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**TITLE: Maple Syrup Urine Disease and Other Disorders of Branched-Chain Amino Acid Catabolism**

**PRESENTER: Stephen Roper**

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## Slide 1:

Hello, my name is Stephen Roper. I am an Assistant Professor of Pathology& Immunology at Washington University School of Medicine. Welcome to this Pearl of Laboratory Medicine on "Maple Syrup Urine Disease and Other Disorders of Branched Chain Amino Acid Catabolism".

## Slide 2: Branched Chain Amino Acids (BCAAs)

The branched chain amino acids, leucine, isoleucine, and valine, are essential amino acids containing aliphatic side chains of various lengths. The term "branched-chain" refers to their structure because each contains a methyl group branching off from the central carbon chain. The primary biological role of the BCAAs are as building blocks for protein synthesis and thus, they comprise a significant portion [of the] amino acid content in muscle and body protein. In addition, these amino acids function in protein turnover and nitrogen disposal, cell signaling, and carbohydrate and ketone homeostasis. Recent research suggests the BCAAs may also play a role in the development and treatment of certain pathologies, such as type 2 diabetes.

During times of metabolic stress or in states of protein excess, the BCAA's can be catabolized to ketones or TCA cycle intermediates for energy production. This process requires the coordinated effort of both cytosolic and mitochondrial enzymes, some of which share specificity, and some of which catalyze reversible reactions. Thus, enzyme defects in this pathway result in reduced capacity to produce metabolic fuels and can lead to phenotypes ranging from mild to lethal. To gain an appreciation of the disorders associated with defective BCAA catabolism, we will first review normal BCAA metabolism.

## Slide 3: Catabolism of BCAAs

The BCAAs share a common set of enzymes for the first two steps of their catabolism, which occurs primarily in peripheral tissues such as the brain, skeletal muscle, and adipose tissue. BCAAs in circulation are taken up by a membrane bound transporter system and are reversibly deaminated by pyridoxine-dependent branched-chain aminotransferases (BCATs), producing branched chain-2-oxoacids. Next, the branched chain-2-oxoacids undergo irreversible oxidative decarboxylation in the mitochondria catalyzed by the branched-chain ketoacid dehydrogenase complex (BCKDHc). The products of this reaction are the branched-chain acyl-CoA thioesters. These thioesters are subsequently metabolized by a set of enzymes unique to each of the BCAA's from which they were derived. Defects in the branched chain ketoacid dehydrogenase complex are responsible for maple syrup urine disease. Defects in other enzymes more distal in the pathway are responsible for several organic acidurias.

### Slide 4: The Branched-Chain Ketoacid Dehydrogenase complex (BCKDHc)

BCKDHc is a large, multienzyme complex situated at the outer-face of the inner mitochondrial membrane. It is composed of 3 subunits designated as E1, E2, and E3 and is a member of the short chain oxo-acid dehydrogenase family along with pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase. The E1 subunit of the complex is responsible for the oxidative decarboxylation of 2-oxoacids and requires thiamin pyrophosphate as a co-factor. It is composed of two subunits: E1 alpha and E1 beta. The E2 subunit is a dihydrolipoamide acyltransferase with a lipoic acid cofactor. E2 forms the core of the enzyme complex and is responsible for the formation of the acyl-CoA products. The E3 subunit is a flavoprotein-containing dihydrolipoamide dehydrogenase which is reduced and oxidized during the catalytic cycle to reset the E2 subunit for additional reactions. Regulation of BCKDHc activity is mediated through an associated kinase (inactivates) and a phosphatase (activates) which are sensitive to the levels of branched chain 2-oxoacids (substrates) and the branched-chain acyl-CoA thioesters (products), respectively.

## Slide 5: Maple Syrup Urine Disease (MSUD): General Characteristics

MSUD is inherited in an autosomal recessive fashion and estimated to have a worldwide prevalence of 1 in 185,000, with an increased frequency in Mennonite populations. It is caused by defects in any of the 3 BCKDHc subunits, resulting in an accumulation of the 2-oxoacids: 2-oxo-isovalerate, 2-oxo-3-methylvalerate, and 2-oxoisocaproate, which spill over into the urine. In addition, the parent BCAAs build up in plasma because the first step of the catabolic pathway is reversible. It is a combination of the excess parent BCAAs (particularly leucine) and the 2-oxoacid metabolites which are responsible for the pathology of MSUD. A distinguishing characteristic of this disorder is elevated levels of a non-protein amino acid called alloisoleucine. This unusual compound is formed by keto-enol tautomerization and amination of the 2-oxoacid

derivative of isoleucine. Although small amounts of alloisoleucine are formed during normal BCAA catabolism, elevated plasma alloisoleucine is pathognomonic for classic MSUD. Finally, the distinct maple syrup odor detected in the urine of affected individuals is produced by sotolone, a chemical thought to be synthesized from excess isoleucine and alloisoleucine. Despite the name of the disorder, the smell of burnt sugar is more consistently found in cerumen than in the urine of affected individuals.

