

Disorders Affecting Protein Metabolism

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Disorders Of Protein Metabolism

Protein is a key constituent of most foods we eat,

Infants with protein metabolism disorders cannot drink human milk because of proteins

Require specialized formulas without the offending amino acid,

And allowing the baby to receive essential nutrients for growth.

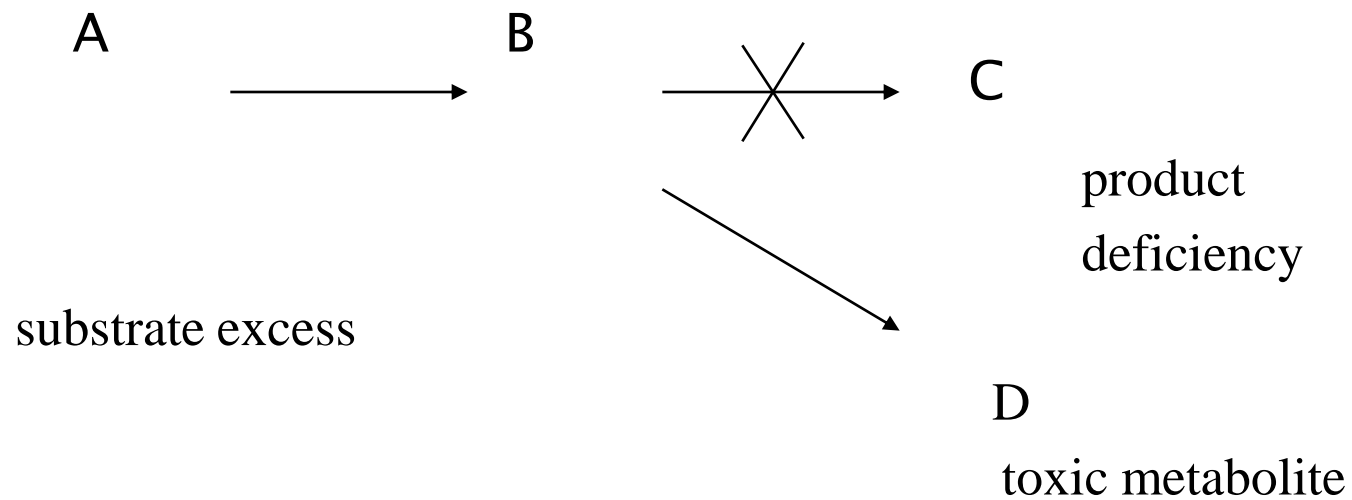
Protein metabolic disorders are inherited inborn error of metabolism.

Disorders of Protein Metabolism

- ▶ An inherited enzyme deficiency leads to the disruption of normal metabolism
- ▶ Accumulation of a toxic substrate.
- ▶ The substance which accumulates interferes with normal metabolism
- ▶ End product deficiency
- ▶ There may be reduced ability to make essential compounds in the body.

What is a metabolic disease?

- ▶ Garrod's hypothesis



What are the types of metabolic disease?

Small molecule disease

- Carbohydrate
- Protein
- Lipid
- Nucleic Acids

Organelle disease

- Lysosomes
- Mitochondria
- Peroxisomes
- Cytoplasm

How do metabolic diseases present in the neonate ??

- ▶ Acute life threatening illness
 - encephalopathy – lethargy, irritability, coma
 - vomiting
 -
 - respiratory distress
- ▶ Seizures, Hypertonia
- ▶ Hepatomegaly (enlarged liver)
- ▶ Hepatic dysfunction / jaundice
- ▶ Odour, Dysmorphism, FTT (failure to thrive), Hiccoughs

Three Types of Presentation

- ▶ Type 1: Silent Disorders
- ▶ Type 2: Acute Metabolic Crises
- ▶ Type 3: Neurological Deterioration

Type 1: Silent Disorders

- ▶ Do not manifest life-threatening crises
- ▶ Untreated could lead to brain damage and developmental disabilities
- ▶ Example: PKU (Phenylketonuria)

Type 2: Acute Metabolic Crisis

- ▶ Life threatening in infancy
- ▶ Children are protected in-utero by maternal circulation which provide missing product or remove toxic substance
- ▶ Example OTC (Urea Cycle Disorders)

Amino Acid Metabolism- Inborn Errors

- **Histidinemia**
- **Maple Syrup Urine Disease, MSUD**
 - MSUD Type I
 - MSUD Type II
- **Methylmalonic Aciduria**
- **Non-ketotic Hyperglycinemia Type I (NKHI)**
- **Hyperlysinemia**

- **Phenylketonuria**
- **Type I Tyrosinemia-Tyrosinosis**
- **Type II Tyrosinemia-Richner-Hanhart Syndrome**
- **Type III Tyrosinemia**
- **Alcaptonuria**
- **Homocystinuria**

PKU

- ▶ **Autosomal Recessive disorder caused by mutation in *PAH* gene**
 - ▶ **Newborn screening started in 1963**
 - ▶ **Incidence: 1 in 15,000/1 in 12000**
 - ▶ **Subtypes and heterogeneity**
 - **Classic**
 - **Moderate and mild**
 - **Non-classical or non-PKU hyperphenylalaninemia**
- % enzyme activity determines clinical severity**

Major Neuropathologic changes

1. Hypomyelination (Phe-sensitive oligodendrocytes)
 2. White matter degeneration (leucodystrophy)
 3. Developmental delay/arrest cerebral cortex
- Microcephaly
 - Mental retardation
 - Seizures

Case I

PKU (Early intervention)_

F/H of PKU

Diagnosed at newborn period
(PA:1100 umol/L)

On PKU diet PA is 395 umol/L at 7 month
with normal mentation

Novel splicing mutation in PAH gene with
parents being heterozygous for the same

Two other affected family members have
the same mutation

PND can be offered to this family.

