The Molecular Basis of Maple Syrup Urine Disease

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Abstract

Maple syrup urine disease (MSUD) is a rare metabolic disorder that is caused by mutations in the branched chain alpha keto acid dehydrogenase enzyme complex (BCKDC). There are three main genes, the BCKDHA, BCKDHB, and DBT, that affect the BCKDC, all contributing to the onset of the disease. MSUD causes encephalopathy, neural deficits, maple syrup scented urine, coma, and even death if not treated due to the aggregation of branched-chain amino acids (BCAAs). There is currently no known cure for patients with MSUD, but the condition can be managed to improve quality of life. This review serves to examine MSUD and understand its pathology, mutations, the populations affected, and explore the treatment methods currently offered while also analyzing new and upcoming research in the field.

The Molecular Basis of Maple Syrup Urine Disease

Introduction

There are thousands of genes in the human genome, each encoding a specific protein and weaved together in such a way that allows the human body to work seamlessly. However, despite the many different checks and balances naturally embedded, coding errors can be made that lead to a dysfunctional gene and disease. Maple syrup urine disease (MSUD) is a metabolic disease that is caused by autosomal recessive mutations in the branched chain alpha keto acid dehydrogenase enzyme complex (BCKDC). Metabolic diseases and disorders encompass a wide range of metabolic imbalances from obesity and diabetes to rarer, lesser-known diseases such as MSUD. Metabolism, at the very basic level, allows the body to convert nutrient intake into energy to fuel cellular processes. These types of diseases affect the homeostasis and normal functioning of the body by disrupting these metabolic processes. MSUD is a rare type of metabolic disease that can be further classified as an inborn error of metabolism disorder that occurs as a result of a singular genetic mutation (1).

It has been discovered that MSUD is caused by mutations that affect all three subparts of the BCKDC with some mutations being more prevalent than others (2). In cases of MSUD, patients experience increased levels of branched-chain amino acids (BCAAs) leucine, isoleucine, and valine due to the abnormal functioning of the BCKDC (3). The disruption of the normal metabolism of BCAAs leads to many symptoms in the patient because of the integral role these amino acids fill. At normal levels, BCAAs play an important function in muscle building and signaling pathways but are toxic when at elevated levels (4, 5). Currently, there are few approved treatments for those suffering from MSUD, and more research is being conducted to better understand the pathophysiology behind the disease.

Function of BCAAs

Branched-chain amino acids are essential to normal metabolism and other processes, including muscle building, cell signaling, and protein structure. In normal functioning, BCAAs are essential amino acids that must be taken in through the diet and cannot be synthesized in humans. It is estimated that BCAAs make up about 20-25% of dietary amino acids. They are found in several different important pathways in normal amounts; beyond this, evidence links high BCAA levels to several different metabolic disorders (6). They are frequently known for their ability to assist in muscle building and have been used in the past as supplements for certain population groups aiming to improve muscle tone and increase muscle mass (4).

Beyond these functions, BCAAs are very well known to be involved in the mTOR pathway, most notably leucine (4). BCAA involvement in the mTOR signaling path contributes to the buildup and breakdown of muscles, as well as the insulin pathway, among other things. Specifically, leucine is a large activator of mTORC1, a subunit of the mTOR complex. Stimulation of the pathway can occur with leucine alone, and decreased levels of this amino acid can solely suppress the activation of the pathway (6). Activation of the mTOR pathway by leucine can also lead to the normal development and function of the immune system, as this signaling pathway contributes to differentiation, activation, and function of both innate and adaptive immune cells (4). Additionally, BCAAs play an important role in normal metabolism involving insulin. BCAAs may both increase insulin release and contribute to resistance to insulin depending on the amounts of BCAAs present. It is hypothesized that the interaction with insulin stems from the role of BCAAs in mTOR pathway signaling (6).

BCAA interaction in neurological development plays an important role as they contribute to the synthesis of the excitatory neurotransmitter called glutamate. Low levels of plasma

BCAAs can lead to several neurological deficits including epilepsy, autism, and other intellectual disabilities (7). Alternatively, too high of BCAA levels can be toxic and lead to severe neurological diseases (6). This becomes a very important concern for those with MSUD, as the buildup of these amino acids is what can eventually cause encephalopathy and death early in life. Varying levels of BCAAs can be detrimental to the patient, so it is important to maintain normal levels to prevent the onset and worsening of mental deficits.

Normal Biology of BCKDC

The BCKDC is essential for the process of BCAA metabolism to continue because it is a rate-determining step that commits the branched amino acids to their respective metabolic pathways. BCAA metabolism catalyzed by the BCKDC is halted in patients with MSUD. Figure 1 shows the typical pathway of BCAA metabolism ending in the products acetoacetate, acetyl-CoA, and succinyl-CoA, all of which are needed for normal cell function (8).

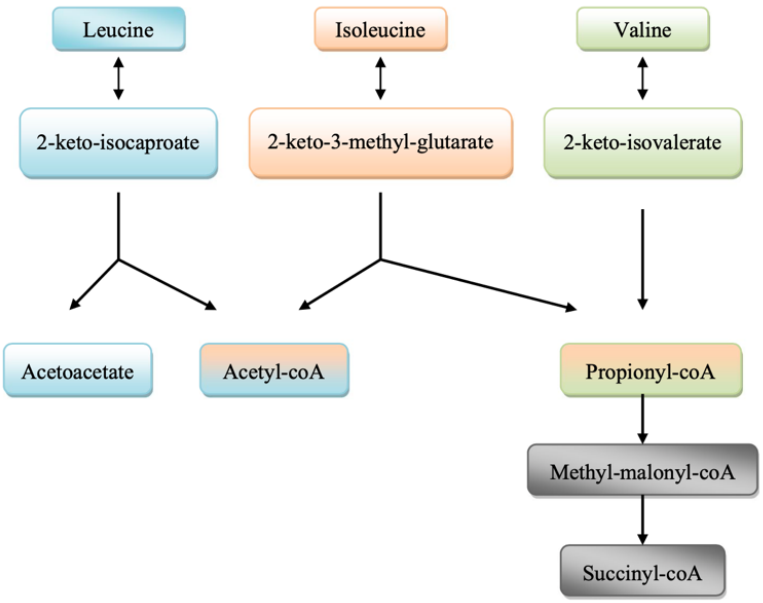

Figure 1. Metabolic pathways of BCAAs. During normal metabolic activity, the BCAAs are metabolized via the BCKDC through a series of processes into acetoacetate, acetyl-CoA, and succinyl-CoA (9).

