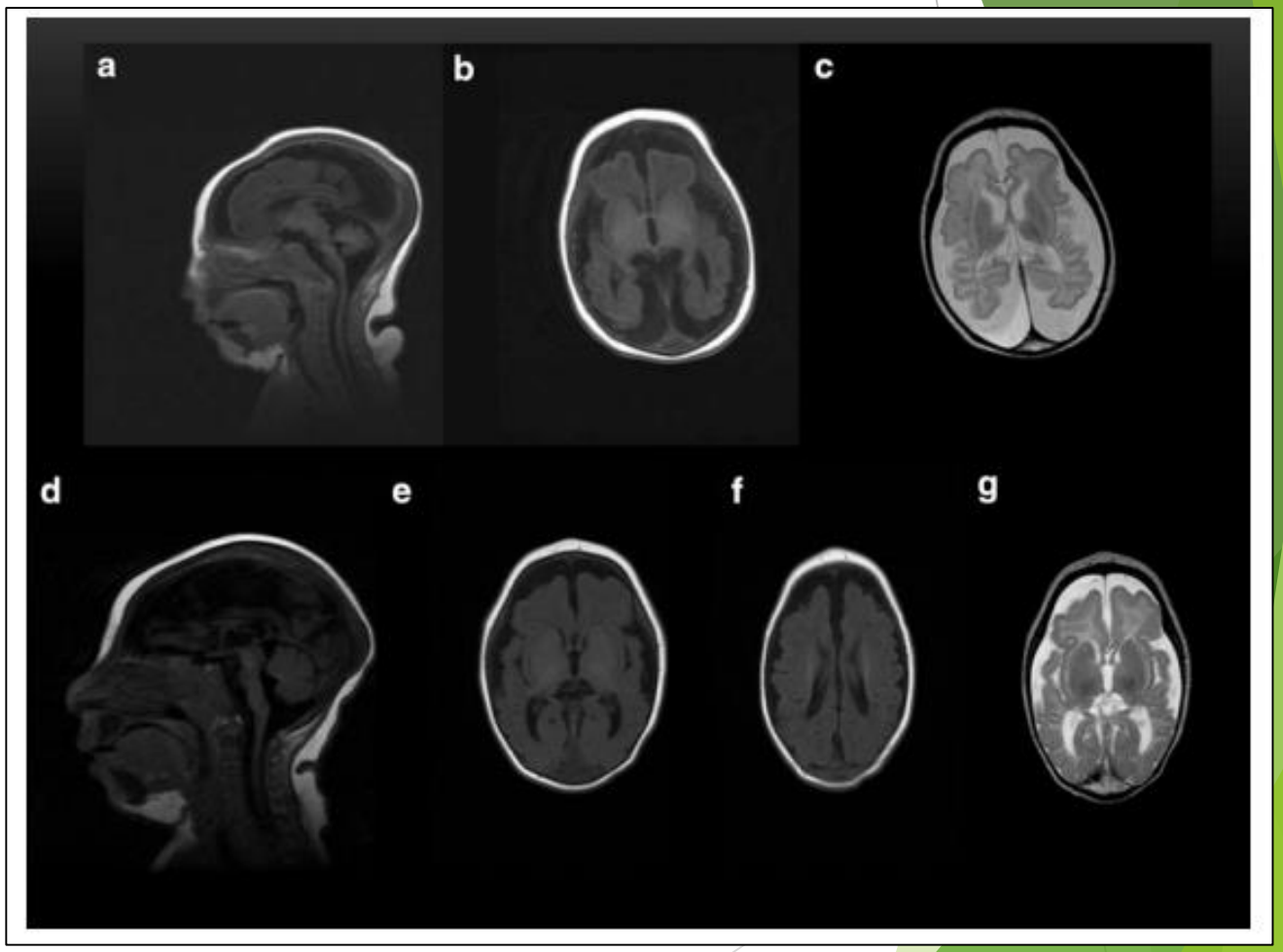
  -All growth parameters <3^{rd} percentile

  -Subtle dysmorphic features: brachycephaly, pear-like head shape, micrognathia

MRI Findings

- Microcephaly
- Brain atrophy
- Delayed Myelination

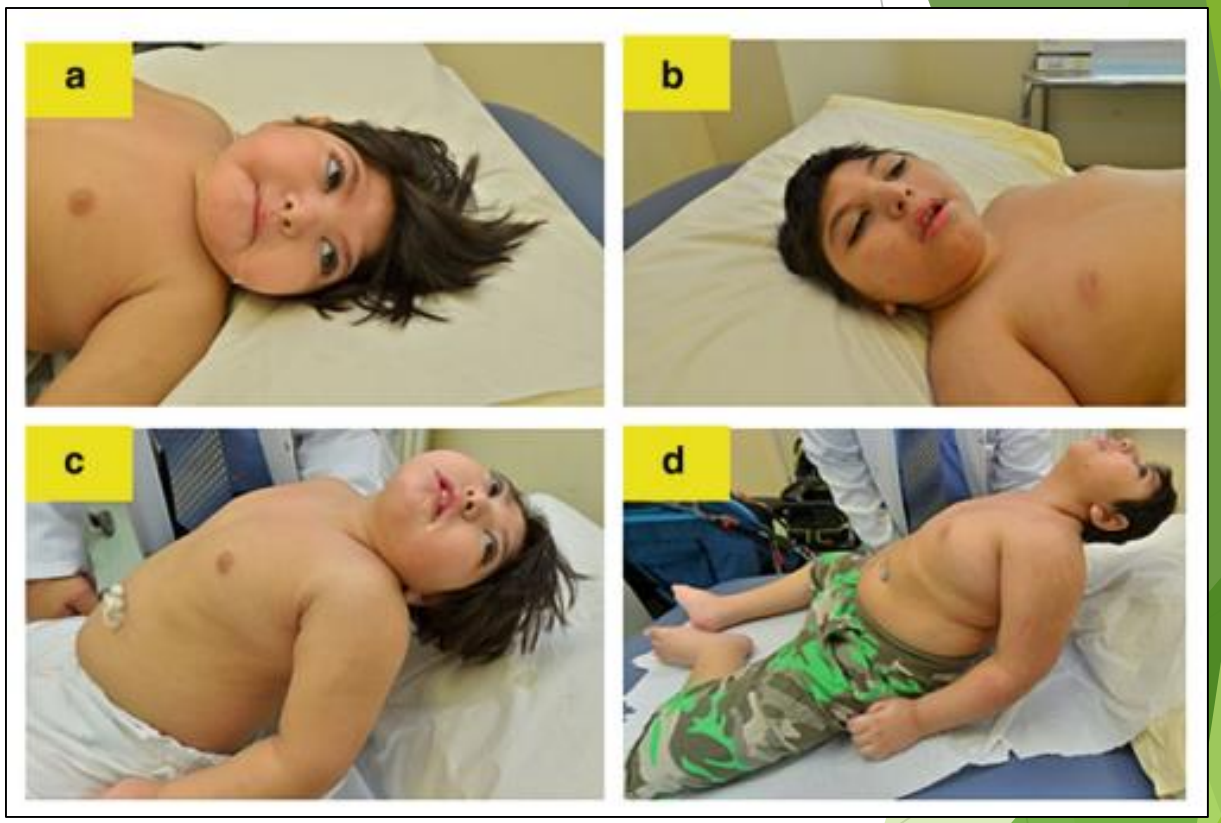
- EEG showed multifocal epileptic discharges and diffuse slowing



Alfadhel *et al* (2014) JIMD Vol 22: 11-16

Clinical Presentation

- Microcephaly
- Brachycephaly
- Pear-like head shape
- Micrognathia
- Axial hypotonia
- Severe developmental delay
- Intractable seizures
- Gastroesophageal reflux



Alfadhel *et al* (2014) JIMD Vol 22: 11-16

Biochemistry

- Normal acylcarnitines, urine organic acids, CK, total homocysteine, lactate and ammonia
- CSF neurotransmitters showed slightly low HVA and 5-HIAA levels

- Amino Acid Results

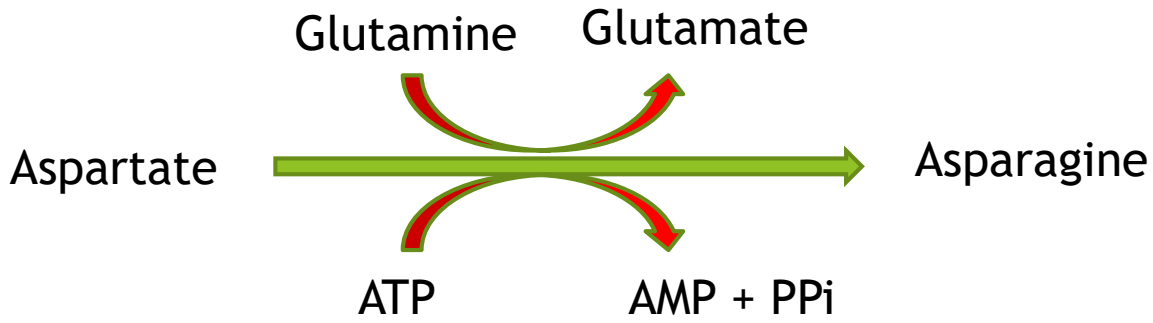
Low plasma asparagine:	10 µmol/L	(33-68.4 µmol/L)
Normal plasma glutamine:	339 µmol/L	(254-823 µmol/L)
Low CSF asparagine:	Undetectable	(1.1 – 6.9 µmol/L)
High CSF glutamine:	922 µmol/L	(356 – 680 µmol/L)

(Low plasma asparagine is not as sensitive a marker as low CSF asparagine)

- Whole exome sequencing identified a homozygous mutation in exon 10 of the asparagine synthetase (ASNS) gene

Asparaginase Deficiency

- Asparagine synthetase in required in the biosynthesis of asparagine



- Defects in Asparagine synthetase lead to low asparagine levels in CSF (note ASNS is highly expressed in the adult brain).
- Asparagine is a key neurotransmitter so low levels lead to neurological impairment
- Very rare – only 20 cases reported worldwide

Asparaginase Deficiency

- Clinical features: Microcephaly (100%)
  Developmental delay (100%)
  Intractable seizures (75%)
  Axial Hypotonia (67%)
- Radiological features: Microcephaly, brain atrophy, delayed myelination (100%)
- EEG features: Multiple independent spike foci (67%)
  Others: Burst suppression pattern, hypsarrhythmia
- Diagnosis: Molecular testing (ASNS gene)
- Treatment: Antispastic medication (Baclofen)
  Clonazepam for hyperkyplexia
  Ventilation support
  NG tube for feeding
- Prognosis: Poor – 50% die within first year of life

Urea Cycle Disorders- An Easy Diagnosis?

Clinical History:

- Two day old infant admitted to PICU with suspected sepsis
- Unremarkable birth history and normal pregnancy
- NH_3 2452 µmol/L
- pH 7.3, HCO_3 18.0 mmol/L, Base Excess -6.7 mmol/L

Observations from amino acid profile:

Pronounced glutamine level

Slightly increased alanine level

Low citrulline level

Increased lysine level

Low arginine

Could this be a urea cycle disorder?