**The longer it takes to get Phe level < 8
mg/dl the lower the IQ of the baby**

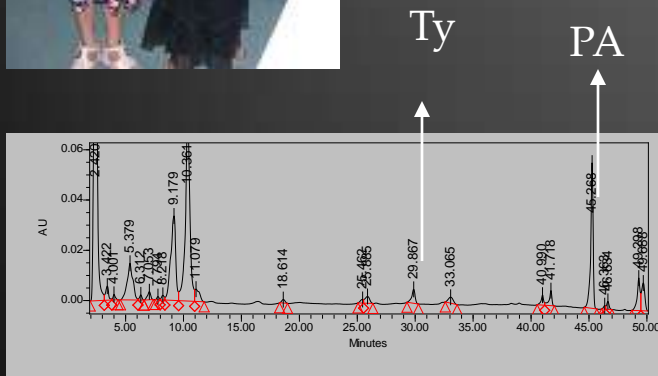


PKU with albinism

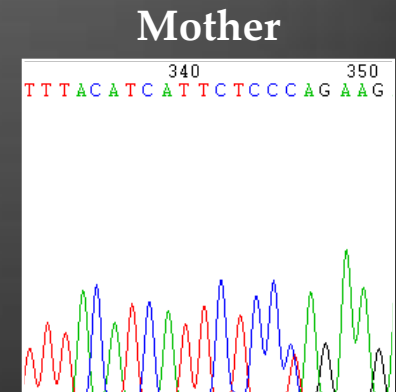
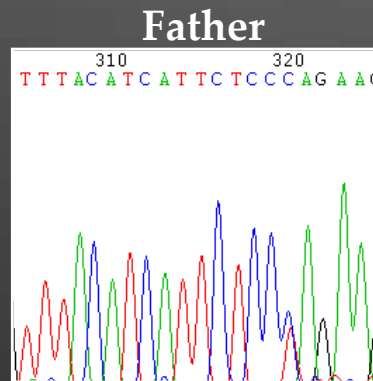
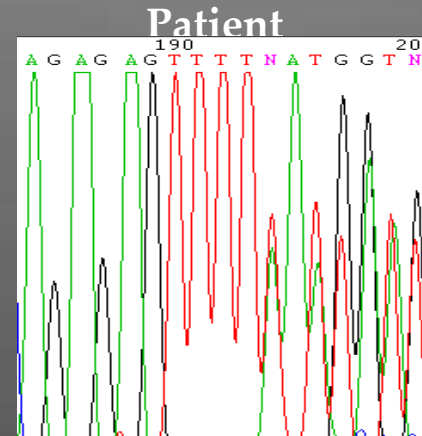
Phenylketonuria (PKU)



Both sisters affected
Autistic
Developmental delay



HPLC chromatogram



Novel splice site mutation

Major Neuropathologic changes

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Major Neuropathologic changes

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PKU

Case 2

- ▶ detected by NBS
- ▶ Phe level by TMS 1400 μ M
- ▶ Phe/tyr ratio >10
- ▶ By HPLC 900nmol/l

- ▶ Confirmed by PAH mutation analysis. Found to be compound heterozygote for a splice site mutation in **IVS8-7A>G** and Novel missense **p.G312R** in exon 9.

- ▶ Started PHE free diet in third week of life
- ▶ Phe level dropped down to near normal level
- ▶ Adjusted protein intake to raise the tyrosine level
- ▶ Now aged 10 months with normal mental development.

- ▶ **This highlights the importance of newborn screening**

Maternal PKU syndrome

- ▶ First mentioned in literature in 1937
- ▶ First mentioned as a complication of PKU in 1956
 - Untreated women
 - 92% risk of mental retardation
 - 73% risk of microcephaly
 - 40% risk of low birth weight
 - 12% risk of congenital heart disease
 - **Reduced risk if maternal plasma phe levels are normalized pre-conceptually**
- ▶ Microcephaly and cardiac defects reported in 1960's

Balancing Metabolic Control

Exposure to normal PHE intake:

Elevations of PHE

Elevations of PHE-ketones

Deficient TYR, DOPA, NE, EPI

Mental retardation / seizures



Elimination of PHE from the diet:

Decreases PHE

Decreases PHE-ketones

Deficient TYR, DOPA, NE, EPI

DEATH from essential AA deficiency

Balancing Metabolic Control

- ▶ Elimination of PHE from the diet:
 - Decreases PHE
 - Decreases PHE-ketones
 - Deficient TYR, DOPA, NE, EPI
 - **DEATH** from essential AA deficiency

Bad

Optimal Therapy of PKU

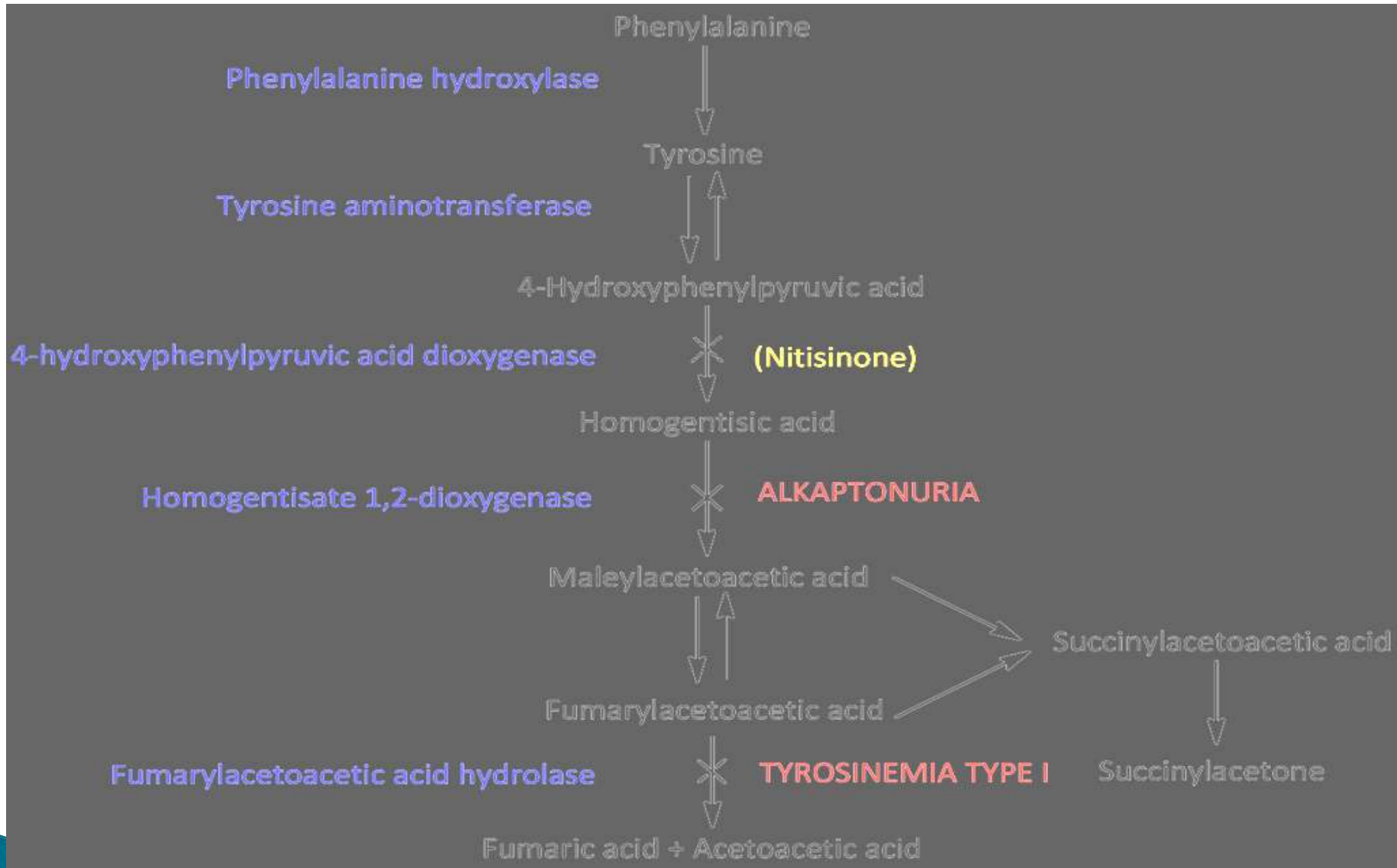
- ▶ Initiate treatment by 7 days of life
- ▶ Phenylalanine levels