A defect in the BCKDC that leads to BCAA accumulation can promote beta cell mitochondrial dysfunction, apoptosis, and stress signaling (10). The BCKDC is involved in the second step of BCAA catabolism by catalyzing the oxidative decarboxylation of the α-ketoacids into branched-chain acyl-CoA esters. This reaction is irreversible and takes place in the inner mitochondrial membrane, where the BCKDC is found. BCKDC activity is most prevalent in the liver, although it is also active in other organs such as the kidneys, heart, brain, adipose tissue, and muscle tissue (6). The properly functioning BCKDC consists of three different enzymes including branched-chain α-keto acid dehydrogenase (E1), dihydrolipoyl transacylase (E2), and dihydrolipoyl dehydrogenase (E3) as seen in Figure 2. It normally contains 12 copies of E1, 24 copies of E2, and six copies of E3.

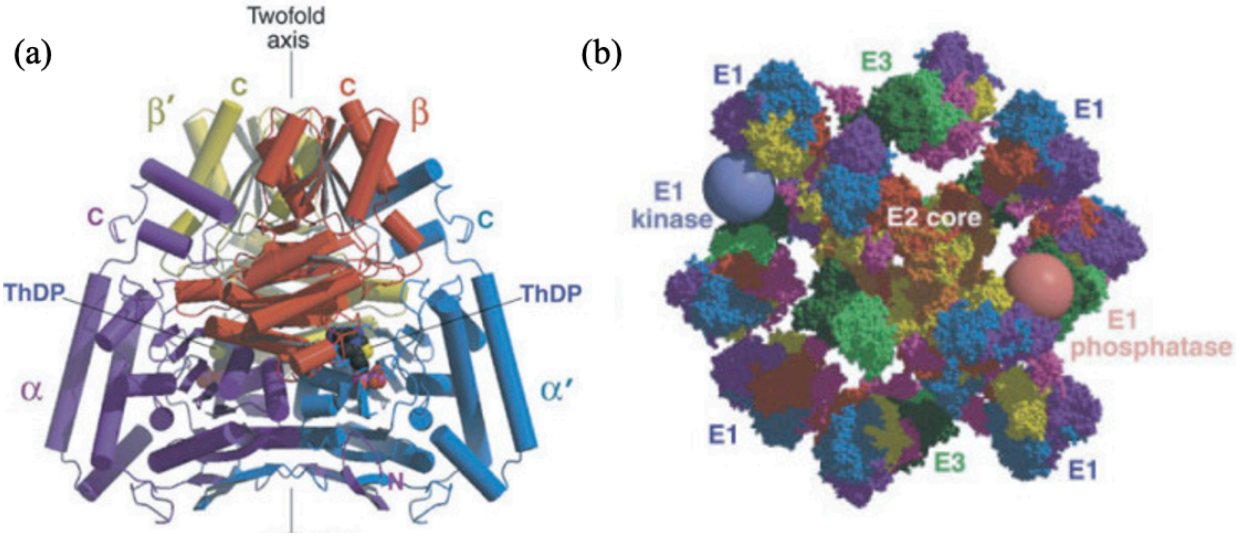

Figure 2. Structure of the E1 complex and the BCKDC. (a) The E1 complex is composed of both alpha and beta subunits that interact around a twofold axis. The binding domain for the E2 complex is on the beta terminal of the tetramer. **(b)** The fully assembled BCKDC consists of the E1 and E3 components around the central E2 component. Figure modified from Aevarsson et al., 2000 (11).

The functioning of the BCKDC is largely controlled via the phosphorylation state of the E1 component (12). The BCKDC is activated by the BCKDC phosphatase, and it is inhibited with the BCKDC kinase. When inactive, the metabolic activity of the BCKDC is shut off (9). The first step of BCAA catabolism involves the reversible reaction of converting the BCAAs into α-ketoisocaproate (KIC), α-keto-β-methylvalerate (KMV), and α-ketoisovalerate (KIV) via the branched-chain aminotransferase (BCAT) (13). After this, KIC, KMV, and KIV can be converted in further reactions involving the BCKDC. The precise protein folding and structure of the E1 complex allows for the BCKDC to properly oxidize BCAAs and branched chain keto acids (BCKAs) into important products needed for daily cell functioning.

Additionally, the BCKDC helps to catalyze the oxidative decarboxylation of BCKAs to prevent the buildup of BCKAs and BCAAs (6). If the BCKDC is nonfunctional, the breakdown of BCAAs is stopped early in the process, leading to their buildup. Because of the major role that the BCKDC plays in the metabolism of the BCAAs, loss of function prevents the formation of products needed for the Krebs cycle by preventing the completion of BCAA catabolism. The metabolism of these essential amino acids also aids in protein synthesis and cell signaling (14). In a fully functioning BCKDC, the activity is closely monitored and controlled. The complex's associated regulatory proteins create a reversible phosphorylation and dephosphorylation mechanism (15). The BCKDC kinase inhibits functioning by phosphorylating three different areas on the branched chain keto-acid dehydrogenase E1 subunit alpha (BCKDHA) (16). This tight regulation ensures that the correct balance of BCAAs is found in the blood at any given moment.

The BCKDHA and branched chain keto-acid dehydrogenase E1 subunit beta (BCKDHB) genes encode both subunits for the E1 component (16). Most commonly, MSUD is caused by a

mutation in the gene that encodes for either of these proteins. This E1 component is a thiamine-diphosphate-dependent enzyme consisting of both the alpha and beta subunit polypeptide chains. These two subunits are closely intertwined, and both serve as integral parts of the BCKDC (11). The alpha and beta subunits are positioned around a central, twofold axis and have other surrounding proteins that bind to and interact with them. Structurally, the E1b itself is composed of alpha and beta domains with a C-terminal domain and N-terminal tail. The alpha subunit of E1b interacts with the beta subunits by grabbing onto them using the C- and N-terminal sequences. The beta subunit is 342 residues divided into two similar sized domains. In its entirety, the E1b subunit has a central beta sheet with several other helices packed closely around it. The E1b also interacts with several of the other components within the BCKDC such as the E2b lipoyl and binding domains, and the protein kinase and phosphatase which controls E1 activity through dephosphorylation. Research has shown that the E1 tetramer only binds one of the E2 copies to the beta subunit binding domain (11). The E1 complex is unique to some of the other components as it requires a thiamine pyrophosphate (TPP) cofactor. The TPP binding to the E1 component opens the binding site for ketoacids, which in turn allows for the release of carbon dioxide (12, 17).

The E2 component of BCKDC is encoded by the dihydrolipoamide branched chain transacylase E2 (DBT) gene and serves to transfer the acyl groups to coenzyme A in the process of converting KIC, KMV, and KIV into isovaleryl-CoA (IV-CoA), α-methylbutyryl-CoA (MB-CoA), and isobutyryl-CoA (IB-CoA) via its lipoate-dependent dihydrolipoyl transacylase activity (16). This is completed by first transferring the acyl group to the lipoamide cofactor bound to E2. It is then catalyzed to move the acyl group to coenzyme A by the active site located on the E2

catalytic domain. The E2 component also forms the core of the BCKDC and attaches to the other components via non-covalent bonds (11).

