

**BIOCHEMICAL BASIS OF  
INHERITED DISEASE  
(INBORN ERROR OF  
METABOLISM) AND  
MANAGEMENT**

# What is inborn error of metabolism?

- Genetic disorders involving disorders of metabolism.
- Most are enzyme defects disrupt conversion of substrates into products.
- In most of the disorders, problems arise due to accumulation of toxic upstream substances, or to the effects of reduced downstream essential compounds.

# Disorders of amino acid metabolism

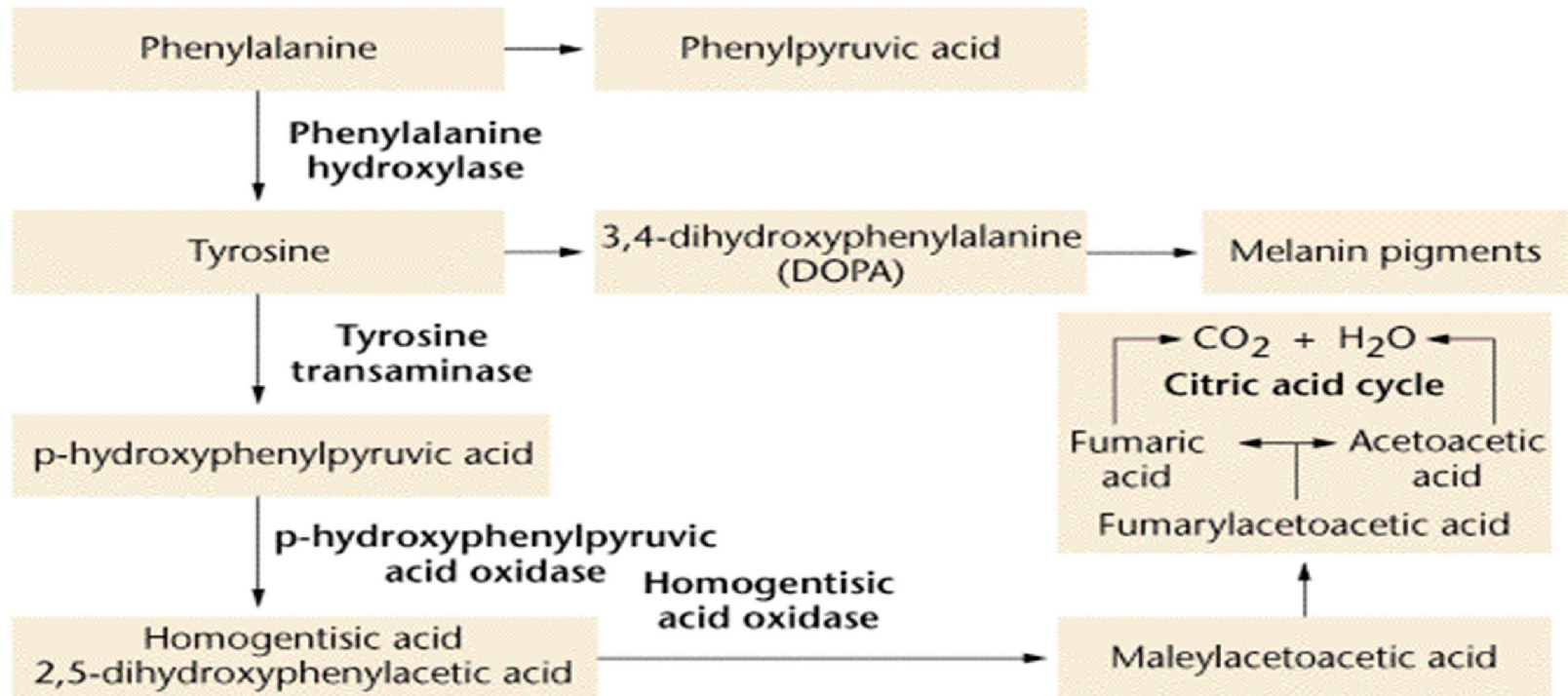
- This is an inborn error of metabolism due to amino acid. It occurs as a result of deficiency or gene mutation of enzyme responsible for the catalysis of a reaction step in a metabolic pathway.
- Examples include alkaptonuria, maple syrup urine disease (MSUD), phenylketonuria, homocystinuria,

# Alkaptonuria

- Alkaptonuria (AKU) is an autosomal genetic disorder caused by a lack of the enzyme homogentisate dioxygenase (HGD).
- This is called an autosomal recessive inheritance, i.e. both parents are carriers of the gene, although they do not suffer any symptoms of the disease. If both parents pass this gene to their child, alkaptonuria results.