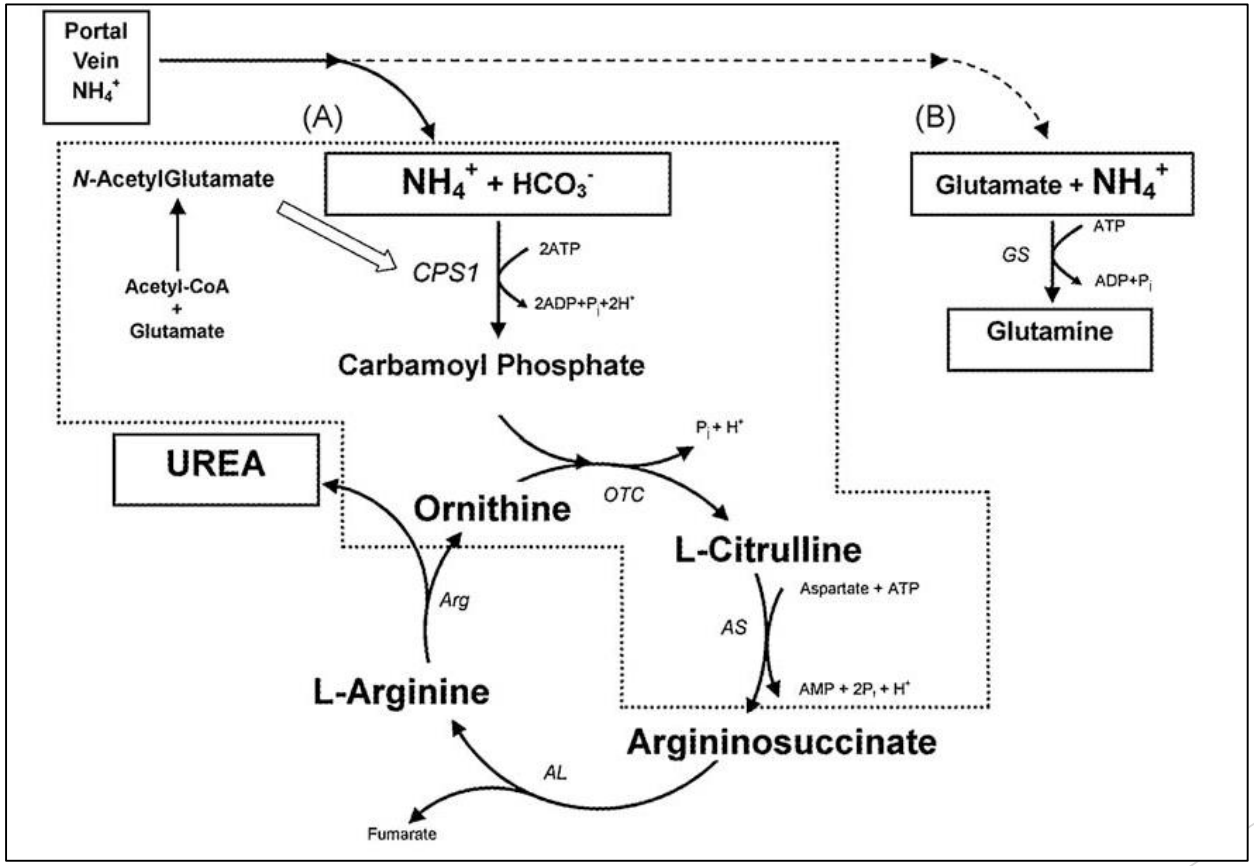
Amino Acid	Concentration (umol/L)	Reference Range
Taurine	143	92-392
Threonine	188	97-350
Serine	293	90-279
Glutamic Acid	178	110-338
Glutamine	1403	248-846
Proline	225	
Glycine	346	220-527
Alanine	630	160-610
Citrulline	0.7	8-36
Valine	152	93-238
Methionine	17	18-62
Isoleucine	41	26-100
Leucine	134	58-162
Tyrosine	73	48-120
Phenylalanine	45	41-111
Ornithine	190	58-251
Lysine	315	92-267
Histidine	97	60-135
Arginine	6	26-216

Amino Acid Interpretation (1)

- Glutamine

  Glutamine synthetase catalyses the condensation of ammonia with glutamate to glutamine.

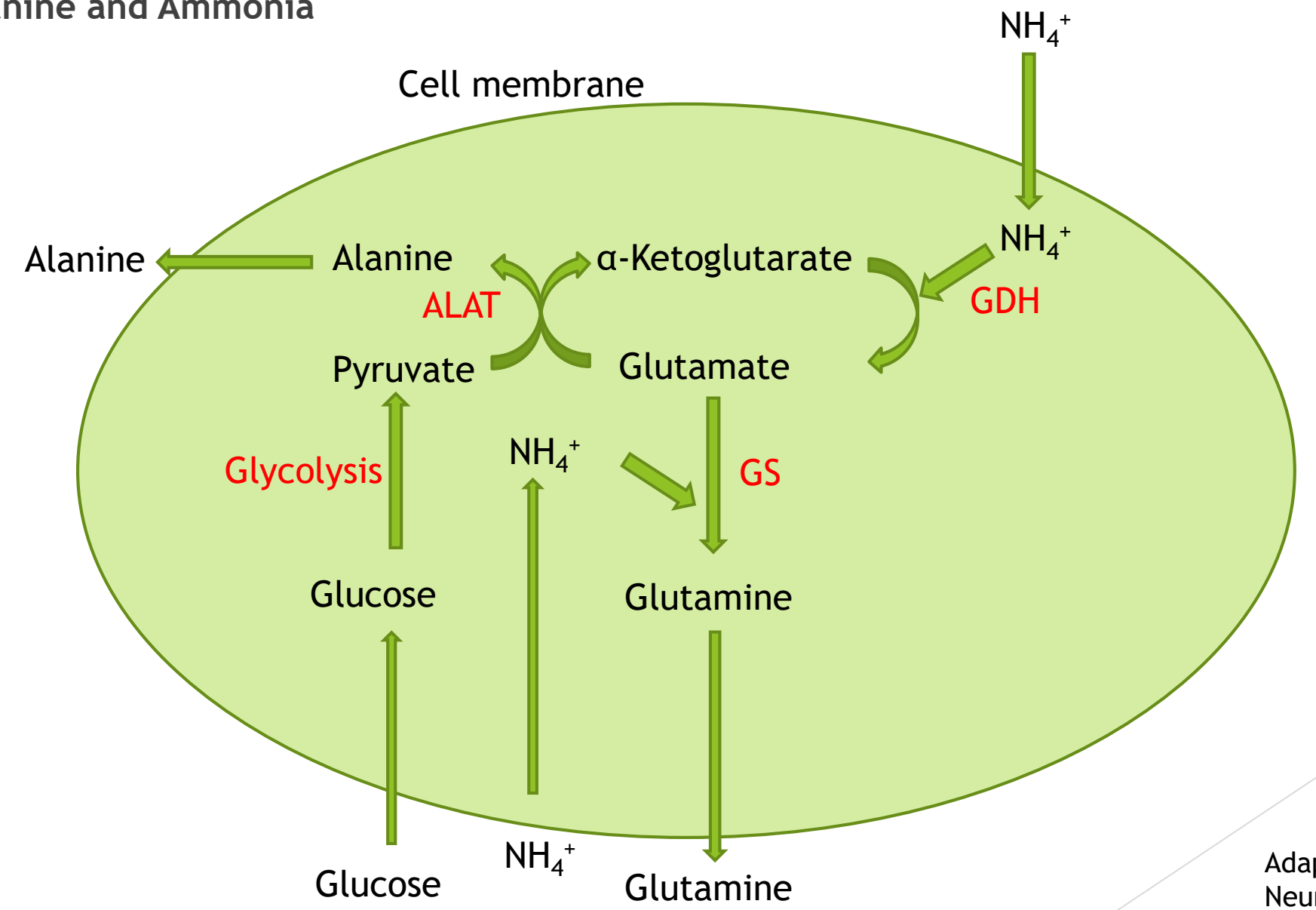
  Glutamine, together with alanine, serves as a non-toxic interorgan ammonia carrier. (Hakvoort *et al* (2016) Hepatology **65**)



Wilkinson *et al* (2010)
Progress in Neurobiology

Amino Acid Interpretation (2)

Alanine and Ammonia

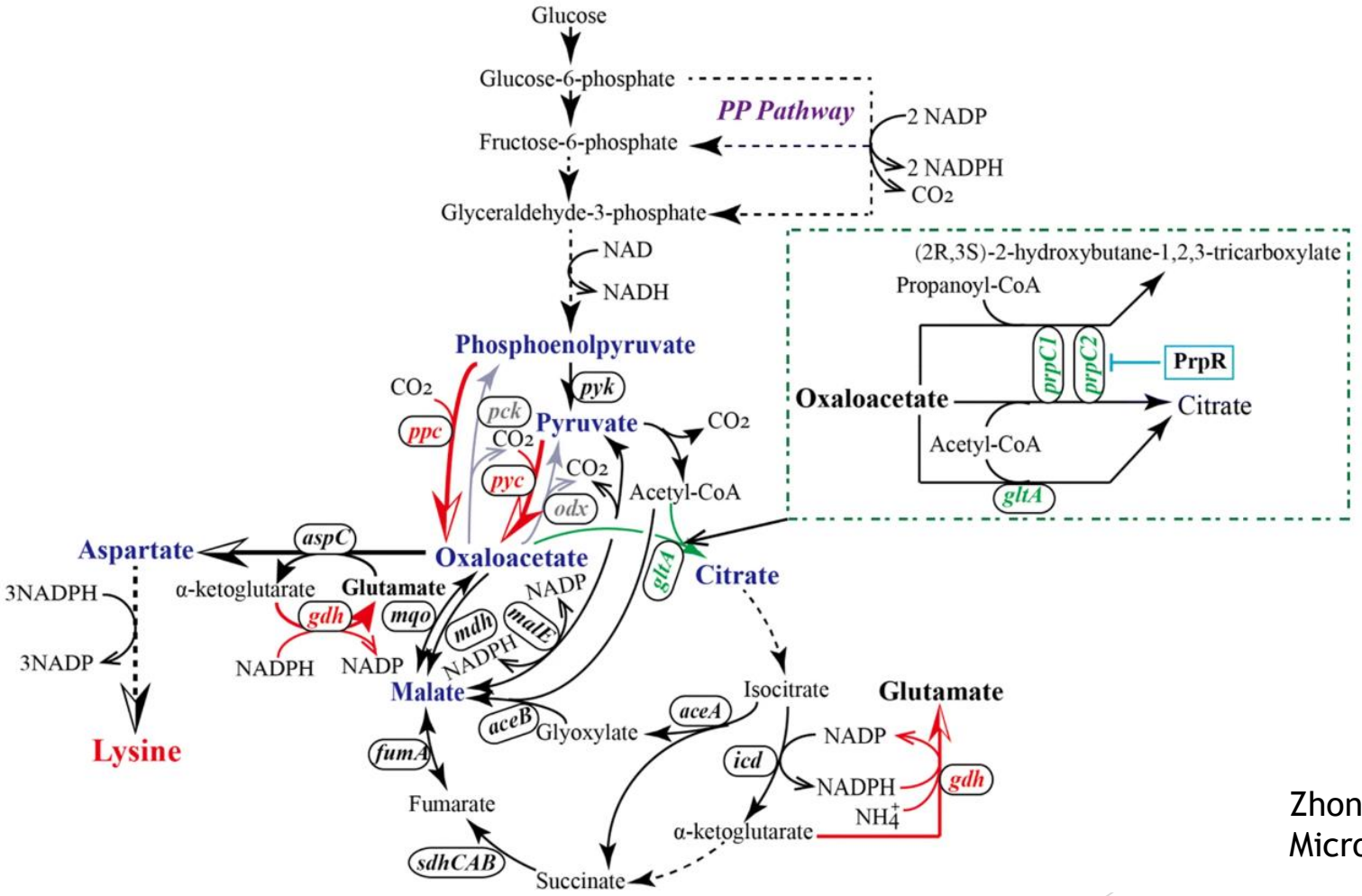


Adapted from Dadsetan *et al* (2011) Neurochemistry International

Amino Acid Interpretation (3)

- **Lysine**

Elevated lysine levels can be associated with urea cycle disorders
Thought to be secondary to shortage of alpha-ketoglutarate
Excess lysine inhibits arginase leading to disruption of the urea cycle and hence hyperammonaemia



Zhong Xhu *et al* (2018)
Microbial Cell Factories **17:** 105

Amino Acid Interpretation (4)

