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Branched-chain amino acid metabolism: from rare Mendelian diseases to more common disorders

Lindsay C. Burrage[1], Sandesh C.S. Nagamani[1], Philippe M. Campeau[2] and Brendan H. Lee[1,3,*]

[1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA, [2]Department of Pediatrics, University of Montreal, Montreal, QC, Canada and [3]Howard Hughes Medical Institute, Houston, TX 77030, USA

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Branched-chain amino acid (BCAA) metabolism plays a central role in the pathophysiology of both rare inborn errors of metabolism and the more common multifactorial diseases. Although deficiency of the branched-chain ketoacid dehydrogenase (BCKDC) and associated elevations in the BCAAs and their ketoacids have been recognized as the cause of maple syrup urine disease (MSUD) for decades, treatment options for this disorder have been limited to dietary interventions. In recent years, the discovery of improved leucine tolerance after liver transplantation has resulted in a new therapeutic strategy for this disorder. Likewise, targeting the regulation of the BCKDC activity may be an alternative potential treatment strategy for MSUD. The regulation of the BCKDC by the branched-chain ketoacid dehydrogenase kinase has also been implicated in a new inborn error of metabolism characterized by autism, intellectual disability and seizures. Finally, there is a growing body of literature implicating BCAA metabolism in more common disorders such as the metabolic syndrome, cancer and hepatic disease. This review surveys the knowledge acquired on the topic over the past 50 years and focuses on recent developments in the field of BCAA metabolism.

INTRODUCTION

The branched-chain amino acids (BCAAs) namely valine, isoleucine and leucine are essential amino acids with hydrophobic side chains that comprise ~20–40% of most dietary proteins (1,2). A large proportion of BCAAs from dietary sources is absorbed from the intestines, bypasses the liver, and is delivered to the peripheral tissues (3). Although the enzymatic machinery for BCAA metabolism is predominantly active in the liver, BCAA catabolism also occurs in other tissues including skeletal muscle, heart and adipose tissue (1,2). The complex interorgan relationships in BCAA metabolism have been summarized elsewhere (1,2).

The role of abnormal BCAA metabolism in human disease was initially recognized with the description and biochemical characterization of maple syrup urine disease (MSUD; MIM: 248600), a Mendelian disorder caused by a deficiency of the rate-limiting enzyme in BCAA catabolism (4–6). Since then, dysregulated BCAA metabolism has been found to be causative or associated with complex neurocognitive phenotypes, insulin resistance, adverse cardiovascular outcomes and liver disease. This review will focus on Mendelian diseases characterized by both elevated and decreased levels of BCAA and dysregulation of BCAA metabolism in more common disorders like diabetes, cancer and liver disease.

BCAA METABOLISM

The initial step in BCAA catabolism is a transamination of these amino acids by branched-chain aminotransferase (BCAT) to generate their respective α-ketoacids (α-ketoisocaproic acid (KIC), α-keto-β-methylvaleric acid (KMV) and α-ketoisovaleric acid (KIV)). These branched-chain ketoacids (BCKAs) undergo oxidative decarboxylation by the branched-chain ketoacid dehydrogenase complex (BCKDC), a large (4.5 MDa) catalytic complex which consists of a heterodimeric E1 decarboxylase component (E1α and E1β subunits), an E2 transacylase component with 24 identical subunits and a homodimeric E3 component (2). BCKDC requires several cofactors, including thiamine pyrophosphate (for the E1 component), Coenzyme A (for the E2 component), lipoamide and flavin and nicotinamide adenine dinucleotides (FAD and NAD) (all for the E3 component). The products resulting from BCKDC-mediated catalysis are isovaleryl-CoA, α-methylbutyrylCoA and isobutyryl-CoA. Through a series of further enzymatic reactions, these products are converted

*To whom correspondence should be addressed. Tel: +1 7137988835; Fax: +1 7137985168; Email: blee@bcm.edu