### Slide 6: Pathology of MSUD

MSUD is primarily a disease of the central nervous system which manifests as acute neurotoxic crises and chronic deleterious effects on brain growth and development. MSUD-associated neurotoxicity is caused by different mechanisms related to increased levels of leucine and the 2-oxoacids. For example, elevated leucine and 2-oxoisocaproate in the brain interfere with neurotransmitter synthesis, cell volume homeostasis, neuron outgrowth, and myelin formation. As well, an overabundance of leucine disturbs the ability of the blood brain barrier to transport other large neutral amino acids, leading to a relative deficiency of some amino acids and abnormal brain development. Dysregulation of transamination reactions in the brain leads to decreased levels of neurotransmitters such as glutamate and GABA and overproduction of 2-oxoglutarate, alanine, and lactate. In addition, elevated BCAA and 2-oxoacid levels alter plasma osmolality and can lead to increased production of antidiuretic hormone. Loss of BCAAs in urine is accompanied by loss of sodium, therefore concurrent ADH elevation (i.e. water retention) can lead to hypoosmolar crisis. It is hypothesized that these hypoosmolar epidsodes, along with the aforementioned mechanisms, may precipitate cerebral edema and lead to coma or death.

### Slide 7: MSUD Phenotypes

Depending on the severity of the BCKDHc defect, manifestations, biochemical findings, and response to therapy, MSUD can be categorized into 5 phenotypes. It is important to recognize that the distinctions between these phenotypes are not absolute and that individuals with a mild form of the disease may present similarly to severe MSUD in times of physiologic stress.

In classic MSUD, which accounts for about 75% of all cases, defects in the E1 or E2 subunits result in <2% of normal BCKDHc activity. These individuals typically present in the first week of life with clinical features including a burnt sugar odor in the cerumen, poor feeding, lethargy, opisthotonus, and fencing or bicycling-like movements. Biochemical findings include ketonuria, 2-oxoaciduria, and elevated plasma BCAA concentrations, including alloisoleucine. Classic MSUD is the most severe form of the disease and delayed recognition of symptoms and treatment can lead to coma or death. Children with classic MSUD who survive early catabolic crises often go on to have cognitive and intellectual disabilities later in life.

The second subtype is called intermediate MSUD. The onset of symptoms for this phenotype are variable and there may be no physical indications of disease in the neonatal period. Like classic MSUD, individuals with the intermediate form have persistent elevation of plasma BCAA,

despite having up to 30% residual activity of the enzyme. Clinical presentation is similar to the classic form when catabolic stress precipitates acute metabolic crises. For individuals with relatively milder forms of intermediate MSUD, the disease may present in late childhood with feeding problems, developmental delays, and/or poor growth.

The third phenotype is called intermittent MSUD. This subtype is unique from intermediate because affected individuals often have no clinical manifestations and have normal plasma BCAA concentrations. Children with intermittent MSUD have normal growth/neurological development and the disorder only manifests in times of increased endogenous protein catabolism. For example, fasting, fever, vomiting, and/or infections can cause saturation of BCKDHc activity and induce crisis in individuals with intermittent MSUD.

The next subtype is thiamine-responsive MSUD. This form of the disorder stems from defects in the E2 subunit of the BCKDHc which decrease the affinity of the enzyme to bind the obligate thiamin pyrophosphate co-factor. These individuals do not present in the neonatal period, but typically later in life with manifestations similar to intermediate MSUD. As the name suggests, thiamin supplementation overcomes the decreased TPP affinity and thereby improves residual activity of BCKDHc.