<u>Age</u>	<u>Level</u>	<u>Freq of Testing</u>
0-12 months	2-6 mg/dl	1x/week
1-12 years	Same	2x/month
> 12 years	2-15 mg/dl	1x/month
Pregnancy	2-6 mg/dl*	2x/week

Tyrosinemia

▶ AAD

- ▶ A deficiency in fumarylacetoacetase (FAH), the final enzyme in the tyrosine catabolic pathway.
- ▶ In tyrosinemia type I, catabolic intermediates maleylacetoacetate and fumarylacetoacetate are converted to the toxic metabolites succinylacetone and succinylacetoacetate.
- ▶ Succinylacetone can also inhibit the porphyrin synthesis pathway leading to the accumulation of 5-aminolevulinate, a neurotoxin responsible for the porphyric crises characteristic of tyrosinemia type I



Tyrosinemia types

- ▶ oculocutaneous tyrosinemia (Type2)
- ▶ Skin and eye symptoms within the first year of life
- ▶ excessive tearing, photophobia, eye pain and redness, and skin lesions.
- ▶ caused by a deficiency of the enzyme tyrosine aminotransferase.

- ▶ Tyrosinemia type III or 4-hydroxyphenylpyruvate dioxygenase
- ▶ deficiency occurs because of an enzyme deficiency of 4-hydroxyphenylpyruvate dioxygenase.

- ▶ The clinical presentation of this form of tyrosinemia is not well known. Develop neurologic problems, mental retardation and ataxia

- ▶ Transient tyrosinemia: Elevated tyrosine levels in a healthy newborn with no liver, renal, and skin abnormalities.
- ▶ Risk factors include prematurity, high protein intake, and
- ▶ deficient intake of Vitamin C.

Tyrosinemia (Type 1)


- ▶ Plasma tyrosine 679micromole/L
- ▶ UOA
- ▶ Hereditary infantile tyrosinemia, or tyrosinemia I,
- ▶ peculiar (cabbagelike) odor, renal tubular dysfunction (Fanconi syndrome), and
- ▶ survival of less than 12 months of life if untreated.
- ▶ Fulminant onset of liver failure occurs in the first few months of life.

S.no	Compound Name	Relative Peak Area of Compound with respect to Internal Standard - Margoric acid			
		Result	Cut-off	Reference Range	Fold Elevation
1	Oxalic-2	1.36%	1.00%	0.00% 0.00%	1.36
2	Malonic-2	1.11%	0.50%	0.00% 0.10%	11.13
3	Glyceric-3	13.13%	2.93%	0.00% 1.60%	8.21
4	Succinylacetone-OX-2(1)	66.36%	0.50%	0.00% 0.00%	132.72
5	Thiodiglycolic-2	0.75%	0.10%	0.00% 0.00%	7.47
6	4-OH-benzoic-2	39.71%	22.38%	0.00% 7.80%	5.09
7	4-OH-phenylacetic(PHPAA)	147.32%	139.99%	8.60% 73.20%	2.01
8	Phenylpyruvic-OX-2	6.01%	0.50%	0.00% 0.00%	12.02
*9	4-OH-phenyllactic(PHPLA)-2	4227.28%	12.51%	0.00% 7.00%	603.90
*10	4-OH-phenylpyruvic(PHPPA)-OX-2	650.48%	1.90%	0.00% 0.90%	722.76
11	Palmitic-1	31.73%	23.34%	0.00% 13.80%	2.30
12	N-Acetyltyrosine-3	6.41%	0.10%	0.00% 0.00%	64.13