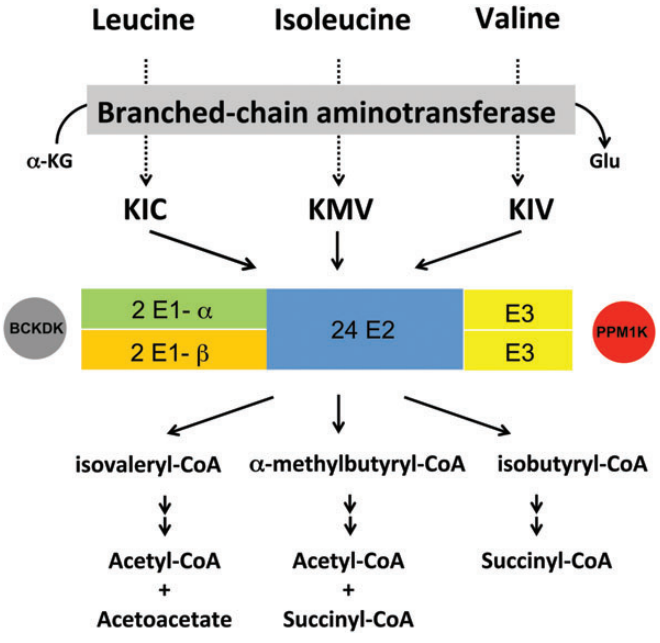

Figure 1. BCAA catabolism. The BCAAs are transaminated by BCAT to generate α-ketoacids (KIC, KMV and KIV). These α-ketoacids undergo oxidative decarboxylation by the BCKDC.

to the end products of BCAA metabolism: acetoacetate, acetyl-CoA and succinyl-CoA (Fig. 1).

MENDELIAN DISORDERS OF BCAA METABOLISM

Maple syrup urine disease

MSUD is an inborn error of metabolism caused by decreased activity of the BCKDC (4–6). Patients with the classic form of MSUD present in the neonatal period with poor feeding and irritability that, if left untreated, may progress to lethargy, coma and death. Elevations of plasma BCAAs and urinary BCKAs are the biochemical hallmark and the presence of L-alloisoleucine in the plasma is pathognomonic for the disorder.

The prevalence of MSUD in the USA is estimated to be ~1:200 000 live births (7) but is much higher is some populations, such as the Mennonites (1:358 live births) (8). Classic MSUD characterized by neonatal presentation is typically a result of biallelic mutations in the E1α, E1β or E2 subunits of the BCKDC. An intermediate or variant form of MSUD presents with milder symptoms or at a later age, and an intermittent form presents with episodic symptoms with normal levels of BCAA between episodes. The intermediate and intermittent forms of MSUD are also due to mutations in the E1α, E1β or E2 subunits; however, the residual activity of BCKDC is higher when compared with classic forms (9,10). A fourth type of MSUD is the thiamine-responsive form which occurs due to mutations in the E2 subunit that produce a full-length mutant form of the E2 protein (11,12). Finally, mutations in the E3 subunit (dihydrolipoyl dehydrogenase), which is shared with pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, result in a severe phenotype distinct from MSUD that is characterized by congenital lactic acidosis and progressive neurologic deterioration (13).

The goal of the initial acute treatment of MSUD is the reduction of plasma leucine levels by discontinuing protein feeds, providing calories from dextrose and intravenous lipids, hemodialysis, if necessary, and eventually introducing BCAA-free formula (14–17). Neurologic status, plasma amino acids, electrolytes and serum osmolarity should be closely monitored as patients are at risk for cerebral edema, a potentially fatal complication. Because prolonged elevation of plasma leucine in the neonatal period contributes to long-term intellectual outcomes, early identification and treatment are necessary (18,19). Thus, MSUD is an ideal disorder for detection via newborn screening (7,20). However, late-onset or intermittent forms may not always be detected by newborn screening (21).