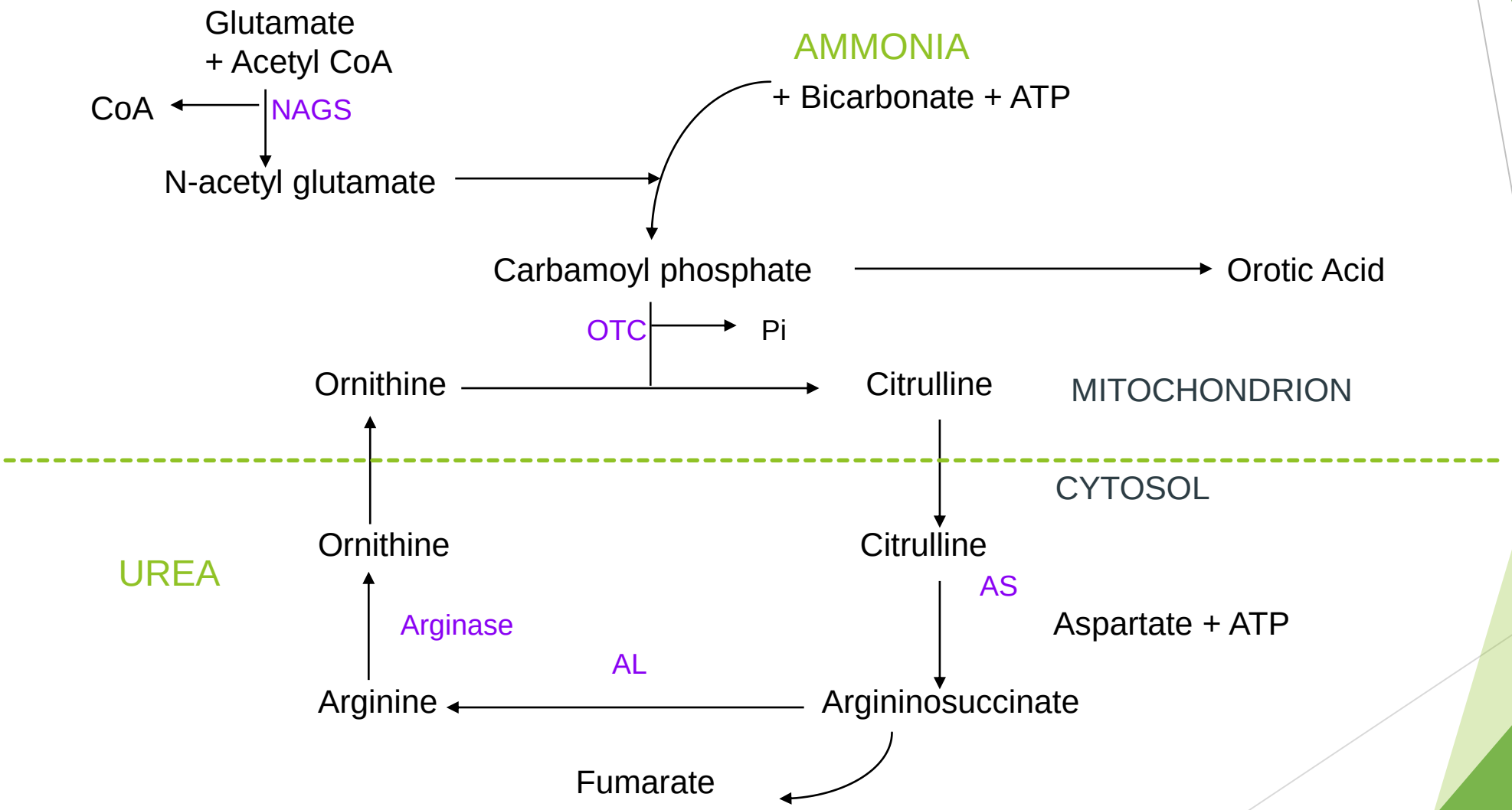
- **Proline**

Proline levels may be elevated in urea cycle disorders as proline synthesis starts with reactions acting on glutamate (raised due to disruption of urea cycle)

- **Arginine**

Low levels of arginine due to disruption of urea cycle (less flux through the pathway)

Urea Cycle


Glutamate
+ Acetyl CoA
CoA
NAGS
N-acetyl glutamate
AMMONIA
+ Bicarbonate + ATP
Carbamoyl phosphate
Orotic Acid
OTC
Pi
Ornithine
Citrulline
MITOCHONDRION
CYTOSOL
Ornithine
Citrulline
UREA
AS
Aspartate + ATP
Arginase
AL
Arginine
Argininosuccinate
Fumarate

CPS/OTC or NAGS?

- **Urea Cycle Disorders Clinical Presentation**

Acute: Acute encephalopathy, Seizures, Ataxia, Vomiting, Multiorgan failure, Peripheral circulatory failure, Psychiatric symptoms

In neonates: Sepsis-like picture, temperature instability, respiratory distress, hyperventilation

Chronic – Confusion, lethargy, headaches, learning disabilities, cognitive impairment, Protein aversion, Recurrent abdominal pain, vomiting, failure to thrive, hepatomegaly, Psychiatric symptoms

- **CPS**

- Most severe of the urea cycle disorders, incidence 1:1,300,000

- Often severe neonatal disease but milder variants can present at any age

- **OTC**

-Males - Severe hyperammonaemia (often lethal in neonate), milder variants common

-Females – Variable presentation: migraine, protein aversion, psychiatric disease (mild symptoms in 75%, severe encephalopathy in 15-20%), carrier females have deficiencies in executive function even if they have never had symptoms of overt hyperammonaemia

-Most common urea cycle disorder (1:14,000)

- **NAGS**

-Rare, <1:2,000,000. Symptoms mimic CPS1 as SPS1 is inactive in the absence of N-acetlyglutamate

CPS/OTC or NAGS?

- All three conditions associated with hyperammonaemia and raised glutamine levels
- **Citrulline**

  -Citrulline aids discrimination between the proximal and distal urea cycle defects as citrulline is the product of the proximal enzymes (CPS1, OTC and NAGS) and a substrate for the distal enzymes (ASS1, ASL and ARG1)

  -Citrulline is absent or present in trace amounts in neonatal-onset CPS1, NAGS and OTC deficiencies - and present in low to low-normal levels in late-onset disease

- **Orotic Acid**

  -Orotic acid can distinguish between CPS1 or NAGS deficiency from OTC deficiency

  Normal/Low levels = CPS1 or NAGS deficiency

  Elevated level = OTC deficiency

(May occasionally get neonates with OTC that don't produce excess orotic acid but they may start producing orotic acid later – suggest repeat testing)

Orotic Acid Results

- Orotic acid/creatinine = 12.5 µmol/mmol (<3.5)

?diagnosis

- Orotic acid/creatinine = 2.2 µmol/mmol (<3.5)

?diagnosis

NAGS or CPS1 Deficiency

- **Amino Acid Profile**

  -NAGS associated with increased alanine and low arginine levels

  -CPS1 associated with increased alanine and ornithine levels

- **Urinary Organic Acids**

  -CPS1 associated with 3-methylglutaconic aciduria

- **Molecular analysis**

  -CPS1 – mutation analysis using DNA from CVS or AFC

  -NAGS - mutation analysis using DNA from CVS or AFC

  - OTC - mutation analysis using DNA from CVS or AFC

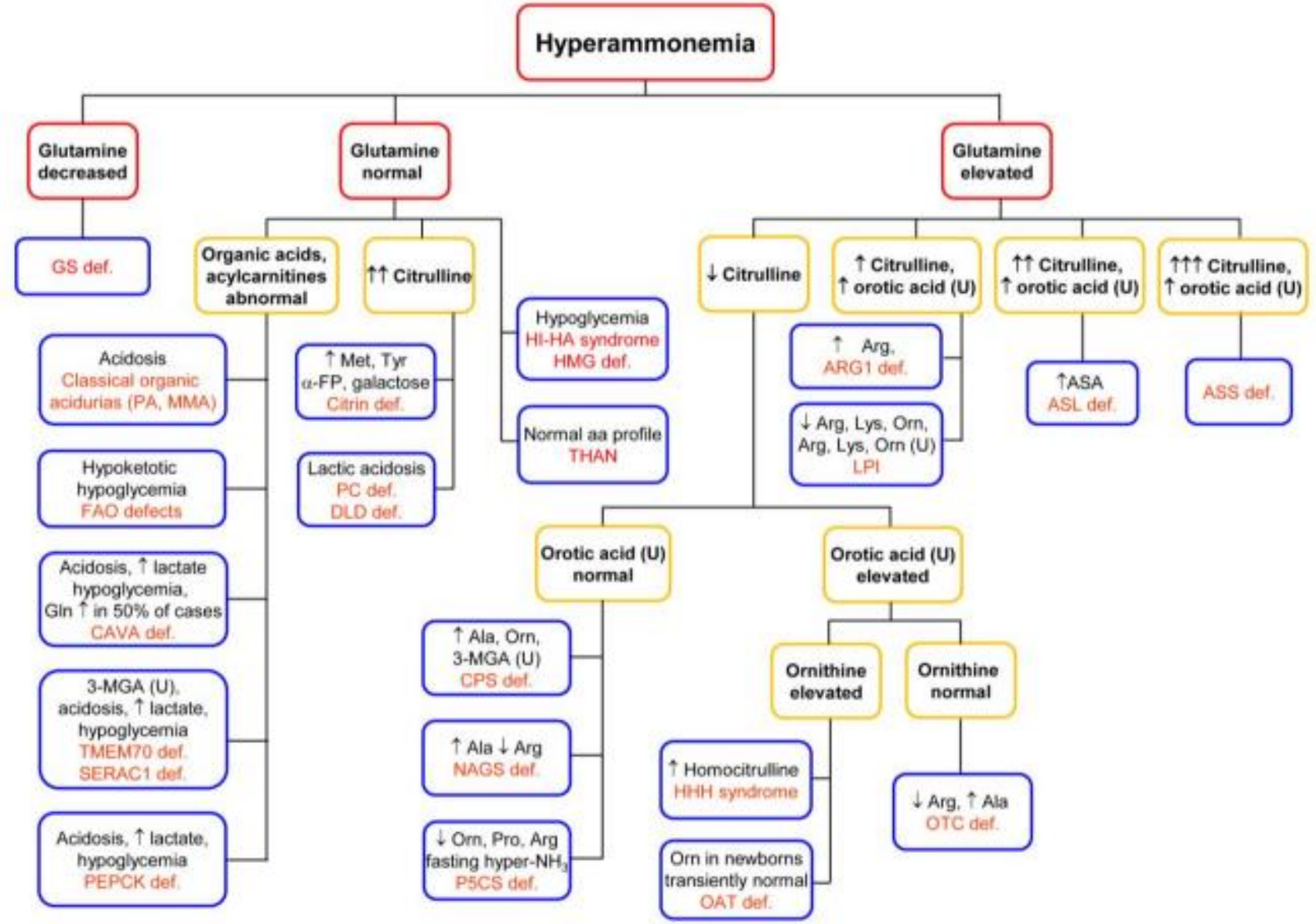
Note: If molecular testing is uninformative, enzyme activity can be completed for each of the disorders.

OTC – enzyme activity in plasma, liver or intestinal mucosa

NAGS – requires at least 10mg of liver tissue, activity decreases rapidly if liver sample is not immediately frozen and activity is dependent on protein intake.