Clinical presentation

UOA in tyrosinemia

Treatment

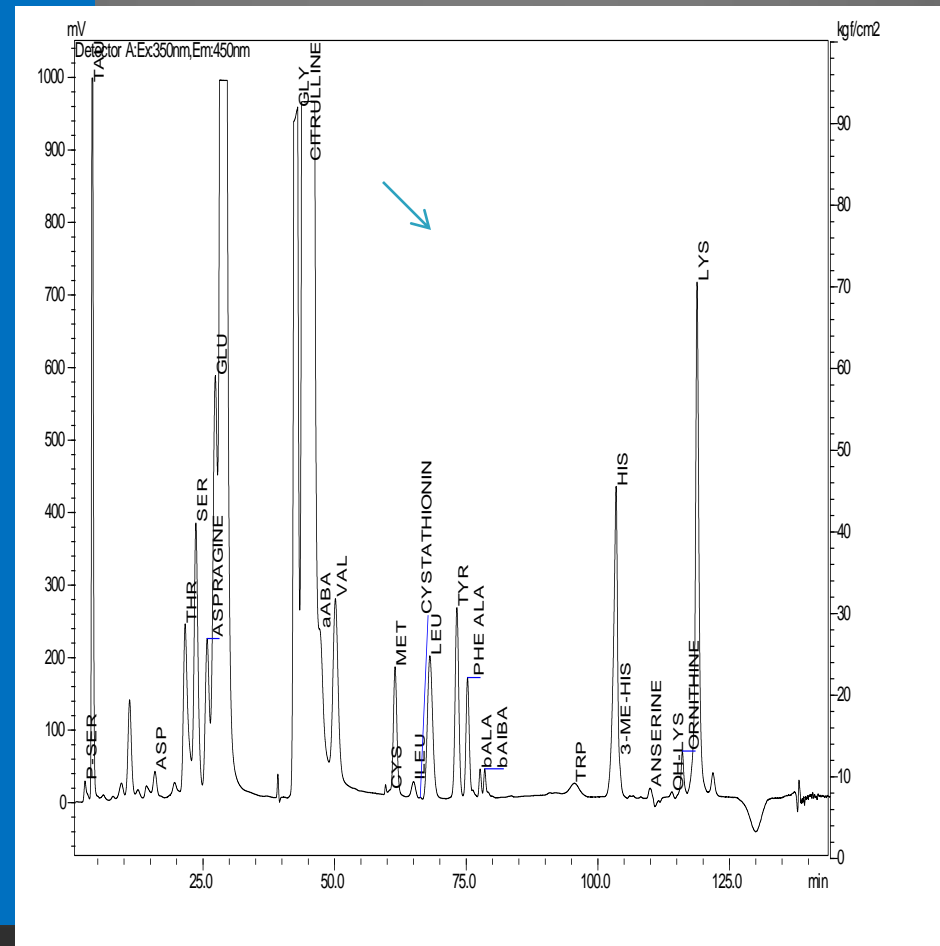
- ▶ Diet, Formula free of phenylalanine and tyrosine
 - ▶ Oral NTBC
 - ▶ The drug 2-(2- nitro-4-trifluoromethylbenzol)-1,3-cyclohexanedione (NTBC) has been successful in the management of tyrosinemia.
 - ▶ NTBC works by inhibiting the proximal tyrosine metabolic pathway.
 - ▶ NTBC, interferes with the production of succinylacetate by stopping the conversion of p-OH-phenylpyruvic acid to homogentisic acid.
 - ▶ Liver transplant.
- 

What are Urea Cycle Disorder

- ▶ Caused by a mutation that results in a deficiency of one of the six enzymes in the urea cycle.
- ▶ These enzymes are responsible for removing ammonia from the blood stream.
- ▶ The urea cycle involves a series of biochemical steps in which nitrogen, a waste product of protein metabolism, is removed from the blood and converted to a compound called urea in the blood.
- ▶ Normally, the urea is transferred into the urine and removed from the body.
- ▶ In urea cycle disorders, the nitrogen accumulates in the form of ammonia, a highly toxic substance, resulting in hyperammonemia
- ▶ Ammonia then reaches the brain through the blood, where it can cause irreversible brain damage, coma and/or death.

Citrulinemia

- ▶ 3day old female with lethargy convulsions, vomiting
- ▶ Hyper ammonemia,
- ▶ no metabolic acidosis,
- ▶ plasma citruline high ,
- ▶ Also in urine and CSF.
- ▶ A case of citrulinemia