The goal of long-term treatment is to maintain the plasma BCAA levels as close to normal as possible using dietary modifications including protein restriction and BCAA-free medical formula. The plasma concentration of leucine, the most neurotoxic of the BCAA, typically guides adjustments to the dietary regimen. Long-term control of leucine levels has been associated with intellectual and neuropsychiatric outcome in this disorder (18,19,22,23). Intercurrent illnesses can result in the release of BCAAs from endogenous protein catabolism and risk for acute metabolic decompensation. Strategies, such as the judicious use of dextrose-containing intravenous fluids, insulin, BCAA-free 'sick day' formula and isoleucine and valine supplements may be necessary in the setting of illnesses to promote anabolism. Although a small proportion of patients respond to thiamine supplementation (12), there are currently no medications for the long-term management of MSUD. Several new therapies for MSUD are being investigated in clinical and preclinical studies.

Liver transplantation in MSUD

The discovery that liver transplantation may be an effective treatment for MSUD was made from observations in two patients with MSUD who required liver transplantation for liver failure secondary to other causes. Both patients had improvement in BCAA levels after transplant (24–26). Since then, numerous liver transplants have been performed. A recent case series described outcomes in 54 patients with MSUD who underwent liver transplantation in the USA between 2004 and 2010 (UNOS registry) with a 98% patient survival and 96% graft survival (27). Of these 54 patients, the course of 37 patients who received liver transplants at a single center has been described (27). BCAA homeostasis normalized within hours of surgery and all patients had increased leucine tolerance after transplantation (27). Interestingly, the finding of one patient with leucinosis in the setting of dehydration indicates that patients may still require careful management in the face of intercurrent illness even after transplantation (27). Pre- and posttransplantation IQ scores were not significantly different in 14 patients (28). However, liver transplantation may prevent future metabolic decompensations and further worsening of cognitive outcomes.

The effectiveness of liver transplantation in MSUD suggests that providing 9–13% of the bodies' BCKDC activity is sufficient to restore BCAA homeostasis (27,29). Furthermore, the livers from several patients with MSUD have been successfully transplanted into patients requiring liver transplant for other reasons (27,30–32). The success of domino transplantation suggests that BCKDC activity in the skeletal muscle and other

tissues is sufficient even in the face of deficient activity in the liver (27,30–32).

Obviously, liver transplantation is not without consequences including postoperative complications and the effects of long-term immunosuppression. Nearly 40% of transplanted patients have needed management for acute rejection (27). Of 54 patients in the UNOS registry, one died and one required a second transplant (27). A larger study ($n = 446$) evaluating pediatric liver transplantation performed for any metabolic disease revealed a 95% survival at 1 year with 89% survival at 5 years (33). Thus, the risks and benefits of liver transplantation must be carefully considered.

Tissue and cell transplantation in preclinical models of MSUD

Although liver transplantation has been shown to be successful in MSUD, the limitations in availability of donor tissue preclude its widespread applicability. Hence, preclinical studies focusing on other sources of cells or tissues with BCKDC activity have gained prominence. Hepatocyte cell transplantation has been evaluated in the iMSUD mouse model (34,35). The iMSUD mouse model was generated by introducing a human cDNA for the E2 subunit that is expressed in the liver of cMSUD (E2 knockout) and has improved survival compared with the cMSUD model (36). Two injections of donor hepatocytes given transdermally into the liver parenchyma in the first 10 days of life resulted in engraftment of cells and BCKDC activity that was 14.36% of control activity when compared with 6.23% in PBS-treated mice (35). The mice treated with hepatocyte transfer had improved body weight, survival and partial corrections of neurotransmitter abnormalities in the brain (34,35). However, although BCAA levels were improved at weaning, they were not significantly different from controls or untreated mice at the time of sacrifice (35). It is thus unclear whether this partial increase in enzyme activity will be sufficient to promote long-term survival in this mouse model for MSUD or be sufficient to prevent metabolic decompensation in the setting of stress.