CPS - enzyme activity in liver

UCD Guideline – 1st Revision 2018 (awmf.org)



Investigations in plasma if not stated otherwise; U: urine; 3-MGA: 3-methylglutaconic aciduria (Rokicki et al. 2017)

OTC Carrier

- 26 year old female presented to labour ward
- No previous medical history
- NH_3 = 78 µmol/L and increased to 94 µmol/L
- Ammonia remained repeatedly elevated following delivery (65-81 µmol/L)

Amino Acid	Concentration (umol/L)	Reference Range
Taurine	38	80-344
Threonine	110	60-231
Serine	92	68-256
Glutamic Acid	54	46-428
Glutamine	651	270-1159
Proline	214	
Glycine	175	185-552
Alanine	580	243-778
Citrulline	19	13-52
Valine	158	117-359
Methionine	18	9-52
Isoleucine	51	35-127
Leucine	97	80-229
Tyrosine	54	57-110
Phenylalanine	46	65-160
Ornithine	58	117-279
Lysine	292	165-378
Histidine	90	81-193
Arginine	47	30-198

OTC Carrier

- OTC is an X-linked disorder of urea synthesis
- Heterozygous females are typically spared from the early-onset lethal presentation but can present with non-specific manifestations of the disease
- The pattern of X-chromosome inactivation in the liver determines OTC enzyme activity
- A proportion of asymptomatic heterozygous females are at risk of hyperammonaemic espisodes, particularly in the postpartum period.
- Triggering events to unmask OTC deficiency can occur at any time, eg. infection, fever, fasting, surgery, pregnancy
- Diagnosis is by molecular testing

  -Identification of a mutation in an affected male or female proband allows determination of carrier status in the mother.

  -Genetic testing does not identify pathogenic variants in OTC in 20% of cases

- Alternative tests:

  1) Allopurinol testing: Oxypurinol ribonucleotide (a metabolite of allopurinol) is used to inhibit orotidine-monophosphate decarboxylase. Carbamoylphosphate accumulates in the mitochondria and spills over into the cystosol, resulting in an increase in oritidine and orotic acid in the urine of OTC deficient patients. (Note there are FP and FN results and ASSD, HHH and LPI can also result in positive tests).

  2) Pedigree analysis

OTC Carrier- Confirmation

- Genetic Confirmation

  -5.8Mb paracentric inversion with the proximal breakpoint between the promoter and coding sequence of the OTC gene. The inversion is thought to disrupt the promoter-enhancer interaction, disrupting OTC expression in liver tissues

OTC Carrier Follow-Up

- No protein restricted diet but followed up in adult metabolic clinic
- Birth plan for subsequent pregnancy:

  -On admission, U&E's, Ammonia, FBC, G&S

  -10% dextrose infusion at 125ml/hr throughout labour to avoid catabolic state

Time from starting Dextrose	Action
0	Commence 10% dextrose at 12ml/hr
+ 4 hrs	Check U&Es, BM/blood glucose
+ 8 hrs	Check U&Es, BM/blood glucose
+12 hrs	Check U&Es, BM/blood glucose
+16 hrs	Check U&Es, BM/blood glucose
+20 hrs	Check U&Es, BM/blood glucose
+24 hrs	**Repeat ammonia** Check U&Es, BM/blood glucose
Continue above pattern until delivery	

Note hyponatraemia is a risk with a dextrose only regimen

Separation of Argininosuccinic Acid (ASA)

09:15:00

BioSys

13/05/2023

Sample: Physiological Fluid Standard

Amount Loaded: 10 nmol

Column Type: Peek

Column Number: H-1551

Resin Batch: 132-101

Bed Length (mm): 200

Diameter (mm): 4.6

Instrument Serial Number: 116448

Test Number: N/A

	Buffer	Nin
Flow Rate (ml/h):	25	20
Back Pressure (bar):	81.9	114

Buffer

		Molarity	pH	Batch No.
Buffer 1 -	Lithium Buffer 1	0.20	2.80	R27823
Buffer 2 -	Lithium Buffer 2	0.30	3.00	N27649
Buffer 3 -	Lithium Buffer 3	0.50	3.15	R27799
Buffer 4 -	Lithium Buffer 4	0.30	3.50	R27809
Buffer 5 -	Lithium Buffer 5	1.50	3.55	N27669
Buffer 6 -	Lithium Buffer 6	0.30	n/a	P27751
Reagent	Ninhydrin			T27837
	Ultrosolve			

Title: LiHP

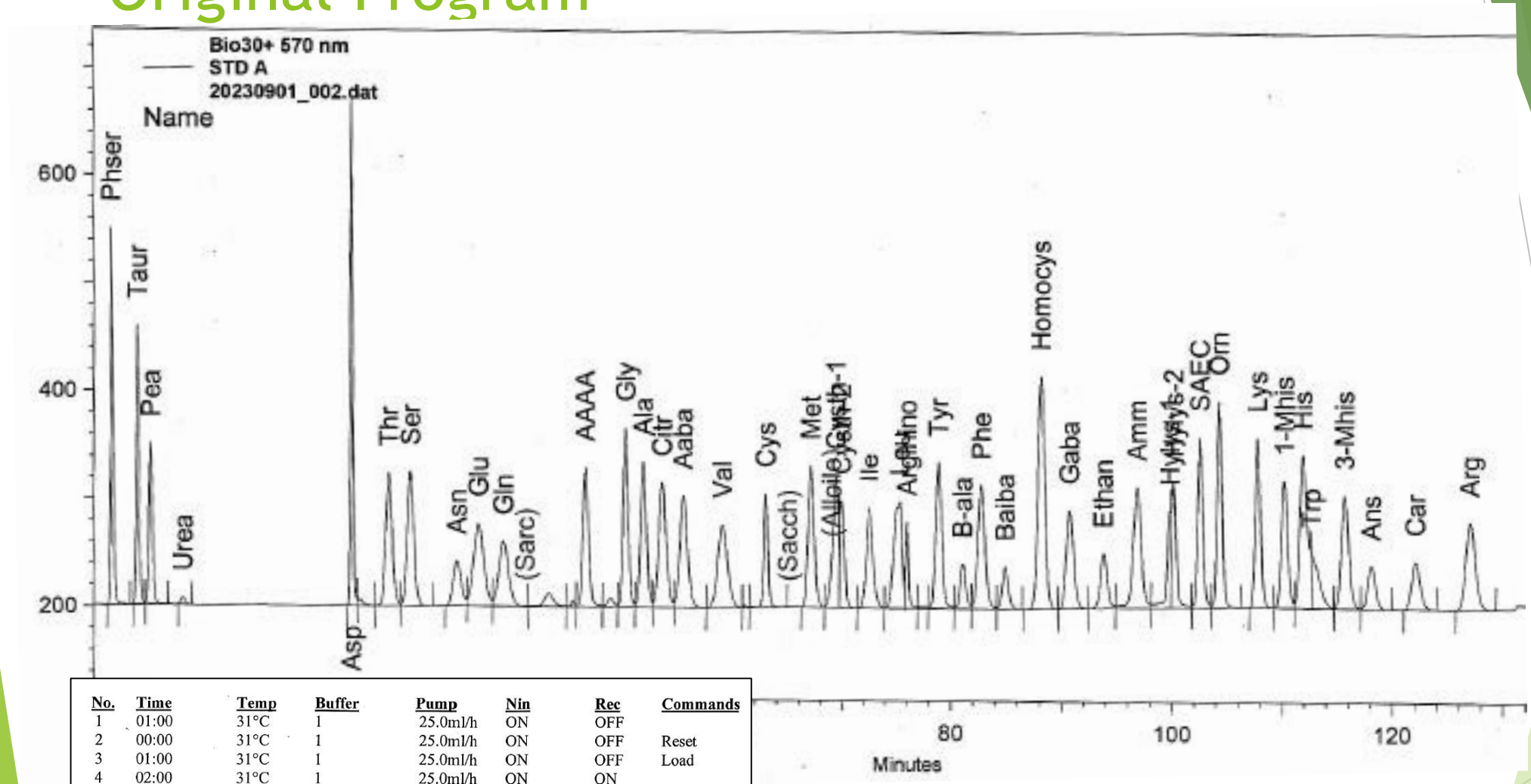
Filename: C:\Biochrom\BioSys\Programs\Column Testing\2023\H-1551\LiHP\116448 LiHP H-1551.prg

Comments: Standard program. Resin 132-101

Nin Flow Rate: 20.0 ml/h

No.	Time	Temp	Buffer	Pump	Nin	Rec	Commands
1	01:00	31°C	1	25.0ml/h	ON	OFF	
2	00:00	31°C	1	25.0ml/h	ON	OFF	Reset
3	01:00	31°C	1	25.0ml/h	ON	OFF	Load
4	02:00	31°C	1	25.0ml/h	ON	ON	
5	00:00	31°C	1	25.0ml/h	ON	ON	Reset
6	06:10	31°C	1	25.0ml/h	ON	ON	
7	28:15	31°C	2	25.0ml/h	ON	ON	
8	20:00	43°C	3	25.0ml/h	ON	ON	
9	02:00	55°C	3	25.0ml/h	ON	ON	
10	24:50	68°C	4	25.0ml/h	ON	ON	
11	38:00	79°C	5	25.0ml/h	ON	ON	
12	06:00	79°C	6	25.0ml/h	ON	ON	
13	06:00	79°C	1	25.0ml/h	ON	ON	
14	02:00	50°C	0	OFF	OFF	OFF	
15	37:00	50°C	1	25.0ml/h	OFF	OFF	
16	06:00	31°C	1	25.0ml/h	ON	OFF	

Original Program



No.	Time	Temp	Buffer	Pump	Nin	Rec	Commands
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2	00:00	31°C	1	25.0ml/h	ON	OFF	Reset
3	01:00	31°C	1	25.0ml/h	ON	OFF	Load
4	02:00	31°C	1	25.0ml/h	ON	ON	
5	00:00	31°C	1	25.0ml/h	ON	ON	Reset
6	06:10	31°C	1	25.0ml/h	ON	ON	
7	28:15	31°C	2	25.0ml/h	ON	ON	
8	20:00	43°C	3	25.0ml/h	ON	ON	
9	02:00	55°C	3	25.0ml/h	ON	ON	
10	24:50	68°C	4	25.0ml/h	ON	ON	
11	38:00	79°C	5	25.0ml/h	ON	ON	
12	06:00	79°C	6	25.0ml/h	ON	ON	
13	06:00	79°C	1	25.0ml/h	ON	ON	
14	02:00	50°C	0	OFF	OFF	OFF	
15	37:00	50°C	1	25.0ml/h	OFF	OFF	
16	06:00	31°C	1	25.0ml/h	ON	OFF	