While both the E1 and E2 components are unique to the BCKDC, the E3 component is common between the BCKDC, pyruvate dehydrogenase, and α-ketoglutarate dehydrogenase, all of which function as ketoacid dehydrogenases (17). In the BCKDC, the E3 component is responsible for completing the catabolism of BCAAs by transferring the released electrons from the previous step to NAD^+ with assistance from a FAD cofactor bound to it (6, 11). Mutations within the E3 component can cause more severe MSUD types because of its commonality between all ketoacid dehydrogenases. Decreased function of these dehydrogenases causes lactic acidosis which can be fatal for the patient. It also progresses the worsening condition of neural function, leading to encephalopathy and neural deficits (6). The E3 component of the BCKDC is encoded by the dihydrolipoamide dehydrogenase (DLD) gene (16). Mutations in any one of these genes can potentially cause the onset of MSUD in patients.

MSUD as a Genetic Disease

Originally, MSUD was identified and described as a progressive neurodegenerative disease by Menke in 1954 before later being discovered that it is caused by a deficiency in the BCKDC (18). MSUD is an autosomal recessive disease that only affects about 1 in 198,000 babies (19). Since MSUD is a genetic disorder, it cannot be passed from person to person except vertically through blood relations. The genome is the genetic information that every person has that encodes all genes. Each of these genes functions to ensure the body is working properly, and the human genome contains anywhere from 50,000 to 100,000 genes (20). Slight alterations in this genetic information can cause catastrophic damages, such as those experienced by patients with MSUD. Within the genome, there are three specific genes that mainly cause MSUD:

BCKDHA, BCKDHB, and DBT (21). These three genes all play important roles in the proper formation and functioning of the BCKDC, and minor conformational changes render the entire complex ineffective in the metabolism of BCAAs.

Types of MSUD

Depending on the origin of mutation, different forms of MSUD can present varying clinical symptoms. The most common and accepted types of MSUD include classic, intermediate, intermittent, and thiamine-responsive type. In addition to these four, there is also a type of E3 deficiency with lactic acidosis, though there is less research on this specific type (22). Other forms of MSUD have also been suggested, but it is unknown whether these types differ enough from the current types to warrant the identification of a new category. MSUD is diagnosed based on severity, symptoms, time of onset, and thiamine-responsiveness (17). An in-depth comparison of these types helps to differentiate and classify the severity of MSUD.

Classic Type

Classic MSUD is the most common and most severe type of the disease, accounting for approximately 75% of MSUD cases (11). It is caused by mutations in any three of the BCKDHA, BCKDHB, or DBT genes (3). BCKDC activity in classic MSUD is recorded to be between 0-2% of normal functioning activity (21). In this form, symptoms present in the neonatal period as irritability, poor feeding, maple syrup scent in the urine and cerumen, apnea, unusual “bicycling” movements, muscle spasms causing arching in the head and spinal area, and lethargy. Further tests can be completed to confirm the diagnosis of MSUD including elevated BCAA and alloisoleucine counts in the blood, as well as elevated branched-chain keto acids found in the urine (3). When left untreated, cerebral edema can worsen and lead to coma and even premature death, commonly occurring within the second week of life (23). The

characteristic maple syrup scent is a result from the buildup of excess isoleucine in the plasma (18). As isoleucine accumulates, a rare catabolic product is formed called solotone, directly resulting in the sweet, maple scent. Its production is a byproduct of the competing reactions of hydroxylation, cyclization, and oxidative deamination to the normal decarboxylation of isoleucine (6). If not treated, this scent can appear within 12-24 hours post birth in the earwax, and 48-72 hours post birth in the urine (24).

After the neonatal period, patients with classic MSUD experience additional symptoms as they age. During the infant and toddler stages of growth, it is common for symptoms such as anorexia, loss of coordination because of ataxia, dystonia causing involuntary muscle contraction, and nausea to present (3). As the patient continues to age with classic MSUD, the severity of the symptoms worsens and impacts cognitive ability at a higher degree. It is common for patients to suffer from cognitive impairment, sleep disturbances, and hallucinations among other symptoms (3). Cognitive impairment occurs because of a lack of glutamate synthesis, an important molecule in the brain that acts as a neurotransmitter (25). BCAA metabolism is integral to this process as the BCAT converts BCAAs into BCKAs, directly leading to the synthesis of glutamate that can then pass the blood brain barrier (BBB) for use in cognitive functions (26).

Intermediate Type

The intermediate type of MSUD is less severe than the classic type, with the onset of symptoms usually appearing in infancy and early childhood (24). Compared to newborns with classic MSUD, those with intermediate type often appear healthy during the neonatal period. The scent of solotone may still be present in the cerumen, and this could be one of the only telling signs of illness during this period (3). Many symptoms are consistent in both types, such as

seizures, difficulty with balance and walking, neurological developmental delay, and other mental struggles. However, patients with intermediate type MSUD do not suffer from the severe decomposition and shut down of organs early in life such as those with classic type (24). Intermediate type MSUD often goes undiagnosed at newborn screenings because of the partial BCKDC activity that is observed. Levels of leucine, isoleucine, and valine can be measured to be within normal ranges in the neonatal period. Typically, intermediate type MSUD is diagnosed when other growth milestones such as gross motor, speech, and language development are not met as expected (27).

Patients with intermediate type MSUD have been identified to have mutations in all three BCKDC genes (3). The range of activity that the BCKDC shows for intermediate type MSUD falls within 3-30% of the normal activity. Distinguishing symptoms include poor growth and encephalopathy specifically during illness (21). Other than this, there are not many other distinguishing characteristics that differentiate it from the classic type, thus making it difficult to clearly diagnose. Individuals who have this type of MSUD can usually prevent worsening symptoms in many of the same ways as those with classic type MSUD, but the treatment can be slightly laxer because it is a less severe type of the disease (21). Although it is less severe, encephalopathy can be a life-threatening consequence if the person is undergoing levels of catabolic stress that are sufficient to prompt serious symptoms (27).

Intermittent Type

Intermittent type MSUD is often regarded as one of the least severe types and does not present in the same ways as classic and intermediate types. Unlike classic MSUD, intermittent type can present at any point in life and does not always show symptoms during the neonatal period (3). It is most typical for symptoms to show between 5 months of life and 2 years (24).

Children who are diagnosed with intermittent type MSUD grow typically and experience far less severe of symptoms. The onset of symptoms pertaining to cerebral deficiencies is more delayed, and studies have shown that alterations in the brain parenchyma in patients with intermittent type MSUD are much less extensive (22). Learning delays are typically not observed until later childhood, and patients can generally tolerate normal levels of leucine intake (21).