- Homogentisate dioxygenase or oxidase (HGD) catalyzes the conversion homogentisic acid, an intermediate in the degradation of phenylalanine and tyrosine to the TCA cycle.
- Lack of HGD leads to a build up of homogentisic acid in the bones, cartilage and urine.
- Alkaptonuria is characterized by the presence of black urine, ochronosis (black bones and cartilage) and a degenerative arthritis of the joints.

- HGD deficiency results in the accumulation of homogentisic acid in the blood and is eliminated in large amounts in the urine.
- Upon contact with air, HGA oxidizes and turns the urine black. This is caused by a black pigment called “alkapton”, and leads to the name alkapton-uria.



## Phenylalanine metabolism

# Clinical manifestations

- Early diagnosis of the disease is black urine.
- Homogentisic acid builds up in the connective tissue causing black and brittle bones and cartilage (osteoarthritis).
- Black spots in the eyes, discoloured ears and dark earwax also affect AKU patients. However these symptoms do not affect vision or hearing, and are often used for diagnostic purposes later in life. This process of ochronosis may also occur under the nails, on the face and hands.
- Kidney, prostate and bladder stones may occur due to the build-up of HGA in the genito-urinary tract, during the production of urine.
- The build-up of HGA can also cause heart complications. In severe cases, it may cause heart disease and patients may require heart valve replacements.