ASA co-eluting with Leucine

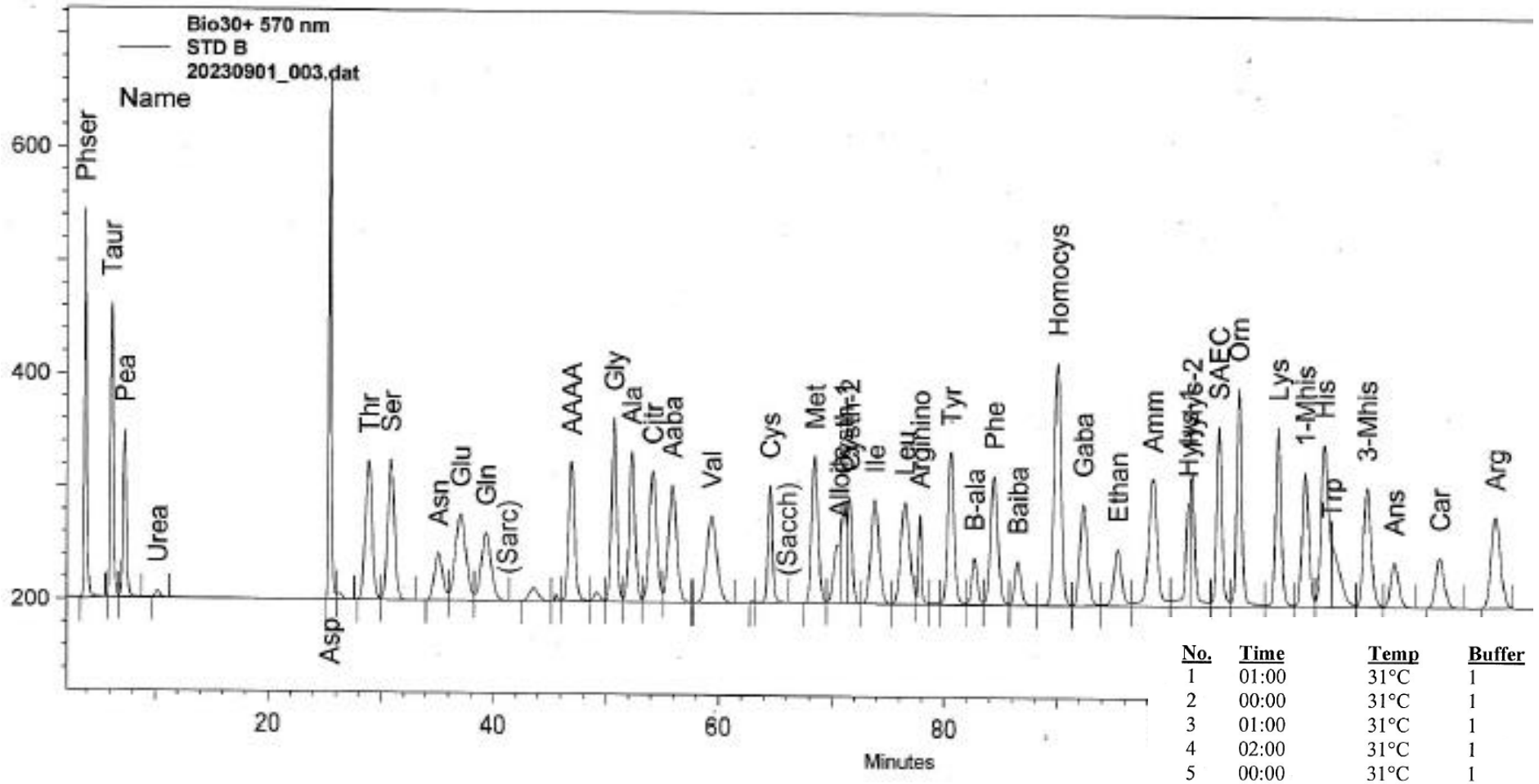
Locating “Missing” ASA

- ASA Mix: 18.14 mg ASA and 8.20 mg L-alloisoleucine in 50ml loading buffer (1.25 mmol/L)
- 25mM Tryptophan (12.76 mg in 25ml loading buffer)

- Test solution: 1ml ASA mix
  1ml 2.5mM Tryptophan solution
  Made up to 5ml with loading buffer

- Run mix with original program and “adapted” programmes to correctly identify amino acids

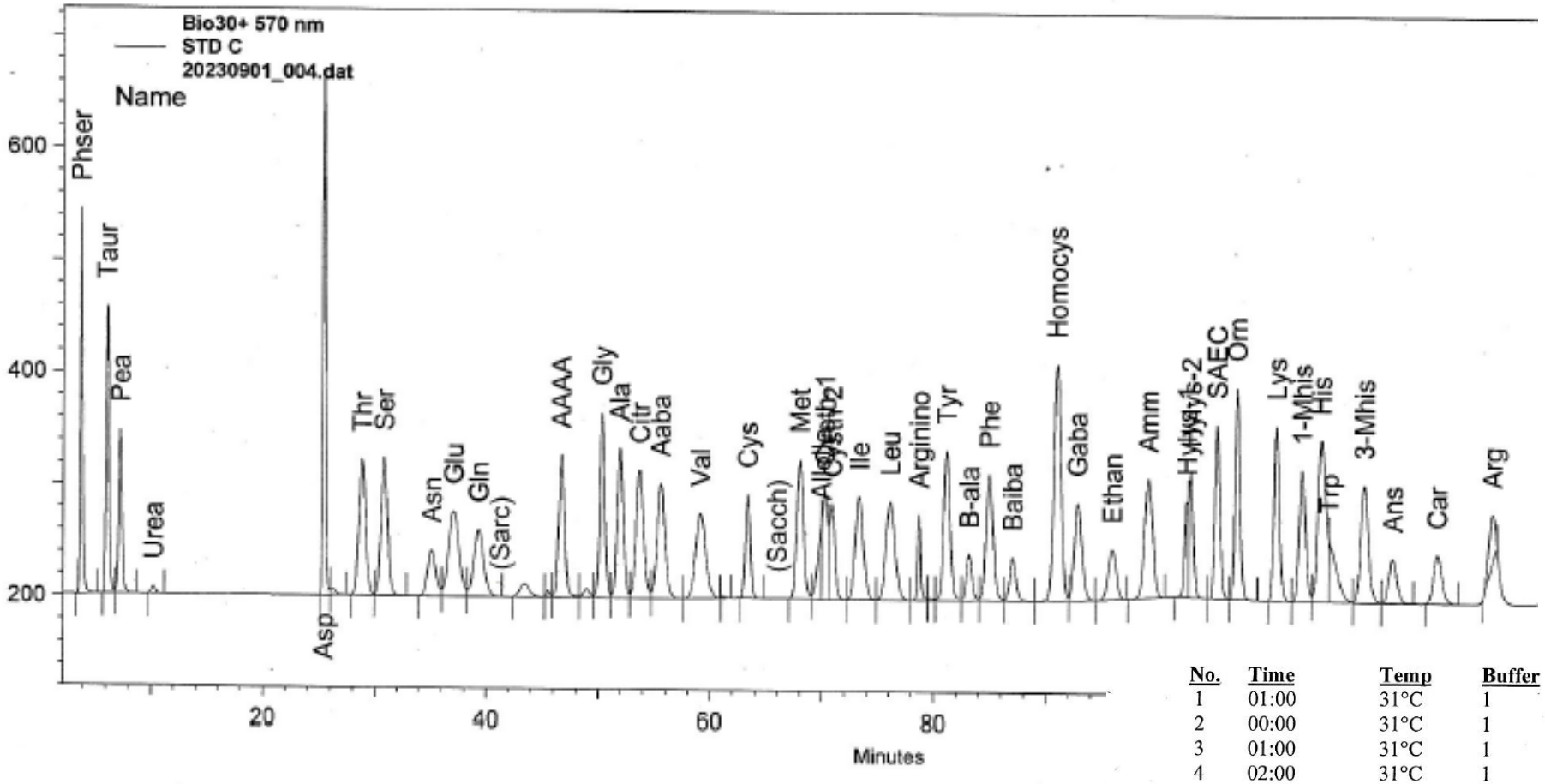
Modified Program 1



No.	Time	Temp	Buffer	Pump	Nin	Rec	Commands
1	01:00	31°C	1	25.0ml/h	ON	OFF	
2	00:00	31°C	1	25.0ml/h	ON	OFF	Reset
3	01:00	31°C	1	25.0ml/h	ON	OFF	Load
4	02:00	31°C	1	25.0ml/h	ON	ON	
5	00:00	31°C	1	25.0ml/h	ON	ON	Reset
6	06:10	31°C	1	25.0ml/h	ON	ON	
7	30:00	31°C	2	25.0ml/h	ON	ON	
8	20:00	43°C	3	25.0ml/h	ON	ON	
9	02:00	55°C	3	25.0ml/h	ON	ON	
10	24:50	68°C	4	25.0ml/h	ON	ON	
11	38:00	79°C	5	25.0ml/h	ON	ON	
12	06:00	79°C	6	25.0ml/h	ON	ON	
13	06:00	79°C	1	25.0ml/h	ON	ON	
14	02:00	50°C	0	OFF	OFF	OFF	
15	37:00	50°C	1	25.0ml/h	OFF	OFF	
16	06:00	31°C	1	25.0ml/h	ON	OFF	

ASA separation improved by increasing Buffer 2 time from 28:15 to 30:00 minutes.

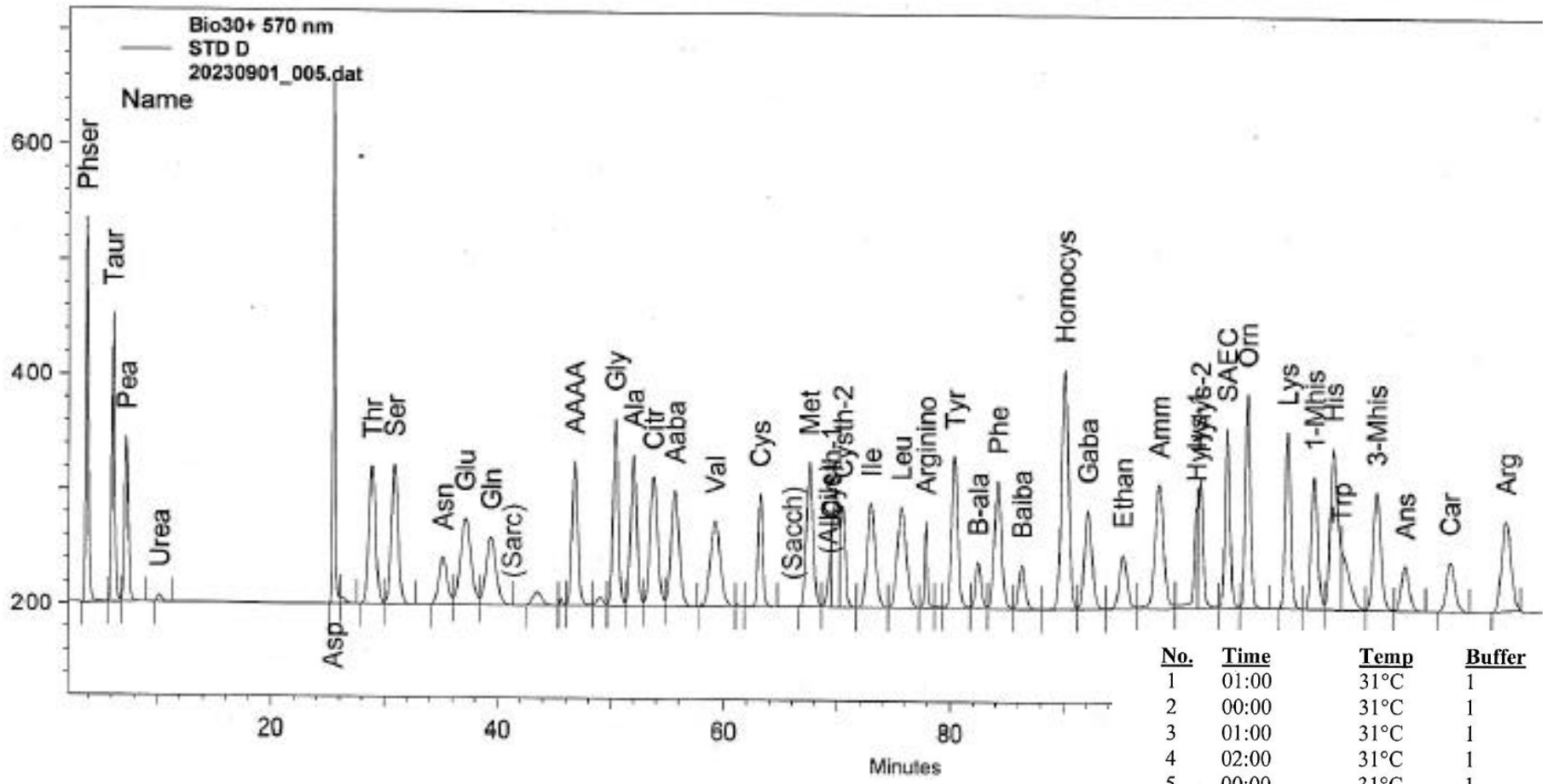
Modified Program 2



No.	Time	Temp	Buffer	Pump	Nin	Rec	Commands
1	01:00	31°C	1	25.0ml/h	ON	OFF	
2	00:00	31°C	1	25.0ml/h	ON	OFF	Reset
3	01:00	31°C	1	25.0ml/h	ON	OFF	Load
4	02:00	31°C	1	25.0ml/h	ON	ON	
5	00:00	31°C	1	25.0ml/h	ON	ON	Reset
6	06:10	31°C	1	25.0ml/h	ON	ON	
7	28:15	31°C	2	25.0ml/h	ON	ON	
8	23:00	43°C	3	25.0ml/h	ON	ON	
9	02:00	55°C	3	25.0ml/h	ON	ON	
10	24:50	68°C	4	25.0ml/h	ON	ON	
11	38:00	79°C	5	25.0ml/h	ON	ON	
12	06:00	79°C	6	25.0ml/h	ON	ON	
13	06:00	79°C	1	25.0ml/h	ON	ON	
14	02:00	50°C	0	OFF	OFF	OFF	
15	37:00	50°C	1	25.0ml/h	OFF	OFF	
16	06:00	31°C	1	25.0ml/h	ON	OFF	

ASA separation further improved by increasing Buffer 3 time from 20:00 to 23:00 minutes (Step 8).