Although hepatocyte cell transplantation has given some promising results in preclinical models, the utility of such an approach in humans may be complicated by limited availability of donor hepatocytes and the need for immunosuppression. An alternative approach is the use of stem cells, such as human amnion epithelial cells, that can be differentiated into hepatocytes. The possibility of human amnion epithelial cell therapy is enticing because these cells are less immunogenic. Transdermal hepatic injections of human amnion epithelial cells in iMSUD mice, doubled the BCKDC enzyme activity and decreased the BCAA levels in the serum and brain (37,38). Furthermore, these biochemical improvements were associated with improvements in body weight, survival and bioenergetic parameters in both brain and serum (37,38).

Recent studies have also investigated adipose tissue as a source of BCAA activity in MSUD mouse (39). Subcutaneous transplantation of adipose tissue from wild-type mice into two different mouse models of MSUD (BCATm knockout or branched-chain dehydrogenase phosphatase (PPM1K) knockout models) resulted in decreased BCAAs by 52–81% compared with non-transplanted mice (40).

Targeting leucine neurotoxicity in MSUD

Despite therapy, patients with MSUD typically have intellectual and social impairments (23,41,42). However, the exact mechanisms underlying the neurotoxicity of BCAAs, particularly leucine and BCKAs are likely complex and incompletely understood. The neurotoxicity of leucine and BCKAs has been discussed elsewhere (43,44) and here, we will focus on aspects of neurotoxicity that are targets of new therapeutic strategies.

One mechanism that explains leucine toxicity is interference with neurotransmitter biosynthesis. Leucine competes with other large neutral amino acids for transport across the blood–brain barrier using the large neutral amino acid transporter (45). Given that some of these amino acids, such as phenylalanine and tyrosine, are precursors of neurotransmitters, this competition for transport likely interferes with neurotransmitter synthesis. Optimization of neutral amino acid transport across the blood–brain barrier has hence become a treatment strategy in MSUD. Strauss and colleagues designed a metabolic formula that enhances the transport of amino acids (e.g. tyrosine, tryptophan, histidine, methionine, threonine, glutamine and phenylalanine) which compete with leucine for entry into the brain (46). In patients using this formula, plasma leucine levels were improved and decreased variation in plasma leucine measurements was noted (46). However, no randomized controlled trial comparing this formula with standard MSUD formulas has been performed.

Likewise, norleucine, a BCAA analog which competes with leucine for transport across the blood–brain barrier has been explored as a therapy in preclinical models (47,48). The administration of norleucine resulted in improved survival, reduced levels of leucine and KIC, and normalization of glutamate, GABA and aspartate levels in the brain (47). However, levels of tyrosine and dopamine in the brain were still low suggesting that although norleucine restores some aspects of energy metabolism in the iMSUD mice, normal amino acid transport across the blood–brain barrier is not restored with this treatment (47).

BCAAs and BCKAs may also contribute to neurotoxicity by increased lipid peroxidation and oxidative stress (49–51). Measures of oxidative stress have been found to be higher in the plasma of patients with MSUD when compared with control subjects even in the setting of low plasma leucine levels (52,53). Carnitine is a quaternary amine that is hypothesized to have antioxidant properties in addition to its well-known role in the transport of long chain fatty acids into the mitochondria. Mescka *et al.* showed that the plasma levels of free carnitine were lower and that measures of free-radical mediated peroxidation (e.g. malondialdehyde) were higher in patients with MSUD when compared with healthy subjects (54). Levocarnitine therapy increased the plasma free carnitine (54) and decreased malondialdehyde levels (54) which suggest that levocarnitine may have antioxidant effects in MSUD (54,55). Long-term studies are necessary to evaluate whether levocarnitine supplementation could affect neurocognitive outcomes in MSUD.