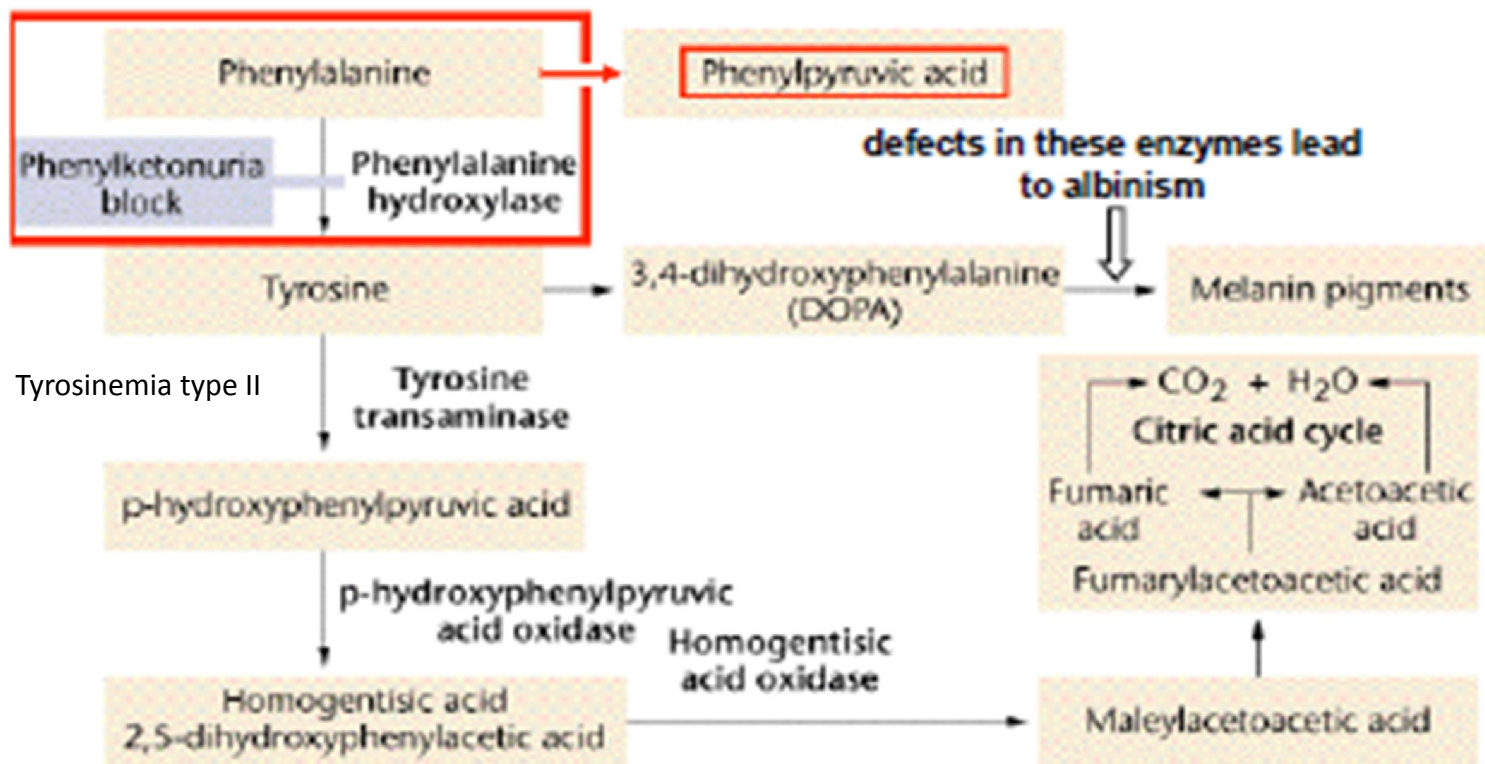
# Management

- Alkaptonuria is an inherited disease which can lead to severe consequences. Early diagnosis and new treatments can enhance the quality of life of patients suffering from this disease.
- Patients often need surgery such as joint replacements or repair of tendon rupture. Some patients may also need heart valve replacements.
- Reduction of protein intake can also help but extremely difficult to do.

# Phenylketonuria

- Phenylketonuria (PKU) is also genetic disorder inherited in autosomal pattern. It is an inborn error of metabolism of phenylalanine. It can also be environmentally induced i.e. over exposure to phenylalanine diet.
- It results from the deficiency of phenylalanine hydroxylase, PAH, which converts phenylalanine to tyrosine.

- The symptoms of the disease vary from mild to severe.
- In the severe form called classic PKU, the infants develop reduced mental ability, behavioural problems, seizure, retarded growth, and movement disorder.
- The mild PKU is characterized by cognitive loss.
- Also, there is non-PKU hyperphenylalaninemia which requires BH<sub>4</sub> as cofactor.



- The main treatment for PKU is a lifelong specialized diet that is very low in phenylalanine, especially during childhood while the patient is growing.

# Homocystinuria

- Homocystinuria is an autosomal recessive inheritance of metabolic derangement of methionine which occurs 1 in 345,000 in population.
- It is due to the deficiency of cystathione  $\beta$  synthase that catalyzes the breakdown of methionine which results in the accumulation of homocysteine and methionine in the blood, cerebrospinal fluid and urine.
- This enzyme catalyzes the conversion of homocysteine to cystathionine by trans-sulfuration pathway of methionine using pyridoxal 5 phosphate as the cofactor.
- The clinical manifestation include cataract, mental derangement, thrombosis (clotting in the arteries which leads to the high risk of stroke and heart attack) and osteoporosis.

# Management

- This disorder can be classically managed in three ways:
- Restriction of methionine and supplementation of cysteine diet.
- Intake of pyridoxine in pyridoxine responsive individuals.
- Intake of betaine, methyl donor, in pyridoxine non-responsive individuals

# MAPLE SYRUP URINE DISEASE

- Maple syrup urine disease (MSUD) can also be referred to as branched-chain ketonuria.
- It is caused by branched-chain  $\alpha$ -ketoacid dehydrogenase enzyme deficiency (BCKD).
- It is associated with the accumulation of the three branched amino acid such as leucine, isoleucine and valine, and their metabolites such as  $\alpha$ -ketoacids in the urine of the affected infant.



- The clinical manifestation include mental retardation, physical disabilities and death within the first few months of the infant.
- Early diagnosis is very helpful as well as dietary control. This includes removal or reduction of amino acids, valine, leucine and isoleucine from the infant's diet.

# LYSOSOMAL STORAGE DISEASE

- The lysosomal storage diseases (LSDs) comprise a heterogeneous group of almost 50 disorders that are caused by genetic defects in a lysosomal acid hydrolase, receptor, activator protein, membrane protein, or transporter, causing lysosomal accumulation of substrates that are specific to each disorder.

- The accumulation is progressive, ultimately causing deterioration of cellular and tissue function. Many disorders affect the central nervous system (CNS) and most patients have a decreased lifespan and significant morbidity.