Modified Program 3

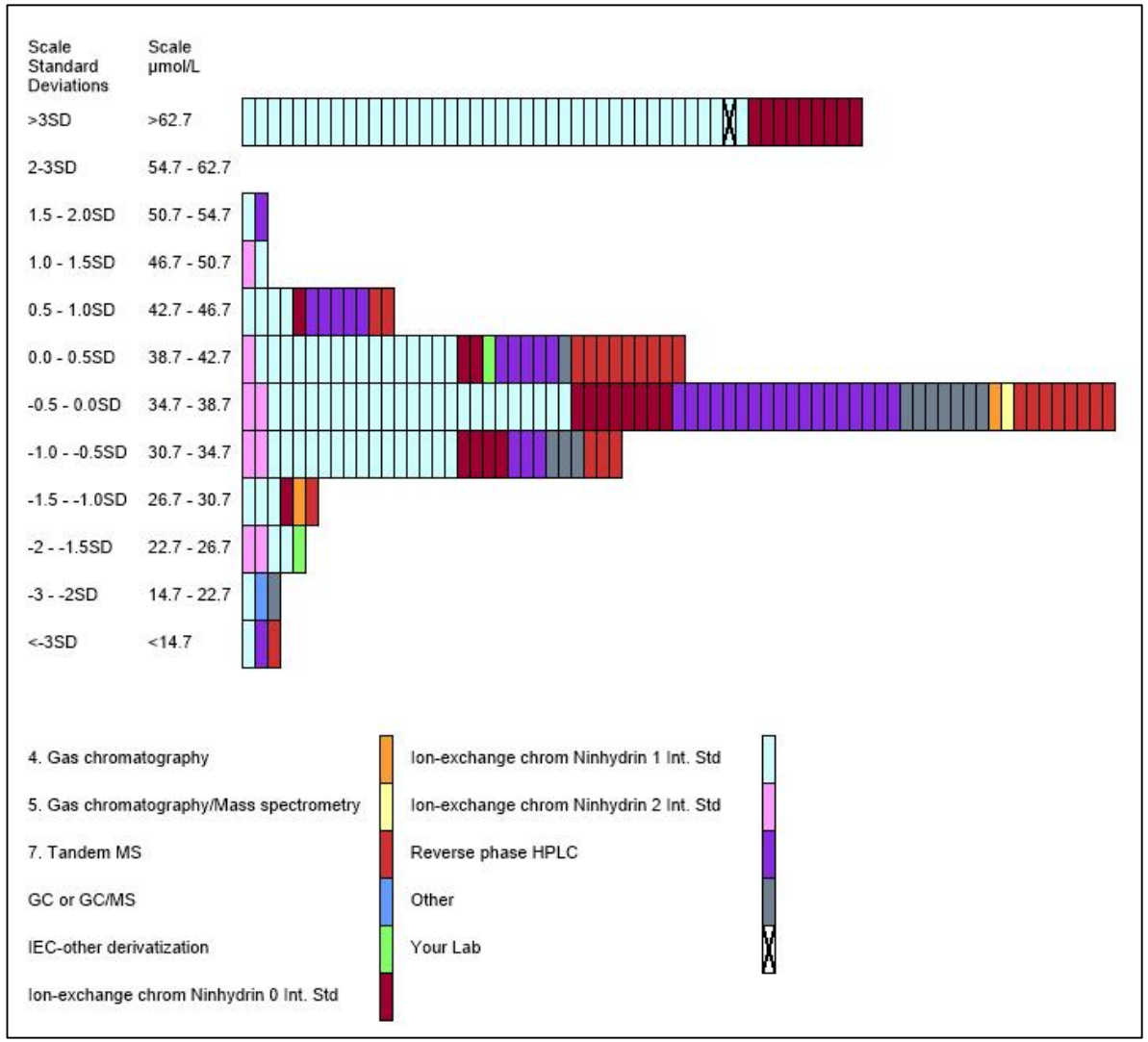


No.	Time	Temp	Buffer	Pump	Nin	Rec	Commands
1	01:00	31°C	1	25.0ml/h	ON	OFF	
2	00:00	31°C	1	25.0ml/h	ON	OFF	Reset
3	01:00	31°C	1	25.0ml/h	ON	OFF	Load
4	02:00	31°C	1	25.0ml/h	ON	ON	
5	00:00	31°C	1	25.0ml/h	ON	ON	Reset
6	06:10	31°C	1	25.0ml/h	ON	ON	
7	28:15	31°C	2	25.0ml/h	ON	ON	
8	20:00	43°C	3	25.0ml/h	ON	ON	
9	04:00	55°C	3	25.0ml/h	ON	ON	
10	24:50	68°C	4	25.0ml/h	ON	ON	
11	38:00	79°C	5	25.0ml/h	ON	ON	
12	06:00	79°C	6	25.0ml/h	ON	ON	
13	06:00	79°C	1	25.0ml/h	ON	ON	
14	02:00	50°C	0	OFF	OFF	OFF	
15	37:00	50°C	1	25.0ml/h	OFF	OFF	
16	06:00	31°C	1	25.0ml/h	ON	OFF	

ASA separation further improved also by increasing Buffer 3 time from 02:00 to 04:00 minutes (Step 9).

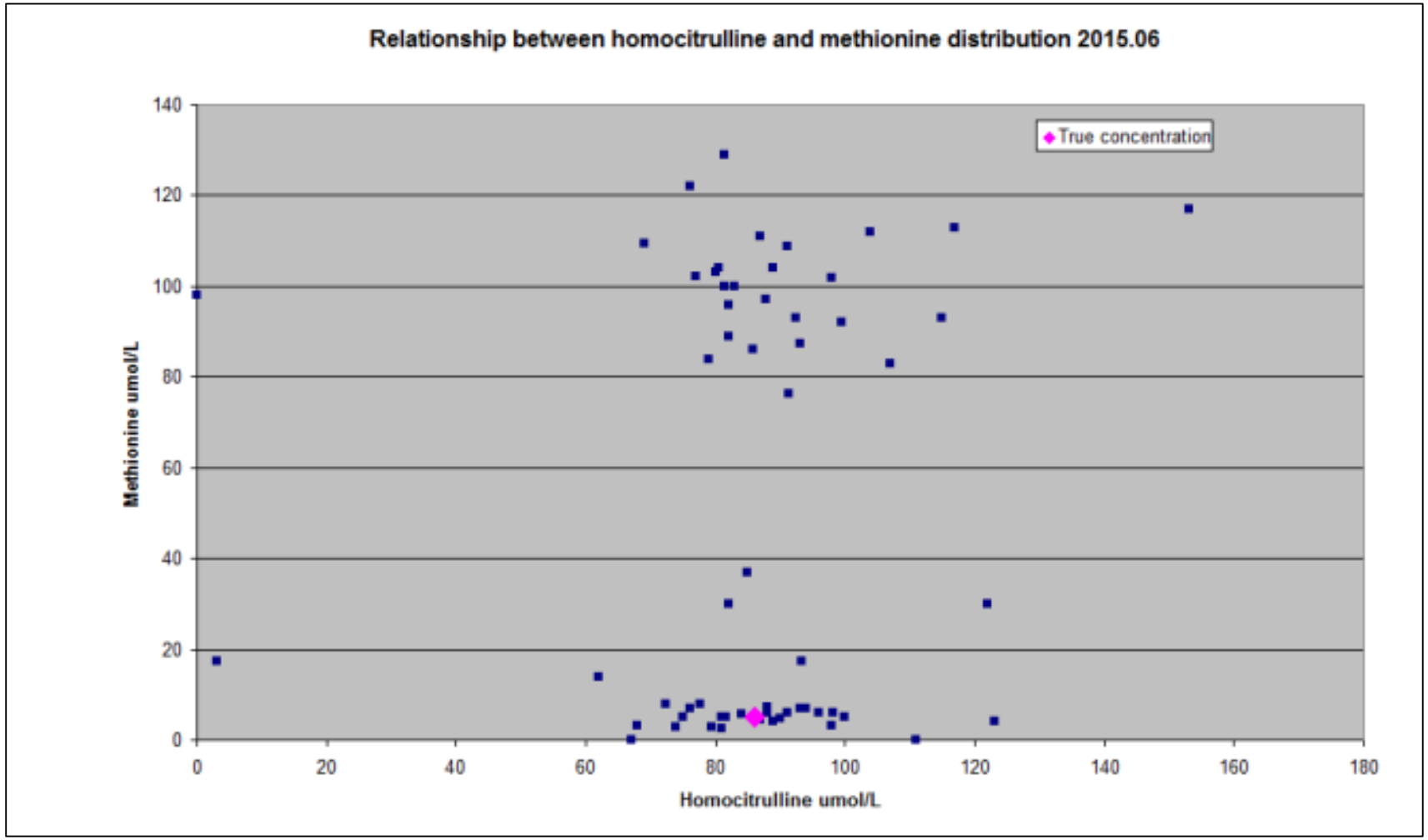
Co-elution of Homocitrulline and Methionine (1)