The last phenotype is E3-deficient MSUD. As previously mentioned, BCKDHc is a member of the short chain oxoacid dehydrogenase family along with pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase. These three DH complexes share a common E3 subunit, thus a defect in the activity of E3 affects all three of these pathways and has a high rate of in utero lethality. Infants that survive to birth manifest with a wide-range of problems, including early-onset neurologic problems, developmental delay, hypotonia, liver disease, encephalopathy, lactic acidosis, and early death.

### Slide 8: MSUD Molecular Pathology

MSUD is an autosomal recessive disorder associated with defects in any of the three subunits of the BCKDHc. The genes associated with the three subunits are designated as *BCKDHA*, *BCKDHB*, *DBT*, and *DLD* for the E1-alpha subunit, E1-beta subunit, the E2 subunit, and the E3 subunit, respectively. Affected individuals may be homozygous or compound heterozygotes for mutations in any one of these genes, of which over 160 disease-causing mutations have been identified. Although a molecular classification system has been proposed to group mutations by the affected subunit, these groupings fail to correlate with clinical severity of the disorder because mutations within a given subunit may be mild, intermediate, or severe.

### Slide 9: Diagnosis of MSUD

Newborn screening programs in all 50 states include amino acid profiles which quantify the combined concentration of leucine, isoleucine, hydroxyproline, and alloisoleucine. A positive newborn screen or recognition of symptoms such as poor feeding, irritability, ketonuria,

opisthotonos, and fencing or bicycling-like movements should prompt biochemical studies such as plasma amino acids and urine organic acid profiles.

While newborn screening is useful for the early detection of MSUD, definitive diagnosis is made by identifying the clinical features of the disease, plasma amino acid quantification, and urine organic acid profiles. Hallmark elevations in BCAAs are observed in the plasma of individuals with classic and intermediate MSUD. In some cases, however, the concentrations of valine and isoleucine may be within the reference interval. Therefore, the identification of persistently elevated leucine, and alloisoleucine concentrations >5 µmol/L, are pathognomonic for the disease. Urine organic acid analysis will reveal elevated levels of the 2-oxoacids: 2-oxo-isovalerate, 2-oxoisocaproate, and 2-oxo-3-methylvalerate as well as hydroxylated forms of these metabolites. Alternatively, a qualitative test to identify 2-oxoacids in urine using dinitrophenylhydrazine (DNPH) can be used in clinical or home settings to aid in diagnosis or identification of acute crises. In the presence of 2-oxoacids, DNPH precipitates and increases the turbidity of the urine.

Molecular studies are not required for diagnosis, but are useful as a supplement to biochemical testing to identify the defective BCKDHc subunit and define genetic variants. Molecular studies aid in confirming MSUD in individuals who do not present with severe metabolic decompensation, as well as prenatal diagnosis, and identification of MSUD carriers. Future development of individualized therapies will likely broaden the utility of molecular approaches in MSUD diagnosis.

Finally, measurement of BCKDHc activity in fibroblasts or lymphocytes can be used to diagnose MSUD, however this is not a preferred method. Studies from the late 1990's showed that there was considerable variation between in-vivo and ex-vivo enzyme activity for individuals with intermediate MSUD. Therefore, the primary approaches for diagnosis are clinical recognition of symptoms and biochemical analyses such as plasma amino acids and urine organic acids.

### Slide 10: MSUD Treatment and Prognosis

The management of MSUD is primarily based on dietary control of BCAA intake and prevention of catabolic crises by nutritional supplementation. The goals of dietary management are to reduce plasma leucine concentration while introducing additional valine and isoleucine to promote anabolism. This approach is therapeutic because valine and isoleucine are typically well-tolerated in MSUD, whereas leucine imparts the majority of neurological manifestations of the disease. Thiamin pyrophosphate supplementation may also be beneficial in individuals with residual BCKDHc activity. Because dietary interventions are the primary therapy, lifetime measurements of plasma amino acids are required to ensure compliance and efficacy of treatment.

In addition to dietary management, orthotopic liver transplant has emerged as a long-term treatment for MSUD. Although the majority of BCAA catabolism occurs in peripheral tissues, the

liver is responsible for approximately 9-13% of total catabolic capacity. Individuals who are transplanted show reduced plasma BCAA concentrations as early 6 hours post-surgery, reduced likelihood of acute metabolic crises, and better cognitive outcomes when transplant occurs at a young age.