# METABOLIC DISORDER OF CARBOHYDRATE

- Galactosemia
- Galactosemia has been used for its toxicity syndrome associated with an intolerance to dietary galactose. This recessively inherited disorder occurs as a result of a deficiency of the enzyme galactose phosphate-uridyl transferase.
- The deficiency of the enzyme causes galactose to be reduced to galactitol and oxidized to galactone. It is the presence of these two intermediate products of metabolism that has direct toxic effect and results in clinical manifestations of galactosemia.
- The classical clinical presentation of galactosemia is failure to thrive in early infancy complicated by vomiting and diarrhea.
- In addition, these infants show deranged hepatic functions with jaundice and hepatomegaly. Severe hemolysis can also occur and cataract may be noted shortly after birth. Retarded mental development may be apparent after a few months of age.
- Galactosemia may not present any symptom in some cases until late infancy or early childhood.
- Early diagnosis is very important. Therefore early removal of dietary sources of galactose can accomplish excellent metabolic control of this disorder with prevention of congenital cataract and normal growth and mental development.
- But removal of dietary sources of galactose cannot reverse mental retardation if it is already established
- The second defect in galactose metabolism occurs secondary to deficiency of enzyme galactokinase. This disorder is recognized mostly based on urine metabolite screening of patient with cataract.
- Deficiency of galactokinase results in the accumulation of galactitol as an end product of galactose metabolism. This compound accumulates within the lenses and is responsible for the congenital cataract. There are no hepatic or other systemic manifestation of this defect.
- This enzyme can be assayed in the RBC and urine for the presence of galactitol and galactose. These two tests are the primary means of diagnosis of galactokinase deficiency. Recognition of this disorder and removal of galactose from the diet will prevent cataract development

# Hereditary fructose intolerance

- This is an autosomal recessive inborn error of metabolism. Incidence of the disease is 1 in 20,000 births, while 1 in 70 persons are carriers of the abnormal gene.
- The metabolic disorder results from the deficiency of fructose-1-phosphate aldolase (aldolase-B) which is responsible for the cleavage of F-1-P into DHAP and glyceraldehyde which leads to the accumulation of F-1-P.
- This occurs when sucrose is introduced in the diet of infants, usually around 6 months of age. Accumulation of F-1-P inhibits glycogen phosphorylase which in turn results in glycogen accumulation in liver and consequent hypoglycemia.
- Other clinical manifestations include vomiting, loss of appetite, and failure to thrive. Hepatomegaly and jaundice may occur. Death may also result if liver damage persists. Fructose is excreted in urine when urine gives positive Benedict's test.
- Withdrawal of fructose from the diet will immediately relieve the symptoms

# Mucopolysaccharidoses

- A group of human genetic disorders characterized of proteoglycans exists, collectively called mucopolysaccharidoses (lysosomal storage diseases). These disorders results from the deficiency of one or more lysosomal hydrolysis responsible for degradation of the dermatan and or heparan sulphate.
- These disorders are characterized by the nature of the macromolecular compounds that accumulate. Varying degree of deficiency or altered activity of hydrolase may result in several of  $\alpha$ -L-iduronidase.
- Deficiency of this enzyme results in the accumulation of GAG (glycosaminoglycan), dermatan sulphate and heparan sulphate.
- In its most severe form, the enzyme deficiency results in Huler's syndrome characterized with severe skeletal, somatic and CNS manifestations and death by 5-8 years of age.
- In its mildest form, this enzyme deficiency leads to Scheie's syndrome accompanied with normal height, no mental retardation, mild skeletal changes, but ocular abnormalities (corneal opacity) and derman sulphate in urine.
- In general, defective degradation of heparan sulphate leads to mental retardation predominantly whereas accumulation of other GAGs lead to mesenchymal abnormalities.
- All mucopolysaccharidoses are inherited as autosomal recessive traits, except hunter's disease which is X-linked (deficiency of iduronate sulphate).

# Organic Acidemia

- Organic acid disorders are inherited in an autosomal recessive manner and affect both males and females. It occurs in infants and children.
- Organic acidemias are characterized by abnormal amounts or types of organic acids in the urine and other body fluids