Mutations in the BCKDHA, BCKDHB, and DBT genes can all cause intermittent type MSUD (3). In periods of wellness, patients are asymptomatic and experience normal growth physically and cognitively. However, episodic decompensations resulting from physiological stress can cause symptoms similar to that of classic type MSUD. Without proper treatment, this can quickly lead to metabolic intoxication, encephalopathy, and, in rare cases, death. BCKDC activity is typically measured between 5 and 20% during acute decompensations, falling between the classic and intermediate type ranges (21). Those with intermittent type MSUD generally have a good prognosis and can lead a normal life.

Thiamine-Responsive Type

Clinically, thiamine-responsive type MSUD presents very similarly to that of the intermediate type (28). Interestingly, this type of MSUD can be caused by mutations in the DBT gene affecting the E2 component of the BCKDC (3). In healthy individuals, thiamine acts as a cofactor to the E1 component of the BCKDC and regulates the enzyme complex's activity. Mutations that cause thiamine-responsive type MSUD can be treated by thiamine supplements because of the positive response observed and lowered BCAA serum levels after treatment (24). While this type of MSUD does respond partially to thiamine, it is still unknown whether a truly responsive-to-thiamine type of MSUD exists (27).

Responsive-to-thiamine type MSUD is much rarer than classic MSUD yet has a brighter outlook. In this type of MSUD, the BCKDC activity that is typically observed lies within 2-40% that of normal activity (21). Similar to intermittent type MSUD, thiamine-responsive type typically experiences delayed onset of cerebral and neurological deficits. Symptoms generally are improved following thiamine supplement treatment (22). The onset of symptoms for the thiamine-responsive type MSUD varies and there has not been a noticeable pattern observed. Typical clinical features follow the intermediate type MSUD patterns with patients exhibiting poor growth and feeding, developmental and mental delays, and encephalopathy during times of stress or illness (21). Thiamine-responsive type MSUD is one of the types that has the best prognosis for patients.

E3-Deficient type (Lipoamide Dehydrogenase Deficiency)

E3-deficient type MSUD, also known as lipoamide dehydrogenase deficiency, is considered the rarest type of MSUD as it is caused by mutations in the DLD gene which are uncommon on their own (3). Research has suggested that mutations that cause this type of MSUD are unproportionally higher in the Ashkenazi Jewish population (2). As a result of its rarity, there is not as much research about it and the causes behind the mutations. BCKDC activity is measured between 0-25% of normal activity (21). The prognosis for patients diagnosed with E3-deficient type MSUD varies based on the level of activity exhibited by the BCKDC (24). However, since the E3 component of the BCKDC is common among all ketoacid dehydrogenases, E3-deficiency causes deficiencies and dysfunction in all three dehydrogenases, often leading to premature death because of lactic acidosis (29).

The differences between E3-deficient type MSUD and the classic and intermediate types are vast, presenting significantly distinct phenotypes that are not seen in the other types (21).

Common symptoms include decreased muscle mass, lethargy, seizures, vomiting, and Leigh-type encephalopathy. Patients also tend to experience issues with the liver including hepatomegaly, nausea, and hepatic encephalopathy (3). Within the understanding of MSUD, not much else is known regarding E3-deficiency. Some suggest that E3-deficiency type should not be considered MSUD, but rather just as dihydrolipoamide dehydrogenase deficiency; however, this is not unanimously accepted (30). Because of this, many studies only examine BCKDHA, BCKDHB, and DBT gene mutations or have limited data regarding the DLD gene. More research is being conducted to better understand how this type of MSUD affects patients.

Mutations in MSUD

Reports about the types and numbers of mutations identified that lead to the diagnosis of MSUD are ever-changing. Currently, MSUD has not appeared to be caused by any one specific type of gene mutation. Mutations that have been documented include missense, frameshift, and nonsense mutations (30). It is far more unlikely for mutations in the DBT and DLD gene to be pathogenic according to recorded reports. The majority of MSUD diagnoses are caused by mutations in the BCKDHA and BCKDHB genes, altering the shape and function of the E1 component of the BCKDC. The E1 complex is very tightly packed to function properly, so any mutation that affects the general protein structure or shape prevents the correct binding of these subunits and inhibits the function of BCKDC. Even missense mutations that only alter one amino acid decrease the stability of the E1 complex, allowing for the inhibition of function (11). As of 2020, 102 BCKDHA gene mutations, 122 BCKDHB gene mutations, 83 DBT gene mutations, and 23 DLD gene mutations have been reported (2).

The rarity of DLD mutations and disagreement as to whether it constitutes as an MSUD classification leave little descriptions of specific mutations being made. Thus, only mutations in

the BCKDHA, BCKDHB, and DBT genes are analyzed further to better understand their role in MSUD pathology. No sources found explicitly claimed that specific mutations in any of these genes were at fault for most diagnoses made of MSUD. Despite this, common trends have been observed and theories made for the exact mechanisms that cause the malfunctioning of the BCKDC. Understanding and identifying key pathogenic mutations could revolutionize the diagnostic process and make newborn screening for this rare disease more effective.

Mutations Within the Genes

There are multiple genes that can be affected that result in a diagnosis of MSUD. Thus, unique mutations within each of these genes have been discovered, and their specificity remains solely within their respective gene. While it is nearly impossible to ascertain the exact cause of mutations within each gene, an understanding of some of the mechanisms that cause it can be reached by examining the mutations. Based on numerous studies, several different mutations have been analyzed in the BCKDHB, BCKDHA, and DBT genes. A few similarities and unique attributes have been identified in each of these genes. Analysis of these specifics provides a basic foundation of interpretation for the underlying mechanisms of the disease.

The BCKDHB gene codes for the E1β subunit that makes up part of the E1 component of the BCKDC. Mutations in this gene are the most frequent type. According to literature, two common point mutations have been identified for the BCKDHB gene that cause the classic form of MSUD. A nucleotide change from C to T at position 853 and from C to T at 331 are both commonly found as the disease causing mutation for MSUD in patients (2). The C to T mutations at both positions change the amino acid from arginine to any amino acid (30, 31). Several other point mutations have been identified as disease-causing mutations in exons 3, 4, 5, 6, 7, 8, most causing either classic or mild variants of MSUD (31). Collected data seems to agree

that most of the disease-causing mutations occurring in the BCKDHB gene are caused by point mutations rather than other types. Many of the mutations recorded are either novel or have only been recorded once or twice in other pieces of literature, making it difficult to draw any conclusions about patterns in data or specific mutations that have a larger effect on clinical outcome compared to others. The widespread positioning of the mutations throughout the entirety of the gene also does not lend to formation of any type of pattern of point mutations.