Methionine 2015.08 ERNDIM Amino Acid EQA Sample

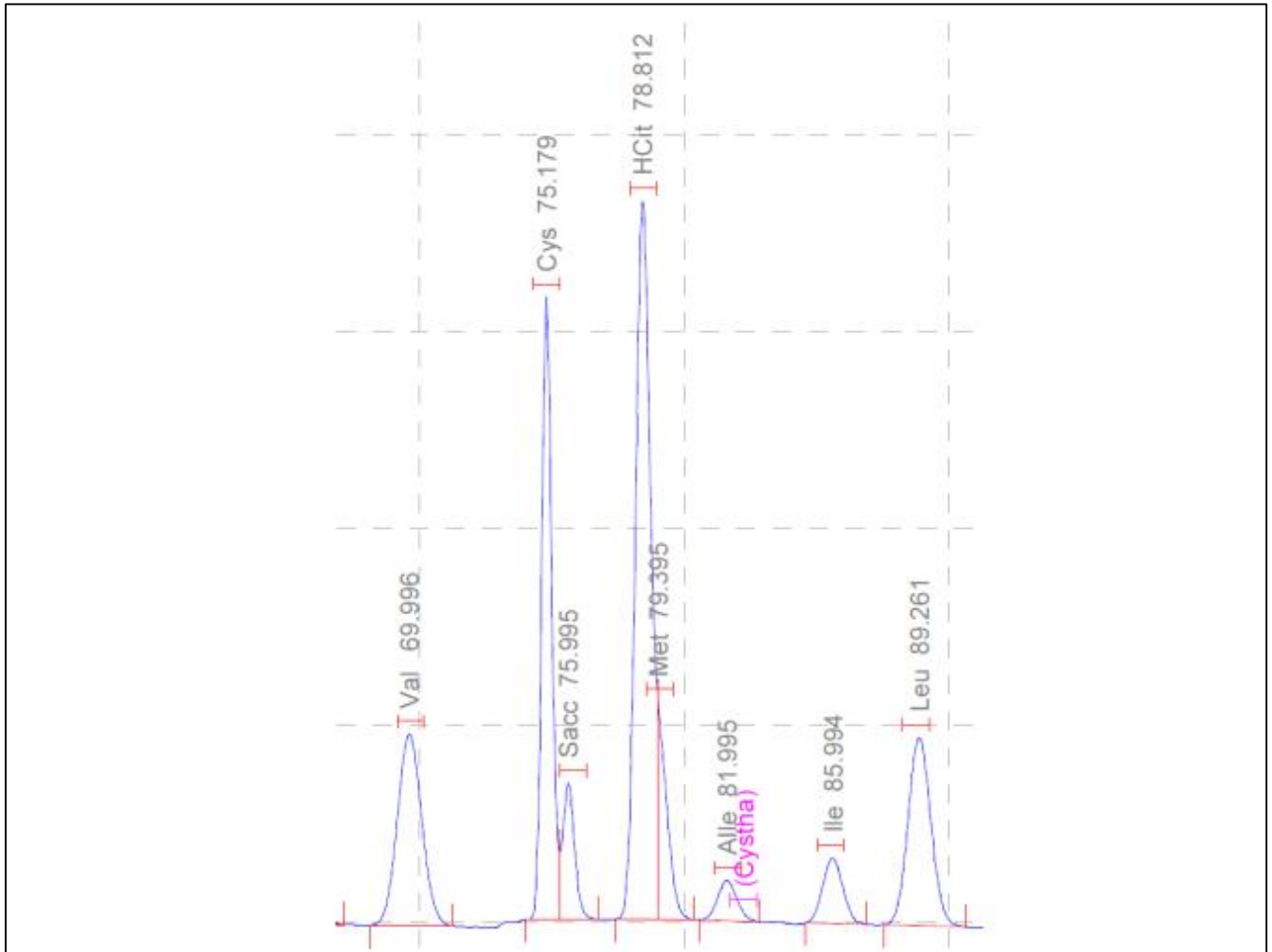


Sample:	2015.08
Analyte:	Methionine
Deadline:	27/11/2015
Unit:	µmol/L
Your Method:	Ion-exchange chrom Ninhydrin 1 Int. Std
Your Result:	240
Method results	
n:	108
Mean:	38.8
Median:	38.1
SD:	7.19
All Labs results	
n:	216
Mean:	38.7
Median:	38.0
SD:	8.01

Co-elution of Methionine and Homocitrulline (2)

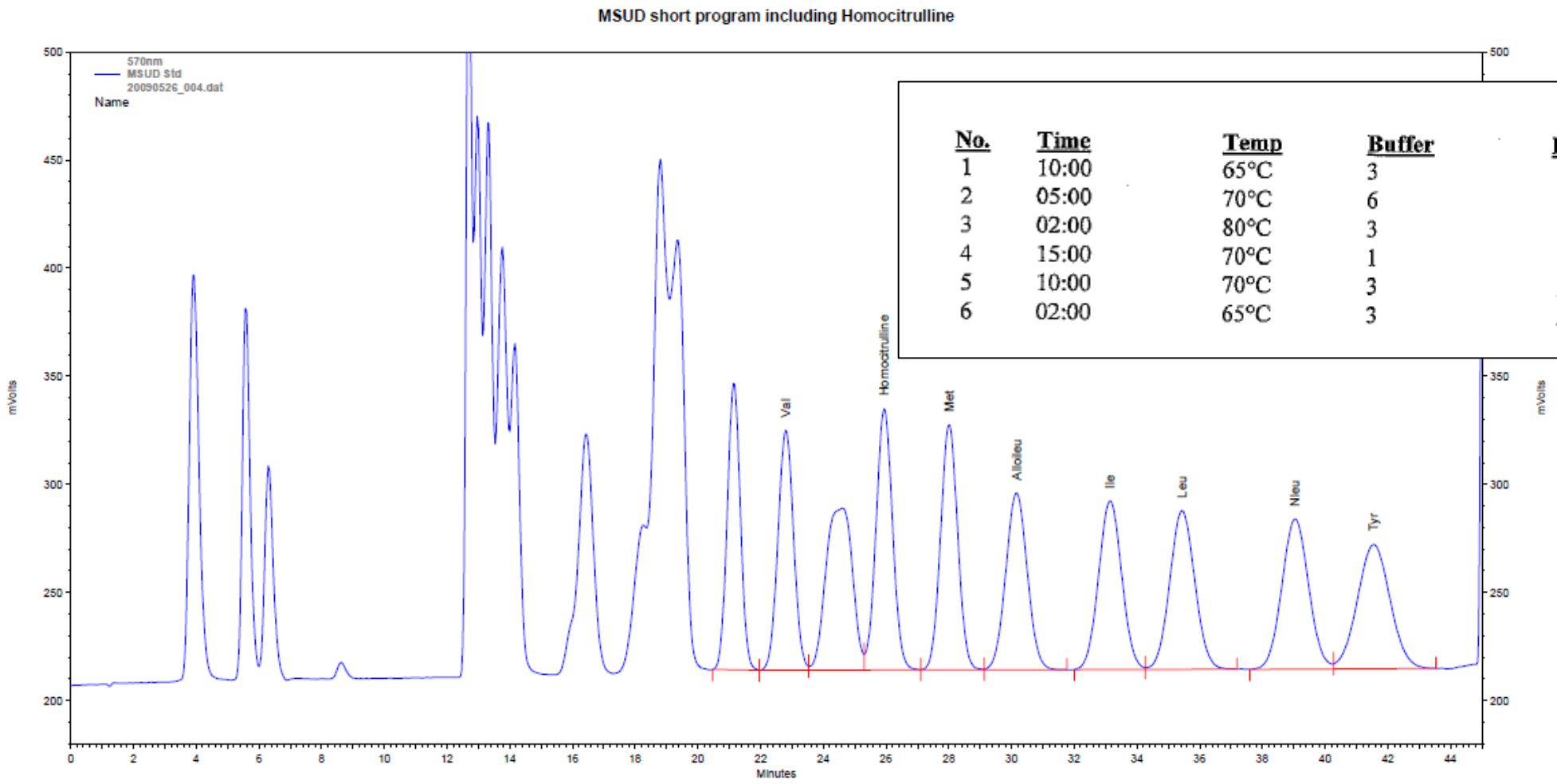


Biochrom 30 and Homocitrulline Separation



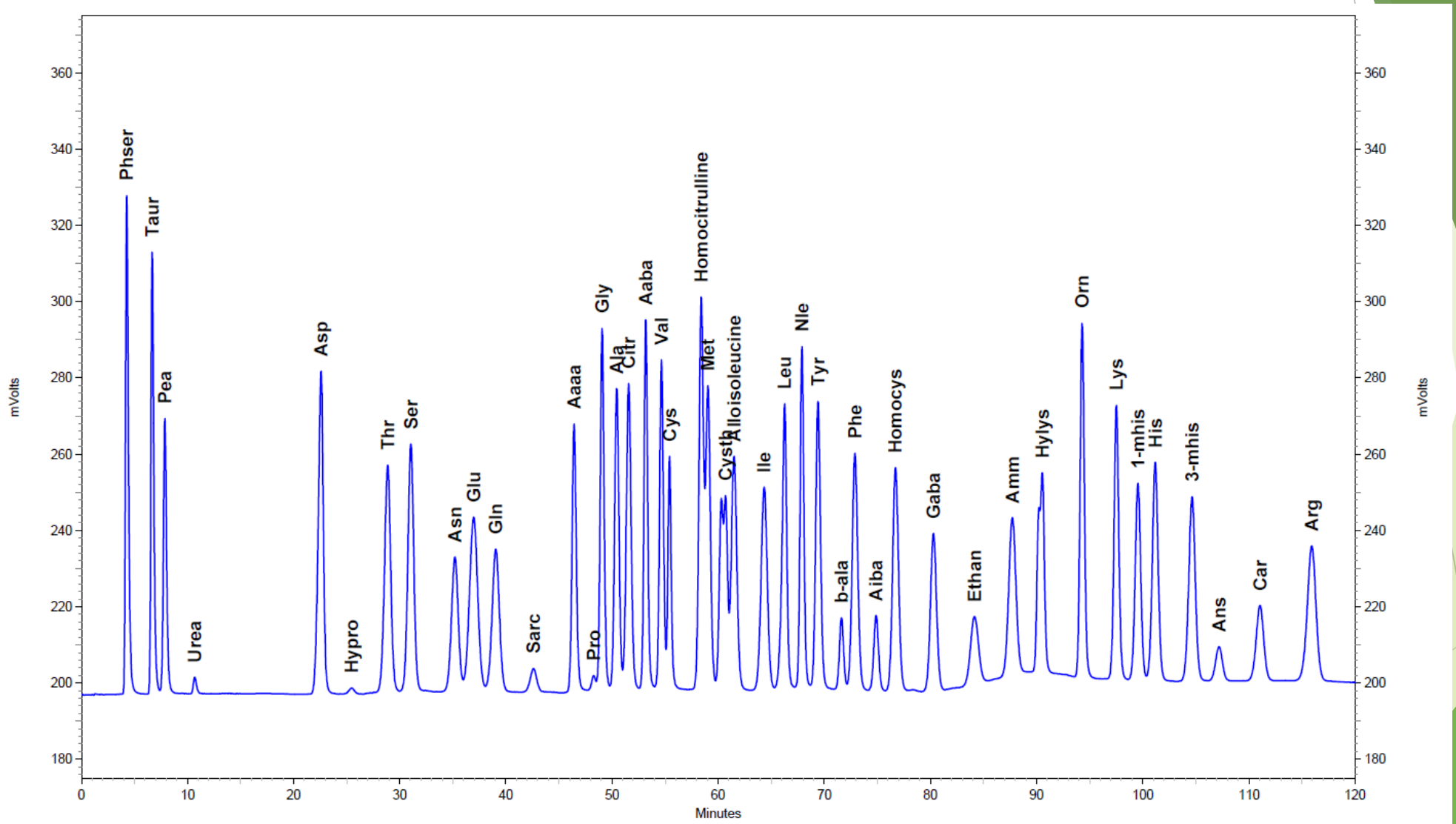
Full run from biochrom 30 with analysis time of 180 minutes. Note the clear peak of homocitrulline with methionine as shoulder

MSUD Program Separates Homocitrulline and Methionine



No.	Time	Temp	Buffer	Pump	Nin	Rec
1	10:00	65°C	3	30.0ml/h	ON	ON
2	05:00	70°C	6	30.0ml/h	ON	ON
3	02:00	80°C	3	30.0ml/h	ON	ON
4	15:00	70°C	1	30.0ml/h	ON	ON
5	10:00	70°C	3	30.0ml/h	OFF	OFF
6	02:00	65°C	3	30.0ml/h	ON	OFF

LiHP Programme and Homocitrulline (1)



LiHP Programme and Homocitrulline (2)