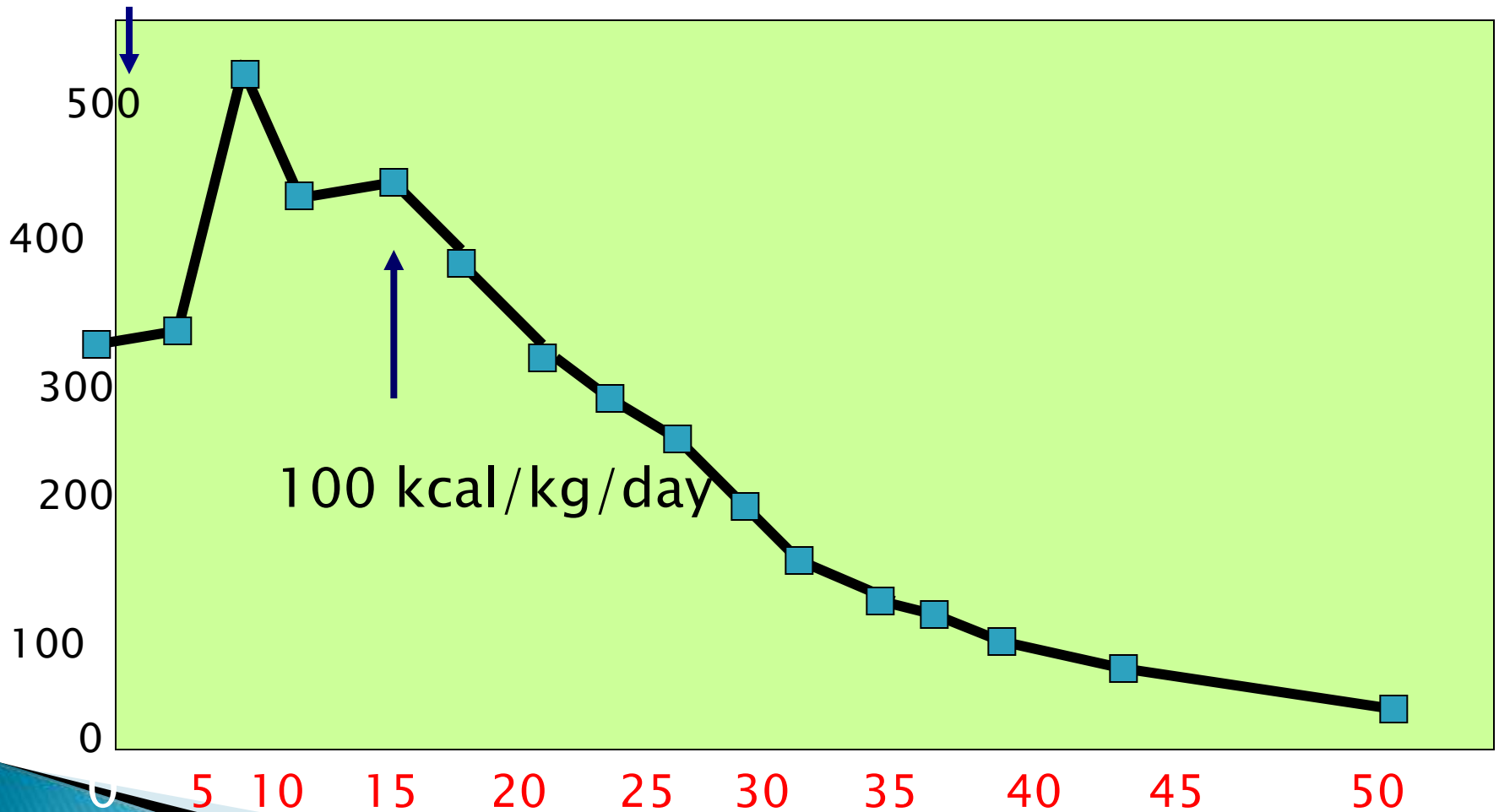
Citrullinemia

- ▶ **Argininosuccinate Synthetase gene (*ASS1*, Chr. 9q34.1)**
- ▶ **Patient**
- ▶ **exon 14: c.1088G>A(p.Arg363Gln)**
- ▶ **exon 15: c.1168G>A (p.Gly390Arg)** **compound heterozygote**
- ▶ **Father**
- ▶ **exon 14: c.1088G>A(p.Arg363Gln)**
- ▶ **mother**
- ▶ **Heterozygous exon15**
 - On Sodium benzoate and formula diet (URCD)
- ▶ Doing well except two episodes of hyperammonemia following ear infection.
- ▶ Two cases presently on formula diet

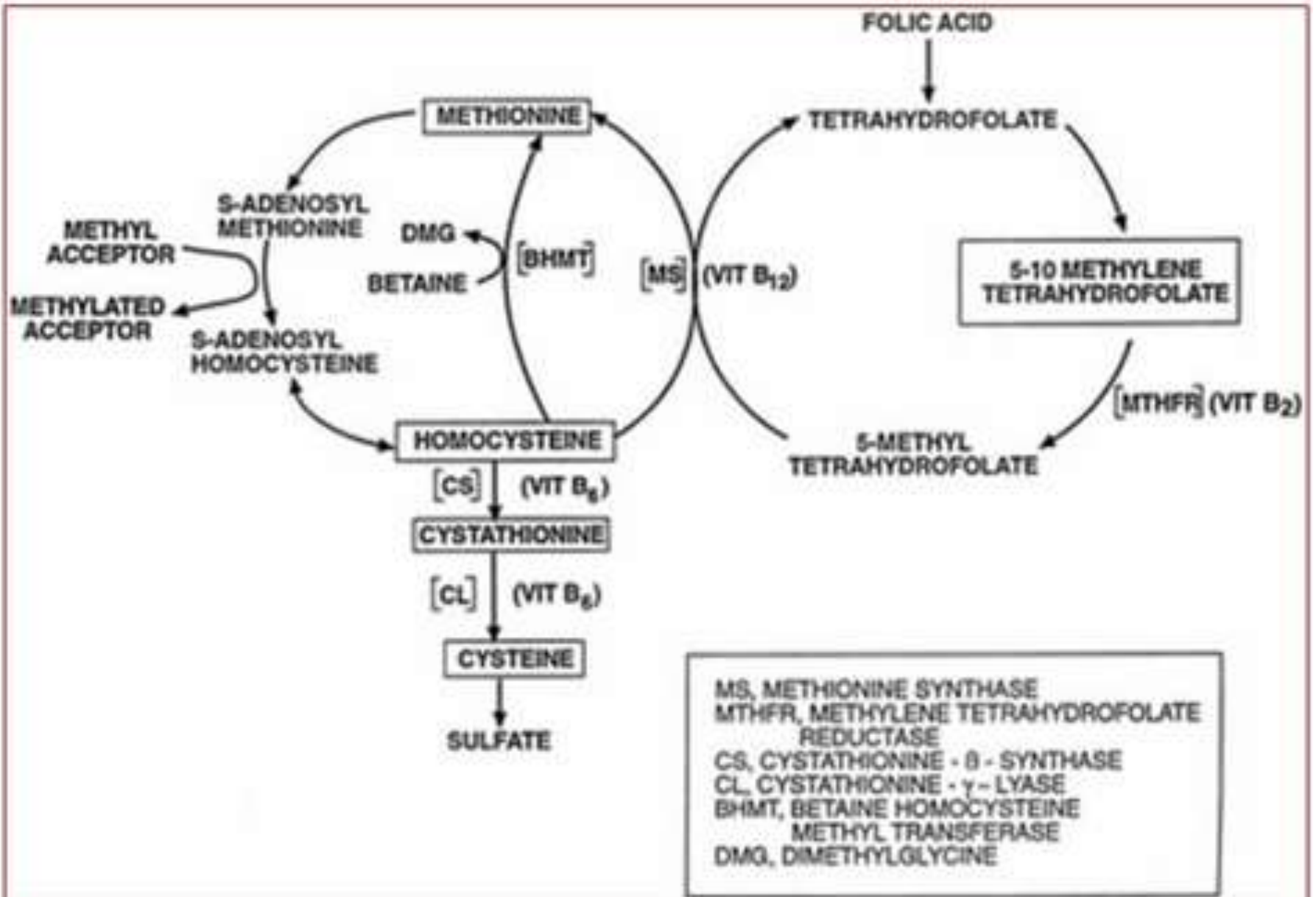
The treatment of urea cycle disorders

- ▶ dietary management to limit ammonia production
- ▶ medications for the removal of ammonia from the bloodstream.
- ▶ Sodium phenylbutyrate is the primary medication being used to treat urea cycle disorders.
- ▶ L-citrulline (for OTC and CPS deficiency) or L-arginine (ASA and citrullinemia) is also required.
- ▶ These are not to be used in Arginase Deficiency.
- ▶ These supplements help catalyze the urea cycle enzymes and promote optimal removal of ammonia.
- ▶ special amino acid formulas (Cyclinex, EAA, UCD I&II), developed specifically for urea cycle disorders to provide approximately 50% of the daily dietary protein allowance.