During times of acute metabolic crises, which can be precipitated by stress, trauma/surgery, or noncompliance with dietary regimens, emergency protocols are focused on quickly promoting return to an anabolic state. This is achieved by increasing BCAA-free formula intake and decreasing leucine consumption beyond normal restrictions. Additional interventions, such as hemodialysis, hemofiltration, and/or parenteral or tube-feedings to reduce BCAA concentrations may become necessary in cases that do not respond to aggressive dietary treatment.

The prognosis and outcome of MSUD are related to the age at diagnosis, how early in life interventions begin, and the quality of long-term metabolic control. Identification and treatment of MSUD prior to 14 days of life results in a less severe disease course and improved intellectual capacities. However, individuals with MSUD who have not received a liver transplant are at risk for death at all ages because of the potential for severe acute metabolic crises.

### Slide 11: Other Disorders of BCAA Catabolism

In addition to MSUD, several other disorders arise from defective catabolism of BCAAs. For example, isovaleric, propionic, and methylmalonic acidurias are caused by enzyme defects more distal in the catabolic pathway than BCKDHc. Because many of the reactions that occur after BCKDHc are irreversible, these disorders do not result in an accumulation of BCAAs in plasma; rather, they are associated with a buildup of intermediates proximal to the enzyme defect and are classified as organic acidurias. Therefore, laboratory diagnosis of these disorders relies on plasma acylcarnitine and urine organic acid profiles rather than amino acid quantification. In the next few slides we will briefly describe the clinical features, diagnosis, and treatment for isovaleric, propionic, and methylmalonic acidemia.

### Slide 12: Isovaleric acidemia

Isovaleric acidemia (IVA) is an autosomal recessive disease caused by reduced function of isovaleryl-CoA dehydrogenase. This mitochondrial enzyme is unique to the catabolic pathway of leucine, where it catalyzes the dehydrogenation of isovaleryl-CoA, producing 3-methylcrotonyl-CoA. In IVA, the build-up of Isovaleric acid and its derivatives are responsible for the manifestations of the disease.

### Slide 13: Isovaleric acidemia

The manifestations of IVA may occur in the neonatal period or later in childhood with variable presentations including mild, intermediate, and severe phenotypes. In the classic form of IVA, clinical features include poor feeding, vomiting, dehydration, seizures, and a characteristic body

odor described as "sweaty feet". In addition, metabolic acidosis, ketonuria, hyperammonemia, and abnormal glucose levels may be observed.

NBS programs provide a means for the early detection of IVA- in some cases pre-symptomatically. Dried blood spot MS/MS analyses of affected individuals show a characteristic elevation in C5 acylcarnitine and follow-up urine organic acid profiles will contain 3-hydroxyisovaleric acid and isovalerylglycine. In addition, molecular diagnostics and enzyme activity assays may aid in classification of disease, but are not required for diagnosis.

Therapy goals for IVA include preventing metabolic decompensation, long term reduction of leucine catabolism through dietary modification, and reducing the accumulation of isovaleric acid by directing metabolism through alternative pathways. Individuals with IVA who are diagnosed and treated early typically have a reasonably long lifespan, however some form of intellectual disability and protein aversion are almost always present.

### Slide 14: Propionic acidemia

Propionic acidemia (PA) stems from dysfunction of propionyl-CoA carboxylase (PCC). This enzyme catalyzes the carboxylation of propionyl-CoA to methylmalonyl-CoA and requires biotin as a cofactor for its activity. Propionyl-CoA is produced through the catabolism of valine, isoleucine, cholesterol, odd chain fatty acids, methionine, and threonine- thus, defects in PCC result in a variety of biochemical disturbances.

### Slide 15: Propionic acidemia

The toxic compounds that accumulate in PA include propionyl-CoA and propionic acid. These substances inhibit the first step of the urea cycle, interrupt anaplerotic replenishment of TCA cycle intermediates, and interfere with oxidative phosphorylation. In addition, methylcitrate, tiglylglycine, propionylglycine, and 3-hydoxypropionic acid are excreted in the urine of affected individuals. Hyperglycinemia is also frequently observed in PA. Newborn screening programs rely on the detection of increased C3 acylcarnitine to identify PA in newborns, however, isolated elevation of C3 acylcarnitine is also observed in methylmalonyl-CoA mutase deficiency, defects in vitamin B12 synthesis and transport, and nutritional B12 deficiency. Thus, the identification of the intermediates listed above by urine organic acid analysis is useful to aid in diagnosis, but molecular testing and/or enzyme activity assays may be needed to clarify the etiology of disease.