No.	Time	Temp	Buffer	Pump	Nin	Rec	Commands
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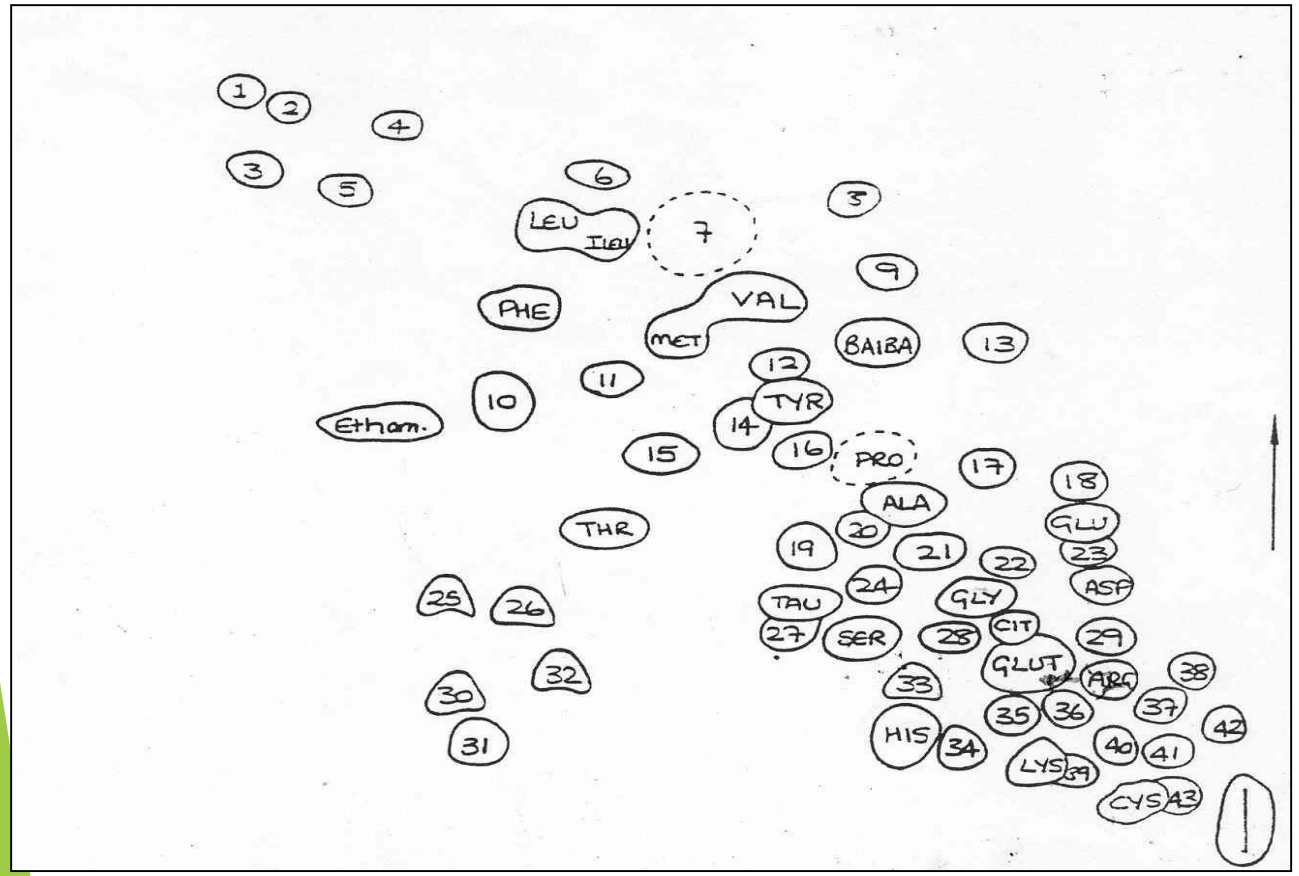
2	00:00	33°C	1	25.0ml/h	ON	OFF	Reset
3	01:00	33°C	1	25.0ml/h	ON	OFF	Load
4	06:30	33°C	1	25.0ml/h	ON	ON	
5	20:00	33°C	2	25.0ml/h	ON	ON	
6	16:00	38°C	3	25.0ml/h	ON	ON	
7	05:00	72°C	3	25.0ml/h	ON	ON	
8	26:00	72°C	4	25.0ml/h	ON	ON	
9	38:00	77°C	5	25.0ml/h	ON	ON	
10	06:00	77°C	6	25.0ml/h	ON	ON	
11	06:00	77°C	1	25.0ml/h	ON	ON	
12	30:00	33°C	1	31.0ml/h	OFF	OFF	
13	06:00	33°C	1	25.0ml/h	ON	OFF	

Typical Optimised Programme for LiHP Biochrom 30

Optimisation Tips:

- Decrease time of Buffer 2 as much as possible so that proline and glycine are still separated
- Adjust temperature of first step of Buffer 3 (decrease by 2°C compared to original programme) and increase the time on the first step of buffer 3 (increase by 2 minutes)

2D-Thin Layer Chromatography: Schematic

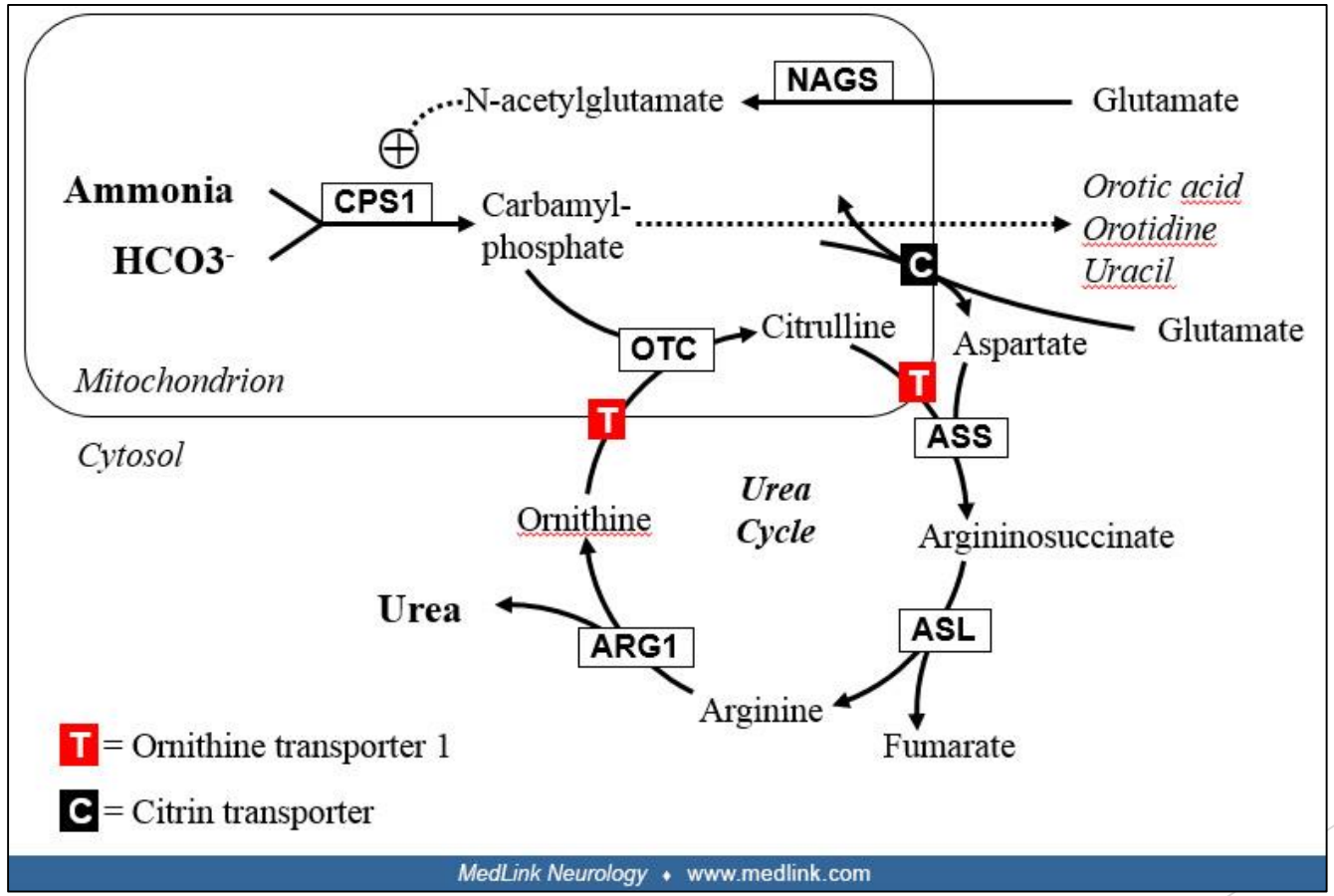


ALA	- Alanine	1. Phenylethylamine
SER	- Serine	2. *p*-Amino phenylacetic acid
GLY	- Glycine (Brown/blue)	3. Tryptamine
CIT	- Citrulline	4. α-Aminooctanoic
GLUT	- Glutamine	5. Tyramine
HIS	- Histidine (Brown/blue)	6. Ethionine
GLU	- Glutamate	7. Urea
ASP	- Aspartate (Light blue)	8. ε-Amino- ncaproic acid
ARG	- Arginine	9. δ-Amino-n-valeric acid
LYS	- Lysine	10. Tryptophan
CYS	- Cystine	11. Felinine
TAU	- Taurine	12.
PRO	- Proline (Pink)	13. γ-Aminon-butyric acid
TYR	- Tyrosine	14. ß-Amino-n-butyric acid
MET	- Methionine	15. δ-Amino-n-butyric acid
VAL	- Valine	16. α-Amino-n-butyric acid
PHE	- Phenylalanine	17. ß-Alanine
LEU	- Leucine	18. α-Amino-adipic acid
ILEU	- Isoleucine	19. Methionine sulphone
ETHAN	- Ethanolamine	20. Sarcosine
THR	- Threonine	21. Homoserine
BAIBA	- ß-Aminoisobutyric acid	22. Homocitrulline

23. S-Carboxymethyl cysteine
24. Hydroxyproline
25. Histamine
26. Putrescine
27. Galactosamine
28. Methionine sulphoxide
29. Asparagine
30. Cadaverine
31. Spermidine
32. Agmatine
33. 3-Methyl histidine
34. Cysteic acid
35. Carnosine
36. Homocystine
37. Argininosuccinic acid
38. Phosphoethanolamine
39. Homocysteic acid
40. Ornithine
41. Hydroxylysine
42. Phosphoserine
43. Cystathionine

HHH Syndrome

- Hyperornithinemia-Hyperammoniaemia-Homocitrullinaemia (HHH Syndrome)
- Disorder of the urea cycle and ornithine degradation pathway



HHH Syndrome: Clinical Presentation

- **Neonatal (8% of cases)**

  -Hyperammonaemia within 24-48 hrs after feeding begins

  -Lethargy, vomiting, tachypnea with respiratory alkalosis and/or seizures

- **Infantile/Childhood/Adult (92% of cases)**

  -Chronic neurodegenerative deficits

  (developmental delay, ataxia, spasticity, unexplained seizures, cognitive deficits)

  -Acute encephalopathy secondary to hyperammonaemia

  -Chronic liver dysfunction (elevated transminases with or without mild coagulopathy)

Neurologic and cognitive abilities can continue to deteriorate despite early metabolic control

HHH Syndrome: Diagnosis

- Episodic or postprandial mild to moderate **hyperammonaemia**

  -Note the degree of hyperammonaemia is typically less significant than other urea cycle disorders

- **Hyperornithinaemia**

  -At time of diagnosis plasma ornithine can range from 200-1915 µmol/L

  -Note levels very rarely normalise with a protein-restricted diet but do decrease significantly

- **Homocitrullinaemia**

  -Key feature but some neonates do not excrete homocitrulline in significant amounts and adults may self-restrict protein and so will excrete minimal or no homocitrulline in urine

  -Lysine in infant formulas can undergo carbamylation during manufacturing processes and be converted to homocitrulline

- **Neuroimaging**

  -Abnormal white matter signal, demyelination, evidence of cortical atrophy

- **Molecular analysis**

  -Pathogenic variants in *SLC25A156* gene

HHH Syndrome: Management

- Treat acute hyperammonaemic episodes
  - -Discontinue oral intake, use ammonia scavengers (sodium benzoate, sodium phenylbutyrate)
  - -Arginine supplementation
  - -Dextrose infusion
  - -Haemodialysis

- Long-term management
  - -Protein-restricted diet
  - -Citrulline supplmentation
  - -Sodium phenylbutyrate
  - -Creatine supplementation
  - -Carnitine supplementation
  - -Occupational/Physical/Speech Therapy