Neonatal Citrullinemia Ammonia Levels ($\mu\text{mol/L}$)



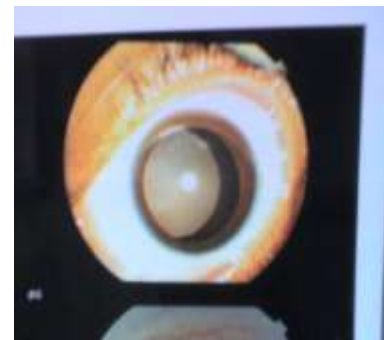
Methionine Metabolism



Homocystinuria

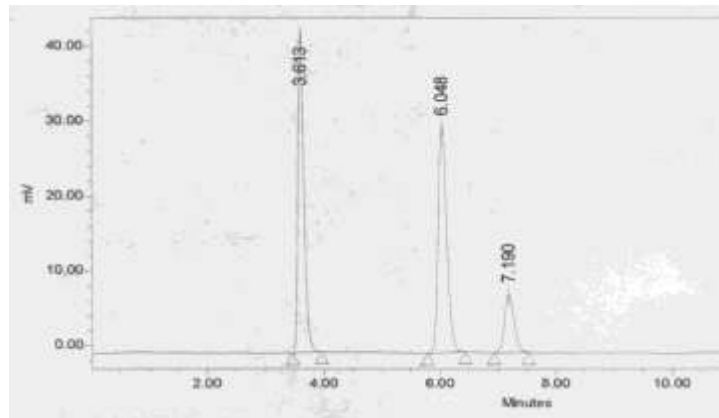
(Methionine metabolism)
CBS deficiency

- ▶ Developmental delay,
- ▶ lens dislocation, behavioral problem, hyperactive attention deficit, scholastic
- ▶ backwardness, stroke (infantile) deep vein thrombosis seizures
- ▶ early coronary artery disease, marfanoid features
- ▶ MRI brain showing infarcts
- ▶ Methionine free diet
- ▶ Supplements with pyridoxin
- ▶ B12