Clinical presentation in classic PA typically occurs shortly after birth and includes sequela such as ketosis, metabolic acidosis, vomiting, dehydration, seizures, cardiac arrhythmias, hyperammonemia, and hypotonia. However, not all phenotypes of PA are severe and late-onset forms of the disease, with mild manifestations, have also been described. Regardless of the age at onset, all forms of PA are at risk for life-threatening metabolic decompensation from infections, stress, or high protein intake.

The majority of individuals with PA who survive the newborn period will have some form of cognitive impairment and developmental delays. Therapeutic strategies include dietary restriction of amino acids and odd-chain fatty acids as well as biotin and carnitine supplementation.

### Slide 16: Methylmalonic acidemia

Methylmalonic acidemia (MMA) is a rare, autosomal recessive disorder most often caused by defective methylmalonyl-CoA mutase production or function. The reaction catalyzed by this enzyme, isomerization of methylmalonyl-CoA to succinyl-CoA, requires vitamin B12 as an obligate cofactor. Thus, defects in the synthesis or transport of vitamin B12 also cause MMA and must be considered in diagnostic workup.

### Slide 17: Methylmalonic acidemia

Clinical presentation of classic MMA usually occurs in the first week of life with poor feeding, metabolic acidosis, nausea and vomiting, and encephalopathy. Because of the potentially life-threatening nature of this disorder, recognition of these symptoms should prompt rapid laboratory work-up including plasma amino acids, acylcaritines, and urine organic acids, which will show increased plasma alanine and glycine, elevated propionyl carnitine, and increased excretion of methylmalonic acid, propionic acid, 3-OH-propionic acid, and methylcitrate during times of acute decompensation. Individuals who do not present in the first days of life may be recognized through newborn screening with elevation of propionyl-carnitine.

Chronic manifestations of MMA include movement disorders, poor growth, epilepsy, renal failure, intellectual disability, vision loss, and immunodeficiency. Therapeutic approaches for MMA are similar to those used for other branched-chain organic acidurias: prevention of crises through the promotion of anabolism and avoidance of catabolism, dietary management, and supplementation of enzyme co-factors. Prognosis of MMA is related to the severity of the enzyme defect and the age at which interventions began.

### Slide 18: Summary

The BCAAs are catabolized to ketones and TCA cycle intermediates during times of dietary stress. In order to utilize this abundant energy source, a series of sequential reactions are carried out in the cytosol and mitochondria of peripheral tissues. Defects in the second enzyme of this pathway, BCKDHc, result in MSUD- a disorder characterized by increased excretion of the 2-oxoacids, elevated plasma BCAAs, and formation of excess alloisoleucine. The primary manifestation of MSUD is neurotoxicity and patients are at risk for acute metabolic crises during times of increased stress such as fasting, infection, or surgery. Importantly, these episodes can

be life threatening and require rapid identification and intervention to prevent encephalopathy, coma, and/or death.

Early diagnosis and intervention are associated with better outcomes in MSUD. Consequently, the disorder is included in newborn screening panels in the United States. However, definitive diagnosis requires clinical correlation and confirmatory biochemical testing, including plasma amino acids and urine organic acid analyses. Treatment options for MSUD include dietary restrictions and orthotopic liver transplantation, both of which have the potential to dramatically improve the quality and length of life for these individuals. .

In addition to MSUD, enzyme defects in the distal BCAA catabolic pathway also result in disease. Depending on the exact location of the defect, toxic intermediates may build up or be shunted into alternative pathways, however no increases in BCAA concentrations will be observed. Representative disorders, including isovaleric, propionic, and methylmalonic acidemia, are characterized by metabolic acidosis, dehydration, hyperammonemia, ketonuria and typically present in the early neonatal period. Diagnosis requires the identification of characteristic compounds by plasma acylcarnitine, urine organic acid analyses, and adjunct testing to rule out co-factors (e.g. biotin and cyanocobalamin) deficiencies which can mimic true disease. Like MSUD, individuals with branched chain organic acidurias are also at risk for severe acute metabolic decompensation during times of increased catabolic activity and are managed by dietary restrictions and cofactor supplementation.

### Slide 19: References

### Slide 20: Disclosures

### Slide 21: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on Maple Syrup Urine Disease and Other Disorders of Branched Chain Amino Acid Catabolism.