Overall, BCKDHA mutations are the second most common type to cause MSUD, following BCKDHB mutations. Like the BCKDHB gene, the BCKDHA gene encodes a subunit of the E1 component of the BCKDC. Also found within the BCKDHA gene are mutations that occur throughout many of the different exons, with pathogenic mutations being noted in exons 2, 3, 4, 6, 7, 9, and even the promoter (31, 32). For the most part, gene mutations in the BCKDHA appear to have less severe clinical outcomes; however, other studies have found just the opposite, with many classic and severe variant phenotypes stemming from BCKDHA mutations (31). The conflict of data demonstrates the complexity of the disease, making it difficult to draw generalized conclusions that can be applied to the population at whole. Prevalent mutations are difficult to identify because of the seemingly random nature of the disease mutations. Population group studies may provide an answer for specific genetic mutations within subgroups.

Mutations that occur in the DBT gene are some of the least common types of mutations to cause MSUD. These mutations affect the structure of the E2 component of the BCKDC (16). Sometimes, DBT mutations lead to types of MSUD that can respond to thiamine treatments, one example being a Chinese male who presented with the classic symptoms of MSUD such as poor appetite and a maple scent. Treatments with thiamine greatly improved the patient's conditions making the case one of the first to prove that thiamine supplementation for DBT mutations could

potentially improve outlook (25). Despite this, most DBT mutations are attributed to causing classic type MSUD. Since thiamine-responsive type MSUD is rarer than classic MSUD, this may imply that the threshold for differentiation between these two types is much narrower than originally assumed. Pathogenicity of mutations in the DBT gene are likely due to secondary outcomes influencing the TPP binding site. Normally, the TPP is located between the E1α and E1β subunits of the BCKDC. However, when a mutation occurs in the E2 component, it could potentially affect the binding affinity of the TPP to the E1α, causing MSUD and the related symptoms (33). Analyzing the types of DBT mutations that cause MSUD could help to explain the pathogenesis of the disease in greater detail.

Population Group Studies

Due to the varying number of mutations within each gene, studying specific population groups may prove to be beneficial in the understanding of how it can affect different groups. Patterns of mutations arise more frequently within specific population groups and can be identified. Five different groups were studied to determine if there are population differences that exhibit commonalities otherwise unseen in the general population of MSUD patients. Mutations in groups of Saudi Arabian, Turkish, Brazilian, Chilean, and Chinese patients were analyzed for MSUD mutations in all genes that can potentially cause the disease. While is it important to examine trends in all patients with MSUD, it is far easier to make these comparisons within population groups to better understand how the disease affects people in different regions (12).

Saudi Arabian Population

In Saudi Arabia, it is believed that the incidence rate of MSUD is much higher, thus resulting in more possible mutations compared to other population groups. The frequency is estimated to be 1:22,000, significantly higher compared to the worldwide frequency. As MSUD

is a genetic disease, it is likely that many of these individuals came from consanguineous families, which aligns with statistics that recognize more consanguineous marriages in this region (30). In a cohort of Saudi Arabian patients, 8 were found to have the same point mutation in the BCKDHB gene at position 817. This specific mutation caused the original threonine amino acid to be changed into a proline amino acid. The most common type of mutation that was found in the BCKDHA gene for Saudi Arabians was a substitution mutation that changed an adenine to cytosine, thus causing an amino acid shift form asparagine to alanine. DBT gene mutations were the least frequent type found in the cohort, but most of them caused minimal alterations to the structure of the BCKDC (30). Overall, studies of the Saudi Arabian population demonstrate that patterns of mutations fall within the expected frequency with BCKDHB being the most common, followed by BCKDHA and DBT mutations.

Turkish Population

Within a Turkish population study, the same trend was observed where BCKDHB mutations were the most prevalent. However, many of the mutations in the BCKDHB were nonsense mutations. These results differ greatly from other literature because the majority of other mutations recorded are missense mutations rather than nonsense mutations. For the Turkish population, 8 out of 14 BCKDHB substitution mutations led to formation of a stop codon. All BCKDHB mutations were also point mutations (34). In addition to this, it was noted that most of the BCKDHA mutations recorded caused more mild versions of MSUD compared to the BCKDHB mutations. Many of the mutations of the BCKDHA were found in the sixth and seventh exons, suggesting that these are areas that could be monitored more closely for possible mutations. Since these two exons have been identified, it is worth considering the implication and placement values of these coding regions when examining for potential disease-causing

changes. All of the DBT mutations that were found caused classic type MSUD, and some of these mutations affected amino acids that are situated in the inner core of the E2 component. As a result, a conformational change took place, causing the onset of the disease (34). These mutations indicate that there are some specific target areas to be mindful of within the Turkish population during the diagnosis of MSUD.

Brazilian Population

The results regarding a Brazilian population study were less defined, as the mutations that were discovered did not give as clear of insight into common patterns that may be seen. For example, the BCKDHB gene showed mutations of all types, including point mutations, deletions, insertions, and duplications; missense mutations made up the majority (32). Due to the wide range of mutations, no new conclusions could be made regarding patterns or specific, targetable areas in the BCKDHB gene. Similar to other population groups, the Brazilian population also had less mutations in the BCKDHA gene. Many of these mutations resulted in insertions and deletions rather than point mutations as observed in other groups. The DBT gene mutations, however, were all point mutations. The majority also caused an amino acid switch from glutamine to another type of amino acid (32). Based on these mutations, the most interesting finding was the seemingly scattered types of BCKDHB mutations that arose in the Brazilian population group.

Chilean Population

In Chilean patients, evidence agreed with the theory that there is no genotype-phenotype correlation of MSUD. None of the patients tested had consanguineous relationships in the family, different from the observations made in other studies. Interestingly, in the BCKDHB gene, there was one specific type of mutation that was found 11 different times. This mutation

caused a switch from the isoleucine amino acid to a lysine. It was described as a Spanish mutation, potentially suggesting the existence of a founder mutation within the population. Located in exon 6, this section of the gene may be a target of interest for the diagnosis of MSUD in the Chilean population (24). The presence of BCKDHA mutations in this population was less than expected, equal to the number of DBT mutations. The overall presence of mutations in the Chilean population is assumed to be due part in fact to people origin. Historically, Chilean people have genetic homogeneity compared to other populations because of their indigenous nature (24). While there is minimal evidence of consanguineous relationships within the culture, lessened genetic diversity can also play a large role in the prevalence of genetic disorders.

Chinese Population

Studies of a Chinese population demonstrated flipped results regarding the prevalence of BCKDHB and BCKDHA mutations. In fact, the Chinese population group had larger numbers of BCKDHA mutations, unlike any of the other population groups studied. The BCKDHB mutations found identified no types of trends that could be used for future research. One unique mutation found in the Chinese population was in the BCKDHA gene. Two of these mutations were discovered to be duplication mutations which are relatively uncommon. Despite this, no other irregularities were observed. However, there was a high death rate associated with the mutations found in the Chinese population, and 8 out of the 11 patients studied passed away. As in the other population groups, DBT mutations were the least common in the population group (2). Unfortunately, none of the data suggested specific areas that could be monitored going forward.