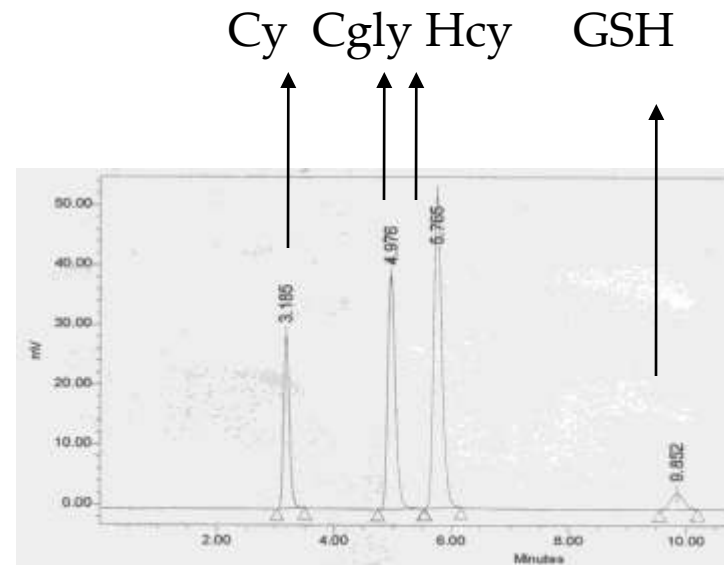


Bilateral dislocation of the lens

HPLC analysis for sulphur containing aminoacids



HPLC CHROMATOGRAM - NORMAL




HPLC CHROMATOGRAM - DEFECTIVE

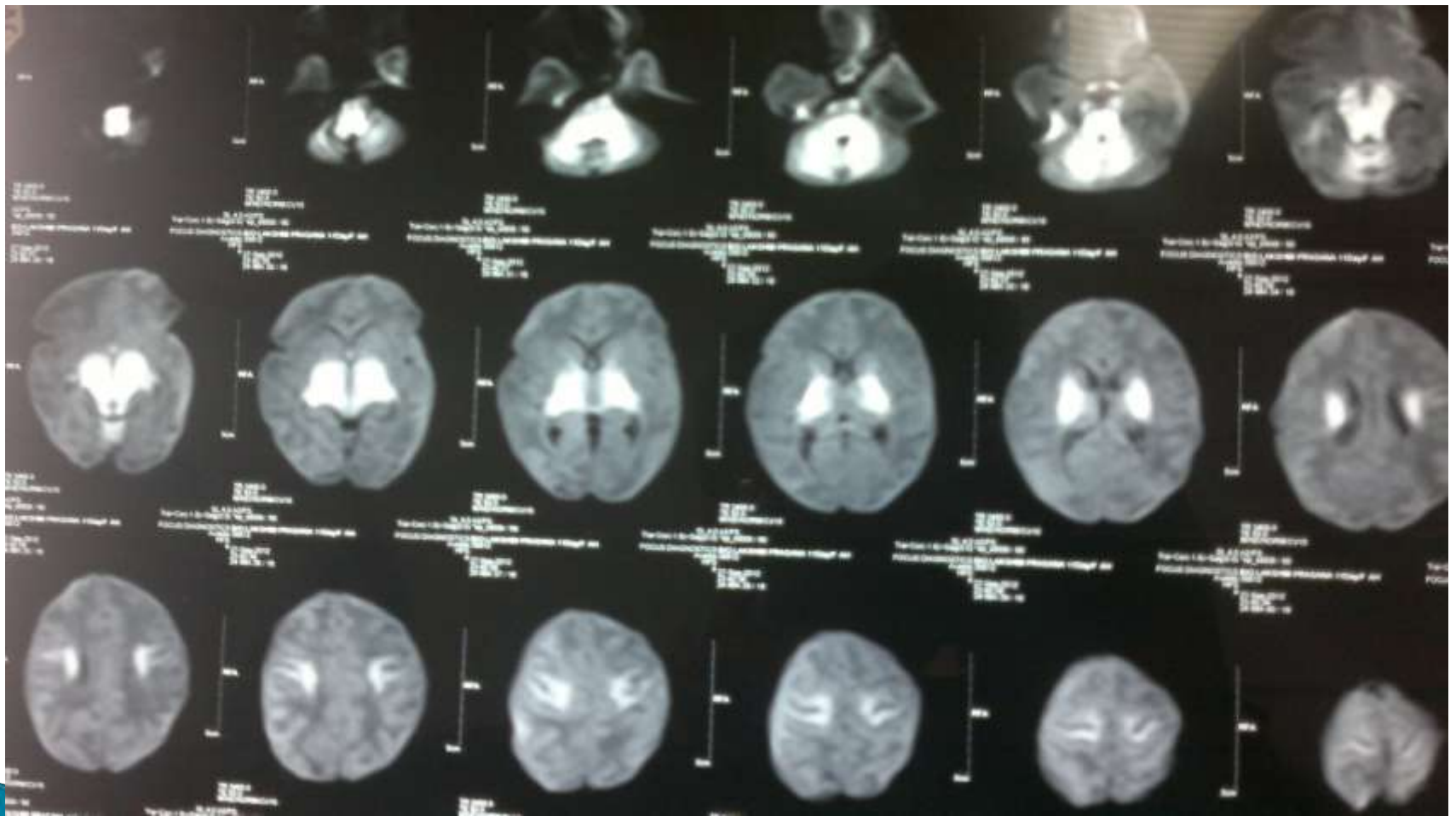
MSUD

- ▶ MSUD is a potentially deadly disorder that affects three amino acids, leucine, isoleucine, and valine.
- ▶ Defects in any of the six subunits that make up the BCKD protein complex can cause the development of MSUD.
- ▶ The most common defect is caused by a mutation in a gene on chromosome 19 that encodes the alpha subunit of the BCKD complex (BCKDHA).

Case

- ▶ Term infant 3kg , normal birth history
 - ▶ D3 of life progressive lethargy, poor feeding
 - ▶ Rapid worsening over 2 days and required intubation
 - ▶ Standard investigations for sepsis normal
 - ▶ EEG – diffuse slowing
 - ▶ MRI diffuse brain oedema
- 

MSUD



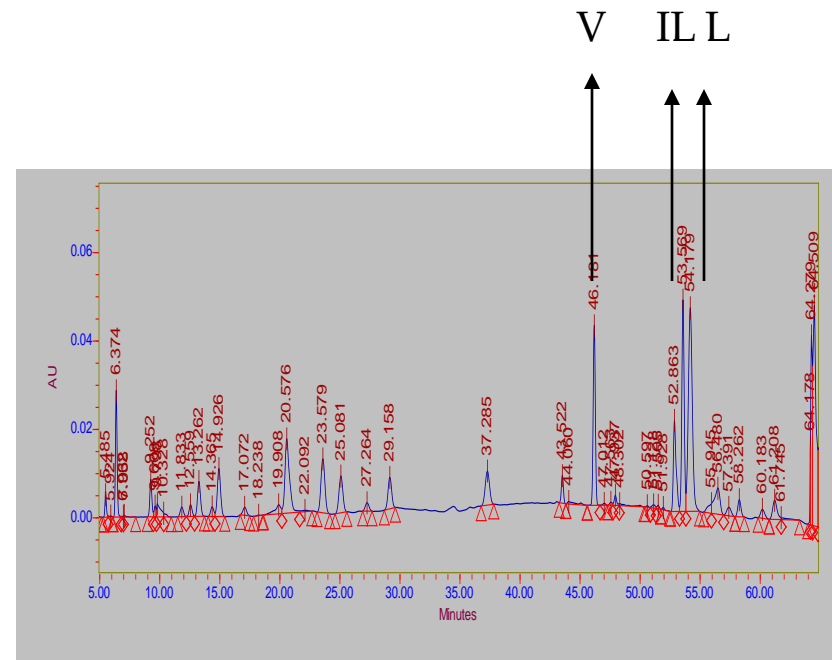
MSUD

- ▶ TMS- Leucine levels – 3000microgram /ml
- ▶ Exchange transfusion
- ▶ NG tube feed
- ▶ Gradually improving on MSUD diet

- ▶ Complications of MSUD diet
- ▶ Valine def
- ▶ Isoleucine def

MSUD(late diagnosis)

- ▶ H/o five infant deaths, 6th child
- ▶ Newborn urine positive for ketone bodies and
- ▶ Aminoacid branched chain aminoacidemia
- ▶ On diet and thiamin supplementation.
- ▶ Child is doing well in spite of delay in treatment.



Early detection by NBS

Case

- ▶ Normal at birth
- ▶ Metabolic acidosis on 5th day of life
- ▶ Confirmed MSUD
- ▶ PND next pregnancy

- ▶ Treatment with MSUD formula
- ▶ Good control by 2 months
- ▶ Liver transplant at 4yrs
- ▶ Now normal, aged 7yrs
- ▶ No diet restriction

- ▶ Organic Aciduria- Disorders of branch chain Amino acid catabolism

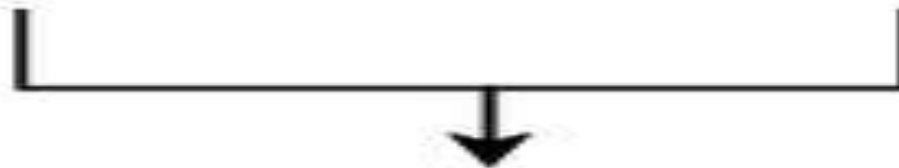
MMA

- ▶ Methylmalonic acidemia is an autosomal recessive disorder of amino acid threonine, methionine and leucine, valine metabolism,
- ▶ involving a defect in the conversion of methylmalonyl-coenzyme A (CoA) to succinyl-CoA. requires the enzyme methylmalonyl-CoA mutase and the cofactor 5'-deoxyadenosylcobalamin
- ▶ Patients typically present at the age of 1 month to 1 year with neurologic manifestations, such as seizure, encephalopathy, and stroke
- ▶ Or with hyperammonemia or Metabolic Acidosis



Odd-chain
fatty acids
Cholesterol

Methionine
Threonine
Isoleucine
Valine



Propionyl CoA
(3 carbons)

*Propionyl CoA
carboxylase
(biotin)*

ATP + CO₂

ADP



Methylmalonyl CoA
(4 carbons)