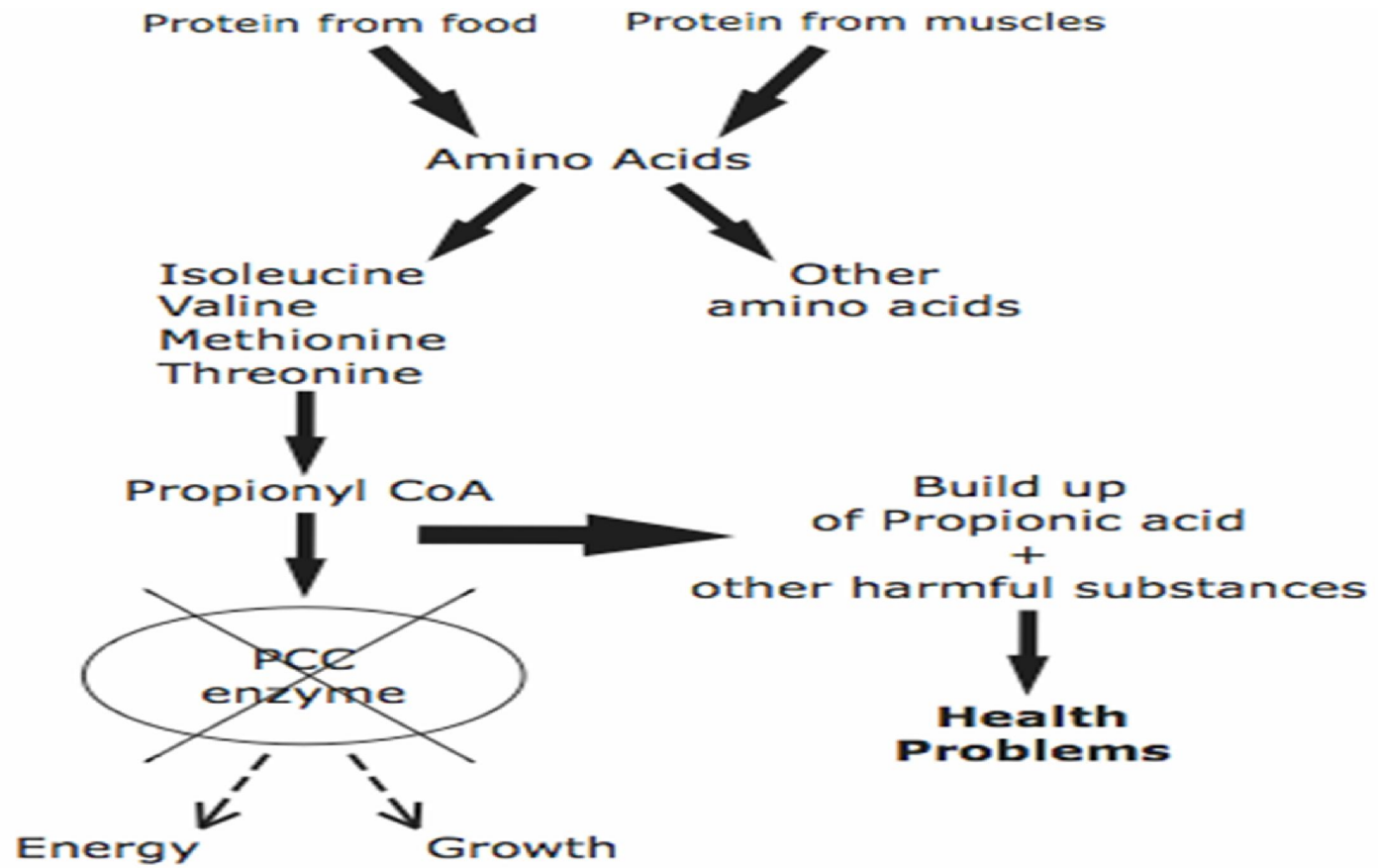
# EXAMPLES OF ORGANIC ACIDURIA

- Methyl-3-Hydroxybutyryl CoA Dehydrogenase deficiency (MHBD)
- 2-Methylbutyryl CoA Dehydrogenase deficiency (2-MBCD)
- 3-Hydroxy-3-Methylglutaryl CoA Lyase deficiency (HMG)
- 3-Methylcrotonyl CoA Carboxyl deficiency (3-MCC)
- 3-Methylglutaconyl CoA Hydratase deficiency (3-MGA)
- Glutaric Aciduria Type I (GA-1)
- Isobutyryl CoA Dehydrogenase deficiency (IBCD)
- Isovaleric Acidemia (IVA)
- Malonic Aciduria (MA)
- Methylmalonic Acidemia (MMA)
- Mitochondrial Acetoacetyl CoA Thiolase – (3-Ketothiolase) (BKT )
- Multiple CoA Carboxylase (MCD)
- Propionic Acidemia (PA)



# Propionic Acidemia (PA)

- Propionic acid occurs when propionyl CoA carboxylase (PCC) enzyme is either missing or not functioning properly. This enzyme's function is to convert branched-chain amino acids to propionyl CoA and subsequently to energy for body use.
- When this enzyme is malfunctioning, substances called glycine and propionic acid, along with other harmful substances, build up in the blood and cause problems.