The population groups studied had mutations located in all three genes; however, most of these mutations were localized to the BCKDHB gene. For all except for two groups, BCKDHA

gene mutations followed, with DBT genes being the least common among the groups. The Chinese population was the only exception to this rule because there were more BCKDHA mutations than BCKDHB mutations. In the Chilean population, there were equal amounts of BCKDHA and DBT gene mutations. Further studies should continue to be conducted to provide information about how MSUD affects different population groups.

Population Susceptibility Due to Limited Gene Variety

Certain populations exhibit much higher rates of MSUD than others, leading to the belief that these groups have founder effect alleles or genes in them. Much like the Chilean population, evidence of founder alleles have been identified in other smaller people groups such as the Portuguese Gypsies, aboriginal tribe Paiwan of Taiwan, Old Order Mennonites, and Ashkenazi Jewish. As it is not uncommon to encounter founder effects within smaller population groups due to less genetic diversity, MSUD mutations are predicted and expected. Understanding the placement and identifying the mutations within these populations can significantly improve the diagnosis process of the disease while lending to a better outlook because of the ability for earlier diagnosis and treatment administration.

Portuguese Gypsies

Within the Gypsy population, a founder mutation has been described based on microsatellite haplotype determination. Gypsy MSUD patients all have the exact same haplotype that leads to the onset of the disease. The mutation's origin within the Gypsy population has also been found to be separate from identical mutations in other non-Gypsy populations, evident that it is indeed a founder mutation. Patients with this mutation have a deletion of a cytosine nucleotide at position 117 in the coding region of the BCKDHA gene. The carrier frequency of

the mutation is calculated to be about 1.4% in healthy individuals, making it a target for genetic analysis during prenatal screening (35).

Paiwan Tribe of Taiwan

Studies in the Paiwan tribe of Taiwan reveal that a large base pair deletion in the E2 component of the BCKDC is potentially a founder mutation. The deletion resulted from a nonhomologous recombination event where an overlapping base pair was identified at the deletion junction. In the gene segment that is deleted, the E2 catalytic domain is disrupted and inactivated, identifying part of the reason for the onset of MSUD symptoms and decreased enzymatic activity of the BCKDC. Additionally, the record of a deletion mutation that is directly resulting from nonhomologous recombination is novel for any of the MSUD genes (36). The Paiwan tribe is yet another population group that exhibits founder effect within their genes.

Old Order Mennonite

Understanding of a founder effect in the Old Order Mennonite population has been around for a longer time and is more well-known than some of the other small populations that also have founder effects. Being able to accurately diagnose MSUD and start treatment as early as possible has proven to be effective in combatting the progression of symptoms by preventing the need for hospitalization and reducing time of illness (37). A carrier frequency of almost 8% and incidence in live births of 1 in 358 are estimated (38). One founder mutation that is well known is a point mutation at position 1312 from thymine to adenine. This mutation was also confirmed with haplotype identification. Additionally, because of the high incidence rate observed, it was concluded that the high frequency of the disease in the Mennonite population is due to random genetic drift complicated by inbreeding within the population rather than solely by inbreeding (38).

Ashkenazi Jewish

The Ashkenazi Jewish population is another group that is well known to have founder effect mutations for MSUD. An allele in the BCKDHB gene that causes a change from the arginine amino acid to the proline amino acid in position 183 has been detected and presented as a founder mutation due to its high frequency. In the study, this particular mutation appears in 10 out of 12 of the mutant alleles. The clinical phenotypes of the patients with this mutation were mostly classic with one case of intermediate MSUD (39). Another mutation has also been identified as a founder mutation and causes a change from guanine to thyine at position 685. The shift changes the amino acid sequence from glycine to cysteine. This point mutation has a very high carrier frequency with estimates being between 1 in 94 or 1 in 110. However, this mutation also occurs in the DLD gene, lending to E3-deficiency of the hepatic variety (40). Some professionals may not agree that this can be specifically regarded as MSUD, but since it occurs in one of the genes that codes for the BCKDC, the effects of the mutation can still limit BCKDC activity and cause some symptoms that are similar to that of classic MSUD symptoms.

The incidences of founder mutations are still relatively unknown within populations because of the difficulty to screen the entirety of a population's genetic material. However, as more cases of MSUD are being found to have the same disease-causing mutations, founder effect in these populations should be more closely examined. The identification of common genetic mutations in at-risk populations is beneficial for the screening and diagnosis of MSUD. While there is still no mutation that is common between all ethnic groups and populations, isolating the specific mutations within population groups is a step in the right direction for improving the prognosis and outcome of patients.

Mutations Causing Conformational Changes

Many mutations lead to the ineffectiveness of the BCKDC due to conformational changes caused by amino acid switches. The pathogenicity of mutations occurs mainly from destabilization due to the amino acid substitutions. Instability in the protein is caused by the altered interactions between surrounding amino acids in sequence. A singular mutation in a gene can be enough to cause a fold-preventing change, indicating that even the slightest change in amino acid sequence is enough to cause disease (30). This further emphasizes the importance of the specific structure of the BCKDC and the sensitivity of the complex's structural integrity.

When evaluating the pathogenic potential of a mutation that causes an amino acid switch, it is important to understand the relationships that each amino acid may have with one another. For example, an alanine to valine substitution would not be expected to cause a dramatic change because of the similar chemical characteristics of the two molecules, and observed data has supported this as two patients with this mutation were diagnosed with a variant form of MSUD that is much less severe than the classic type. However, a change from arginine to tryptophan at a key location for structural integrity of the BCKDC would be expected to have a much larger clinical outcome, partly due to the simple size difference between the two amino acids. Additionally, the change from a positively charged arginine to the uncharged tryptophan alters the normal contact bonds made in the amino acid sequence, contributing to a more severe phenotype which has also been observed (34).

In addition to understanding the normal interactions between amino acids, it is also imperative to understand the overall implication of mutational changes on the whole structure. Most of the gene mutations cause disease by destabilizing the structure of the BCKDC, directly resulting from a broken hydrogen bond. Other pathological causes include interface-damaging

mutations that prevent the proper structure from forming. One mutation that has been found to demonstrate this principle produces a nonsense mutation. The resulting truncated protein due to the nonsense mutation can hardly perform with the same efficacy as required for adequate BCAA metabolism. Analysis of the data has still not uncovered a genotype-phenotype correlation for the disease raising concerns about the ability to effectively diagnose and treat patients neonatally (30). Additionally, missense mutations within critical structural areas of the protein also tend to cause more catastrophic effects on the overall function of the BCKDC (34). The delicate and intricate structure of the BCKDC prevents mistakes in the coding from being tolerated, and these findings are evidence to the claim. Figure 3 demonstrates how the swapping of an amino acid can inhibit the interactions and, subsequently, the conformation of the BCKDC.