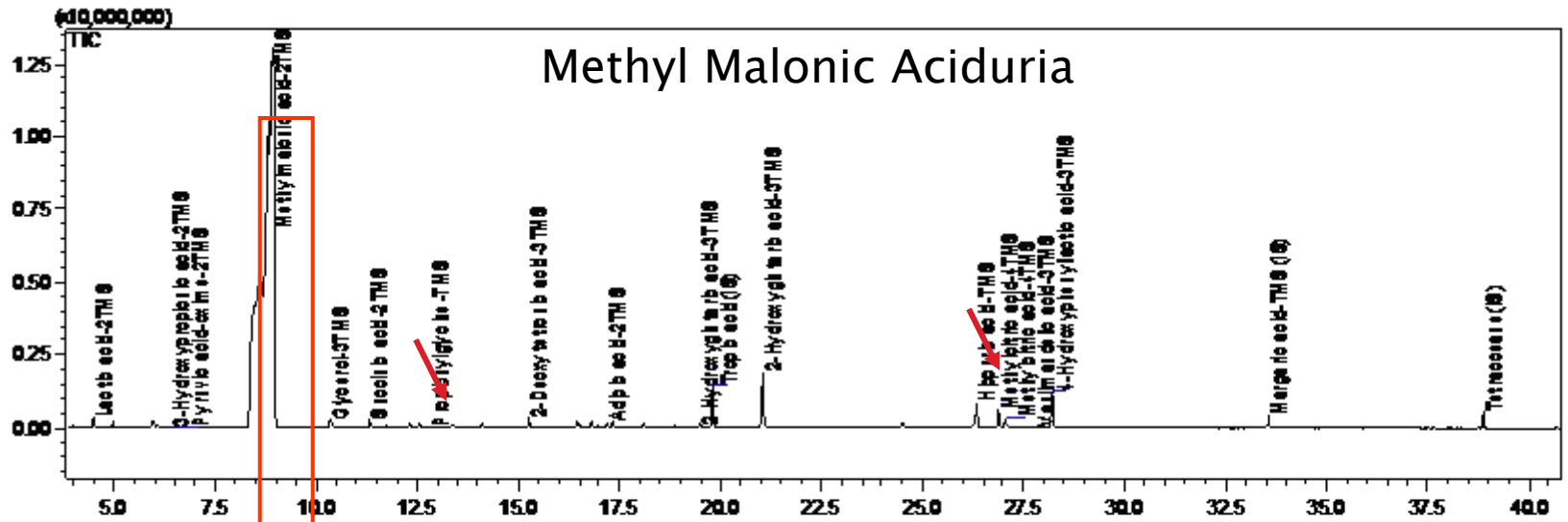
*Methylmalonyl
CoA mutase
(vitamin B12)*



Succinyl CoA



Citric Acid Cycle
&
Gluconeogenesis



3month old

Acute metabolic acidosis

Encephalopathy

Failure to thrive

on special diet

Name	Fold elevation
Methylmalonic acid	~1210
Methylcitrate	~19
3-hydroxypropionic acid	~3
Propionylglycine	~3



At 8months
Normal development

Both sibs affected with MMA

1st diagnosed after metabolic acidosis

2nd by NBS

On formula feeds and low protein diet

Normal development and

Neurological damage

By NBS



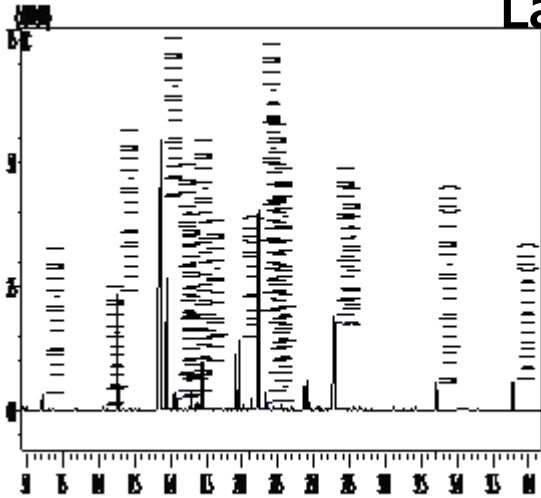
Diagnosed on 9th day of life

Glutaric Aciduria Type I

- ▶ AA's lysine, hydroxylysine and tryptophan are involved.
- ▶ caused by deficiency of glutaryl-CoA dehydrogenase.
- ▶ Excessive levels of their intermediate breakdown products (glutaric acid, glutaryl-CoA, 3-hydroxyglutaric acid, glutaconic acid) can accumulate and cause damage to the brain (and also other organs), particularly the basal ganglia,
- ▶ GA1 causes secondary carnitine deficiency, as glutaric acid, like other organic acids, is detoxified by carnitine

Glutaric aciduria - Type 1

Late diagnosis



GA	3 - OH GA	Glutaconic acid	2 OH GA
1560	32	30	8

Late diagnosis
Movement disorder
ID





MRI
Batwing appearance
Sylvian fissure

Child with Glutaric Aciduria Type I



35 days old female infant born to consang(1st cousin) couple.
Macrocephaly
NBS detected elevated C5DC in TMS(2.0/<0.7)
Neurosonogram Small cyst near the ventricles, minimal internal hydrocephalous
AF wide patent ,bulging
Urine organic acids & mutation study confirm GAI
Diet replacement since 3rd week of life
Normal development
Presently aged 5yrs

Follow-up neonate with Isovaleric aciduria

- ▶ **Premature neonate, 1.2 kg, Earlier two died in the neonatal period with no diagnosis.**
 - ▶ **Severe neonatal crisis**
 - ▶ **TMS was done in view of previous NND**
 - ▶ **TMS and GC-MS and mutation study confirmed**
 - ▶ **Isovaleric acidemia**
 - ▶ **special diet, low in leucine, (leucine free)**
 - ▶ **Add glycine, carnitine supplements.**
 - ▶ **Now aged 6yrs, with normal mental development.**
-
- ▶ **An example of early detection through NBS**
 - ▶ **and management by formula diet**



Goals of dietary management for infants and children are to

- ▶ Support an appropriate rate of growth
 - ▶ Support normal intellectual development
 - ▶ Maintain optimal nutritional status
 - ▶ Provide adequate nourishment

 - ▶ Prevent neurological crisis
 - ▶ Prevent liver and renal function problems
 - ▶ Prevent formation of tyrosine crystals in the eyes (this occurs with elevated plasma tyrosine levels)
- ▶ Adequacy of therapy is monitored by frequent measurement of:
 - ▶ Plasma phenylalanine and tyrosine
 - ▶ Plasma or urine concentrations of succinylacetone in Tyrosinemia

 - ▶ All plasma amino acids (to assure the adequacy of intake and to prevent deficiencies)

 - ▶ Albumin, prealbumin, hematocrit, and hemoglobin (as measures of the overall adequacy of the diet)

 - ▶ Liver function studies

THANK Q