## Clinical presentation includes

- poor appetite
- vomiting
- extreme sleepiness or lack of energy
- low muscle tone (floppy muscles and joints)
- learning disabilities or mental retardation
- delays in walking and motor skills
- rigid muscle tone (spasticity)
- poor growth with short stature
- seizures
- osteoporosis
- pancreatitis
- skin rashes

Biochemically, the following are observed:

- ketones in the urine
- high levels of acidic substances in the blood (metabolic acidosis)
- high blood ammonia levels
- high blood levels of glycine
- high levels of certain organic acids
- low platelets
- low white blood cells

# TREATMENT

- Low protein diet.
- Avoid going a long time without food
- Medication
- ✓ Children with PA may benefit by taking L-carnitine. This is a safe and natural substance that helps the body make energy. It also helps get rid of harmful wastes. L-carnitine is part of the usual treatment for PA.
- Regular blood and urine tests
- ✓ Monitoring ketones

# Isovaleric acidemia

- Isovaleric acidemia is genetic disorder that affects how protein is broken down in the body. It is a metabolic disorder.
- Isovaleric acidemia occurs in less than 1 in 100,000 births.
- People who are born with isovaleric acidemia are unable to properly breakdown an amino acid called leucine in the food they eat. This is because they are missing an enzyme in their cells.

- Mutations in the IVD gene cause isovaleric acidemia. Mutations in this gene reduce or eliminate the activity of the enzyme isovaleryl-CoA dehydrogenase.
- This enzyme is necessary for breaking down isovaleryl-CoA, which is produced during the metabolism of the amino acid leucine.
- This causes the accumulation of isovaleric acid in the blood, which causes symptoms of this condition.

- Symptoms of isovaleric acidemia can begin shortly after birth through childhood.
- Early symptoms of a crisis include: feeding difficulty, lethargy, hypothermia, and a “sweaty feet” odor. If untreated, symptoms include: metabolic acidosis, ketonuria, thrombocytopenia, neutropenia, hyperammonemia, seizures, cerebral hemorrhage, coma, and death.
- Survivors of repeated metabolic crisis can have brain damage. In individuals without symptoms until childhood, metabolic crisis can be triggered by illness, infection, or high protein intake.



## Treatment

- Immediate diagnosis and treatment of isovaleric acidemia is critical to normal development and survival.
- Individuals should follow a low-leucine, low-protein diet, which generally requires medical foods and formulas.
- L-carnitine supplementation and glycine may be recommended.

# UREA CYCLE DISORDER

- The urea cycle disorders (UCD) result from genetic mutations causing defects in the metabolism of the extra nitrogen produced by the breakdown of protein and other nitrogen-containing molecules.
- The urea cycle is composed of five primary enzymes, one cofactor producer and two transport molecules across the mitochondrial membrane. Inborn errors of metabolism are associated with each step in the pathway.

- **N-Acetylglutamate Synthetase Deficiency (NAGS)**

N-acetylglutamate synthetase deficiency affects the body's ability to make N-acetylglutamate (NAG) which is a required cofactor for the function of carbamoyl phosphate synthetase I. Without NAG, CPSI cannot convert ammonia into carbamoyl phosphate.

- Patients with complete NAGS deficiency rapidly develop hyperammonemia in the newborn period. Patients who are successfully rescued from crisis are chronically at risk for repeated bouts of hyperammonemia.

- **Carbamoylphosphate Synthetase I Deficiency**
- Carbamoylphosphate synthetase I deficiency affects the liver's ability to convert nitrogen to urea. This enzyme takes ammonia and through the use of bicarbonate and ATP produces carbamoyl phosphate. This enzyme requires the presence of its cofactor N-acetylglutamate.
- Along with ornithine transcarbamylase (OTC) deficiency and NAGS (N-acetylglutamate synthetase) deficiency of CPSI is the most severe of the urea cycle disorders.

- **Ornithine Transcarbamylase (OTC) Deficiency**
- Ornithine transcarbamylase deficiency affects the liver's ability to convert ammonia into urea. OTC combines carbamoyl phosphate with ornithine to make citrulline which is subsequently processed to urea.
- OTC is X-linked which results in the majority of severe patients being male. Females can also be affected but tend to present outside the neonatal period.