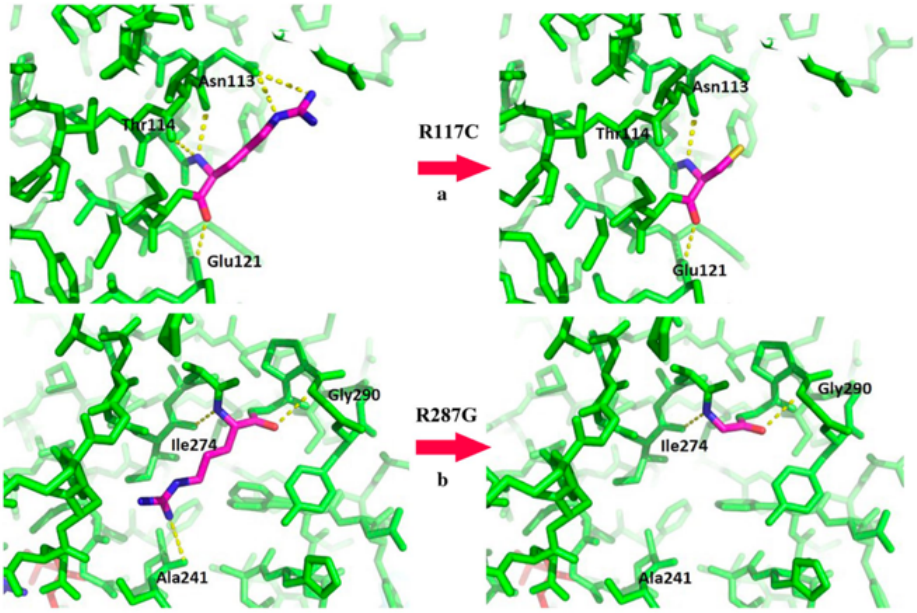

Figure 3. Substitution of amino acids alters the structure of the BCKDC. Mutations that cause amino acid substitutions can greatly alter the number of interactions made with surrounding amino acids. **(a)** In one missense mutation, the amino acid switch from arginine to cysteine reduces the interactions with surrounding amino acids from five to two. **(b)** A missense mutation from arginine to glycine prevents contact with the surrounding alanine amino acid. Figure modified from Sun et al., 2020 (2).

While each case of MSUD is unique, similarities in the mechanism of action provide insight into how these mutations can cause the onset of MSUD. As more data is collected and the interactions between the amino acids within the BCKDC are better understood, it may provide more options for treatment of patients. Additionally, if certain mutations can be found early enough, being able to understand how they will affect the patient will allow for earlier treatment, increasing their prognosis and outlook. The interactions of amino acids and the overall functioning of the BCKDC are integral parts of understanding MSUD.

Initial BCAA Levels

Different sources contradict one another regarding the role that BCAA levels play in the presentation of the disease. It is worth noting that some studies have suggested that the initial BCAA levels of a patient do not have an effect on the symptoms displayed by the patient, while other studies suggest that they do have an impact. In the Chilean population, initial BCAA levels did not correspond with existing literature. Thus, it was concluded that initial BCAA levels do not play as important of a role as metabolic control and intake with evidence demonstrating that starting values did not have a correlation to the phenotype of MSUD (24). This is heavily contrasted with the study of the Chinese population. In this study, individuals who passed away all had higher initial leucine and isoleucine levels than the 3 living patients, supporting the idea that higher initial levels lead to more severe phenotypes (2). As there is no consensus within the scientific community yet, more research should be conducted to determine whether initial BCAA levels contribute to the overall prognosis and phenotypical outcome in MSUD patients.

Treatments

Despite the vast variety of symptoms that can be experienced by patients with MSUD, there are relatively few approved and known treatments to manage the condition. The type of

MSUD and severity of symptoms play an important factor when considering a treatment plan. Also, the time of diagnosis can be crucial for ensuring a positive prognosis and improving quality of life for affected individuals. If the diagnosis of MSUD is based solely on clinical symptoms, efficacy of treatment may be significantly reduced due to the frequency of misdiagnoses since early symptoms are not always indicative of MSUD (28). Occasionally, psychotropic medications are used to treat patients who have mental health issues directly resulting from MSUD. When used, antidepressants are the most common prescribed medication (41). Treatments for individuals with MSUD can have varying results because of the nature of the disease. It is important that patients and their caretakers are diligent with their treatment plans to maximize their effectiveness and be able to manage the disease efficiently.

Diet Management

The most common type of treatment prescribed for MSUD patients of any type is to follow a strict diet that limits the intake of BCAAs. The accumulation of BCKAs, the metabolic product of BCAAs, has detrimental consequences for the central nervous system, so it is imperative that the patients diagnosed with MSUD follow this regimen closely. In the acute stage of treatment, individuals can receive sufficient nutrient intake via BCAA-free milk, intravenous bags, or the administration of fats (17). Biochemical measurements should also be taken and monitored to ensure that BCAA levels do not rise to an unsafe level for the patient. Diet management should be continued into adulthood with calorie monitoring and prevention of BCAA dietary intake (3).

A major concern for patients following diet restriction is the potential of a lack of nutrients. This can be combatted with the administration of nutrient-rich formulas to ensure that other essential amino acids are still being taken in for other metabolic processes. Leucine is most

responsible for causing cognitive deficits and encephalopathy, so it is especially crucial to restrict leucine in the diet. Leucine levels in blood plasma should be closely monitored so the threshold of the tolerance level is not surpassed. Additionally, leucine tolerance changes throughout the lifespan, and this should be taken into consideration when monitoring the amino acid levels. However, the other two BCAAs can be administered in small doses to ensure that the maintenance of both of these amino acids is adequate. Supplements with these amino acids are safe to use within moderation so normal metabolic functioning apart from BCKDC activity is not inhibited (21).

Thiamine Supplementation

Some types of MSUD positively respond to thiamine supplementation, and symptoms can be managed with thiamine. As a cofactor of the E1 component, the intake of thiamine can drastically improve BCAA levels in the blood in MSUD types that are thiamine responsive. Unfortunately, thiamine supplements are only effective for some patients, primarily only those who have residual BCKDC activity or those who have mutations in the DBT gene. The intake of thiamine in the diet can also improve MSUD conditions by increasing the tolerance level of the individual to dietary BCAAs (25). When prescribed treatment with thiamine, daily doses can be between 10 and 1000 milligrams (24). Patients who have thiamine-responsive MSUD type typically have a mutant E2 component that alters the binding site for the TPP, rendering it ineffective for normal function (17). Not all types of E2 mutations are thiamine-responsive, so treatment utilizing thiamine supplements can sometimes be ineffective depending on the nature of the mutation. However, using thiamine to help lessen the effects of symptoms on the patient serves as a promising treatment method for those with MSUD.