- With a deficiency of CPSI or ornithine transcarbamylase the ability to synthesize citrulline is impaired.
- Under these conditions excess ammonia accumulates in the liver and kidneys as glycine and glutamine.
- The challenge is to get rid of these two amino acids. This is accomplished by supplementing a protein restricted diet with incredibly large amounts of benzoate and phenylacetate. Benzoate is converted into Benzoyl CoA which then reacts with glycine to form hippurate.
- Likewise phenylacetate is converted into phenylacetyl CoA which then reacts with glutamine to form phenylacetylglutamine. These two compounds can then be excreted to partially overcome the genetic defects.

- **Argininosuccinate Synthetase Deficiency (ASSD) (Citrullinemia I)**
- Defects in argininosuccinate synthetase (ASS) affect the ability to incorporate ammonia into urea. This enzyme combines citrulline with aspartate to form argininosuccinate.
- Patients with complete ASSD present with severe hyperammonemia in the newborn period.
- The use of arginine in these patients allows some nitrogen (ammonia) to be incorporated into the urea cycle which makes treatment somewhat easier than other defects in the cycle.

- **Citrin Deficiency (Citrullinemia II)**
- Citrullinemia II is an autosomal disorder that results in decreased activity in the liver of a transport molecule for aspartate. This results in limitation of activity for the enzyme argininosuccinate synthetase which combines aspartate and citrulline to make argininosuccinate.
- Citrin (the defective protein) is an aspartate-glutamate transporter across the mitochondrial membrane.
- This defect can present with classic newborn hyperammonemia, intrahepatic cholestasis, jaundice and fatty liver, but is more likely to present with insidious neurologic disorders, hyperammonia, hypercitrullinemia and hyperlipidemia in adulthood.



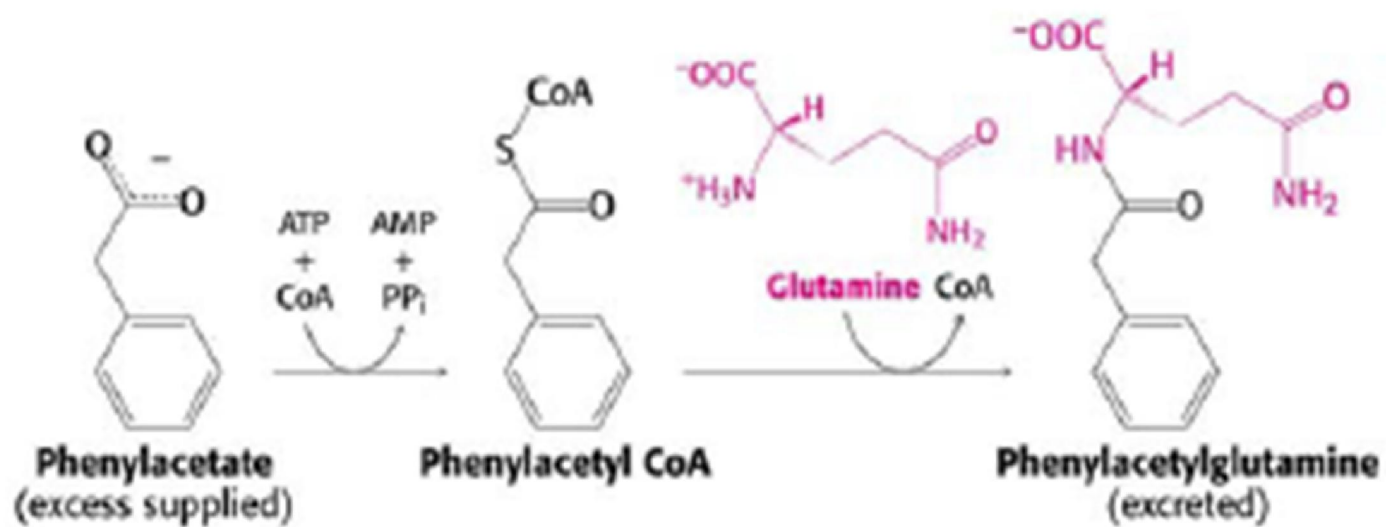
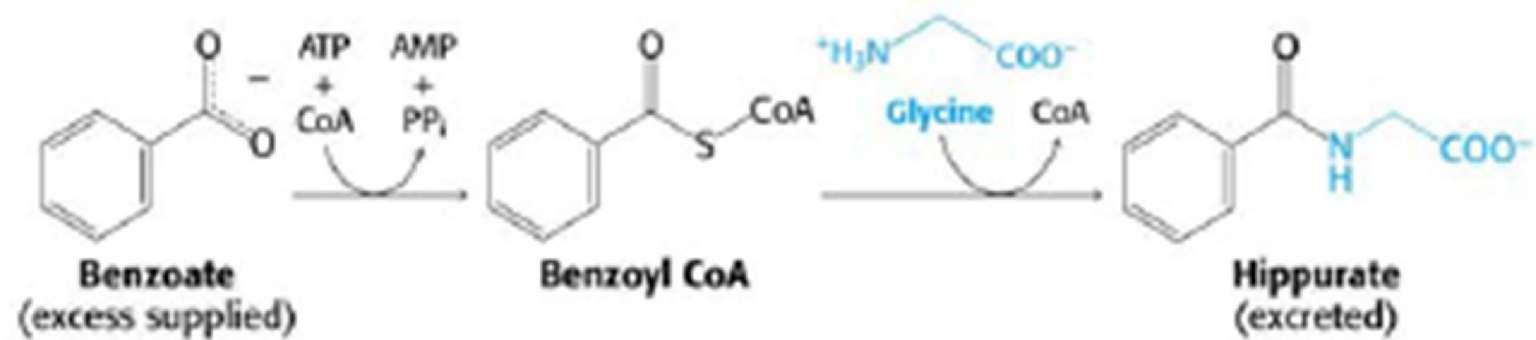
## **Argininosuccinate Lyase Deficiency (Argininosuccinic aciduria)**

- Argininosuccinate lyase deficiency affects the body's ability to clear the nitrogen already incorporated into the urea cycle as argininosuccinate.
- This causes hyperammonemia. Severe defects often present with rapid onset hyperammonemia in the newborn period. This disorder is marked by chronic hepatic enlargement and elevation of transaminases.
- Hepatomegaly observed may over time progress to fibrosis with unclear mechanism. These patients can also develop trichorrhexis nodosa, a node-like appearance of fragile hair, which usually responds to arginine supplementation.
- Treatment of these patients is based on a reduction in nitrogen intake and supplementation with arginine to complete a partial cycle.

- **Arginase Deficiency (Hyperargininemia)**
- Arginase deficiency is not typically characterized by rapid-onset of hyperammonemia. These patients often present with the development of progressive spasticity with greater severity in the lower limbs.
- They also develop seizures and gradually lose intellectual attainments. Growth is usually slow and without therapy they usually do not reach normal adult height.
- Other symptoms that may present early in life include irritability, anorexia and vomiting. Severe hyperammonemia are seen infrequently with this disorder, but can be fatal.

# MANAGEMENT OF UCD

- Emergency management of patients in hyperammonemic coma resulting from a UCD is based on three interdependent principles
- First, physical removal of the ammonia by dialysis or some form of hemofiltration;
- Second, reversal of the catabolic state through caloric supplementation and in extreme cases, hormonal suppression (glucose/insulin drip); and
- Third, pharmacologic scavenging of excess nitrogen.



# MITOCHONDRIAL DISORDER

- **MELAS: Mitochondrial Encephalopathy, Lactic Acidosis and Stroke-like episodes.**
- This is the most common type of mitochondrial encephalopathy.
- Onset: Usually between 2 and 40 years of age.
- Disease characteristics: Hallmark sign is the ‘MELAS’ attack.
- General characteristics are exercise intolerance, seizures, dementia, muscle weakness, hearing loss, blindness, migraine-type headaches, myopathy, gastric dysmotility, polyneuropathy, ptosis, cardiomyopathy, diabetes, renal failure and short stature.

# Causes

- MELAS syndrome has been associated with at least 6 different point mutations, 4 of which are located in the same gene, the tRNA Leu (UUR) gene.
- The most common mutation, found in 80% of individuals with MELAS syndrome, is an A → G transition at nucleotide 3243 in the tRNA Leu (UUR) gene.
- An additional 7.5% have a heteroplasmic T → C point mutation at bp 3271 in the terminal nucleotide pair of the anticodon stem of the tRNA Leu (UUR) gene.
- Moreover, a MELAS phenotype has been observed associated with a mitochondrial 13513 G → A mutation in the ND5 gene and in POLG deficiency.

# Methionine cycle