Liver Transplantation

The primary location of BCKDC activity is the liver tissue, thus making the liver a target organ for possible therapy. Overall, the liver is responsible for about 15% of total BCKDC activity, with the remaining activity spread between other tissue types (24). Normal functioning of BCKDC within the liver alone can lower peripheral amino acid levels enough to reclassify the patient as having a mild MSUD variant, or even maintain safe levels within the patient (17). Despite this, completely normal BCKDC activity and function can never be fully restored in an affected patient because BCKDC activity is not limited solely to the liver. The result is the potential of metabolic intoxication due to accumulation of BCAAs (3) As such, liver transplantation for patients with classic MSUD can lead to much higher quality of life as it reduces a multitude of symptoms normally experienced with this type. In fact, a successful liver transplantation can return BCAA plasma levels to normal ranges, improve psychological conditions, and maintain normal amino acid homeostasis (41). It can also potentially maintain homeostasis of BCAAs well enough that the patient no longer needs a restricted diet (42).

Unfortunately, liver transplantation is not always feasible for patients. It is improbable to assume that every patient diagnosed with classic MSUD is able to receive a transplanted liver. With an already limited supply of donor organs, MSUD patients do not typically have priority for liver transplants, making it an unlikely treatment. However, the severe neurocognitive effects that can result from an MSUD diagnosis can sometimes classify them as high priority on the donor list (41). In the event that there are no donor livers available, it is possible to use liver tissue from an unaffected relative to achieve similar results (3). The ethical implications of giving MSUD patients donor livers over other patients is also widely debated as some can manage the condition with other methods of treatment. However, evidence suggests that liver

transplantation from MSUD patients to another recipient could provide the same results as a normal liver transplantation with no adverse effect to BCAA levels in the recipient (41). Liver transplantation can greatly improve MSUD conditions in patients with classic type MSUD, but there are still many ethical, medical, and clinical implications to consider before making this decision.

Future of MSUD

The lack of a currently known cure for MSUD is garnering further attention into research for potential treatments. The need for understanding MSUD and finding treatments is pertinent as most of those who are diagnosed with the disease have a very poor prognosis. Research in the field currently has been mainly focusing on the improvement of currently known treatments; however, there have been some promising methods that may prove to be more effective and efficient than diet management and liver transplantation. While a general cure has yet to be found, ongoing research in the field gives hope to future of MSUD.

Aggregation into Amyloid-Like Fibrils

Up until recently, there has been a lack of understanding on the exact mechanism of BCAAs that lead to the pathology of the disease. Despite this, one study has demonstrated that BCAAs in high amounts self-assemble into amyloid-like fibrils (1). This discovery is revolutionizing the way that MSUD is viewed. The study also found that the BCAA aggregation fibrils behave and have similar characteristics as amyloid fibrils, suggesting that the current medications used for amyloid fibrils could potentially also be used for MSUD patients. Polyphenols are a current treatment used to manage the aggregation of amyloid fibrils, and their use with BCAA fibrils showed reduction in aggregation in a similar manner. Administration of polyphenols significantly slowed and prevented the aggregation of BCAAs, in turn reducing the

cytotoxicity of aggregation fibrils (1). If BCAA aggregation can be treated before it worsens, the presenting symptoms of the disease may be reduced as a result. While this can only be characterized as a management method rather than a cure, it still opens the pathways for potential treatments. Further research in animal models needs to be performed before the potential of it being used in humans with MSUD is considered.

AAV Gene Therapy

Neonatal gene therapy seems to be another promising option warranting further study. Since the cause of MSUD is known to be a genetic mutation in either the BCKDHA, BCKDHB, or DBT gene, further studies have begun to examine the efficacy of neonatal gene therapy on mice that have knockouts of these specific genes. BCKDHB is the most commonly affected gene, and one recent study's research shows that manipulation of this gene could greatly improve the life expectancy of knockout-gene mice (43). The study utilized AAV8 capsids containing a human transgene of BCKDHB with an EF1α promoter. The transgene used was then discovered in hepatic tissue, cardiac tissue, and the brain, indicating that the supplemental BCKDC activity provided by the capsid was sufficient enough to alleviate symptoms in the mice. A few limitations in transgene therapy do exist, including the concern that proliferating tissues may not be able to express the gene in a high enough level to prove effective in age (43). Even so, gene therapy is becoming a prominent contender for long-term treatment of MSUD. Applying this concept to humans could help to greatly improve life outlook in babies that are diagnosed with MSUD if caught early enough.

Oral Enzyme Therapy to Target Leucine Plasma

In patients with MSUD, leucine plasma levels are of major concern due to their elevation leading to neurotoxicity and death. Recent research into the lowering of these levels has led to

the discovery of an enzyme in *Planctomycetaceae bacterium* that could be used to convert leucine in the gastrointestinal tract into an unnatural byproduct called isopentylamine, thereby preventing the absorption of leucine into the bloodstream. In one particular study, a specific variant was identified that is able to withstand the low pH level of the stomach, making it viable for oral consumption (44). The enzyme is a type of leucine decarboxylase called LDCv10 that can withstand harsh conditions after undergoing protein engineering. This manipulation induces mutations that can then increase the stability of the protein in environments that are found in the stomach and intestines. Benefits of an oral enzyme therapy include improvement of quality of life due to a lesser need for diet restriction (44). This oral medication is much easier to manage and can effectively reduce the plasma leucine levels in mice and nonhuman primates, making it a great candidate for MSUD treatment testing.

Current research on MSUD demonstrates that there are a lot of promising treatment methods that could potentially be used for patients. New methods being researched range from targeting the structure of BCAA aggregation to targeting the specific BCAAs that accumulate as a result of the disease. The combination of these methods with established treatments in the future could significantly improve the lives of those who are diagnosed with MSUD by reducing symptoms and making the condition more manageable. As it is an ongoing research field, much remains to be understood about the metabolic disease.

Conclusion

MSUD is a metabolic disorder that is caused by genetic mutations in the genes that contribute to the normal functioning of the BCKDC. When the BCKDC function is impaired, BCAAs cannot be properly metabolized, leading to their aggregation and accumulation. As a result, symptoms can appear that include encephalopathy, elevated BCAA levels, poor growth,

and syrup scent in the urine. While no singular mutation has been identified as the main proponent of the disease, MSUD is understood to have several different mutations that contribute to the pathology. The main population groups that are affected include smaller people groups with less genetic diversity. Despite having an understanding of many of the different aspects and facets that encompass MSUD, research has still yet to determine a cure for those who are diagnosed. Current treatments involve diet management, liver transplantation when available, and thiamine supplementation. As research continually uncovers the pathophysiology of MSUD and finds more treatment methods that effectively alleviate the symptoms, patients with MSUD can have hope in a positive prognosis and increased quality of life.

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