Another mechanism of leucine and BCKA toxicity is the disruption of energy metabolism in the brain. The BCAAs, particularly leucine and BCKAs inhibit pyruvate dehydrogenase (56), α-ketoglutarate dehydrogenase (57) and mitochondrial respiration (58–62). Leucine also alters creatine kinase activity in the brain with the direction of the alteration dependent on the anatomical location in the brain (63). In addition, elevations in KIC

contribute to glutamate and aspartate depletion (43,64,65). Alternative mechanisms by which the BCAAs and BCKAs may contribute to neurotoxicity include the inhibition of the acetylcholine synthesis (66), induction of apoptosis of glial cells and neurons (67), alterations of various neurotrophic factors (68–70) or intermediate filament phosphorylation (71,72) and decreased α- and β-adrenergic receptor binding (73).

MSUD therapy and BCKDC regulation

Whereas increasing BCKDC activity via tissue or cell transplantation is one therapeutic strategy for MSUD, an alternative strategy is to focus on targeting regulation of BCKDC by altering its phosphorylation. BCKDC activity is inhibited by phosphorylation of two serine residues of the E1α subunit by the branched-chain ketoacid dehydrogenase kinase (BCKDK) (Fig. 2). In contrast, dephosphorylation of the E1α subunit by PPM1K, which is encoded by *PPM1K*, activates the enzyme. Interestingly, the finding of a homozygous mutation in *PPM1K* in a patient with a mild form of MSUD highlights the importance of BCKDC regulation by phosphorylation (74). The regulation of BCKDC has been reviewed elsewhere (1,2,75–77).

Sodium phenylbutyrate (NaPBA) is a nitrogen-scavenging agent that is used for the prevention of hyperammonemia in patients with urea cycle disorders (UCDs) (78–81). Patients with UCDs who are treated with NaPBA have been observed to have decreased plasma BCAA levels, a phenomenon that is replicated in healthy controls (82–84). Furthermore, an open-label pilot study demonstrated a decrease in BCAAs and BCKAs in three of the five patients with MSUD treated with NaPBA (84). Thus, the decrease in BCAAs associated with NaPBA is not unique to patients with UCDs. *In vitro* and animal studies have demonstrated that NaPBA increases the activity of the BCKDC by preventing phosphorylation of the E1α subunit by BCKDK (84). Thus, the resulting increased residual enzymatic activity of BCKDC leads to decreased plasma levels of BCAAs (84). A randomized placebo controlled study to investigate the effects of NaPBA on plasma BCAAs in a large population of patients with MSUD is currently underway (ClinicalTrials.gov Identifier: NCT01529060).

A structurally based design approach has been used to design a more potent and novel inhibitor (S-CPP) of the BCKDK that results in decreased phosphorylation of the BCKDC and thus increased activity of the enzyme complex (85). Single dose of S-CPP in mice demonstrated a decrease in plasma levels of the BCAAs (85). Long-term studies in animals are necessary to test whether these effects on the BCAAs persist with long-term treatment.

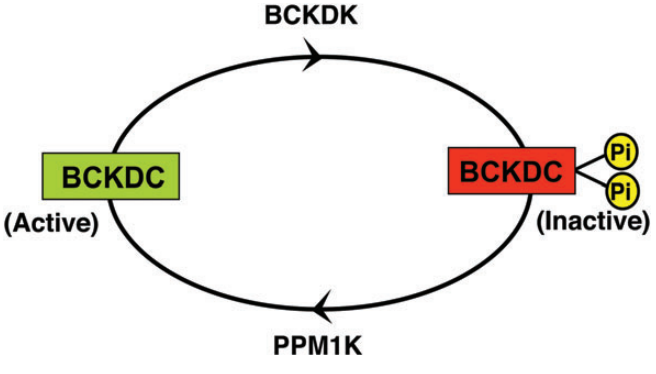

Figure 2. BCKDC regulation. The BCKDC is regulated by phosphorylation. The BCKDK phosphorylates two serine residues of BCKDC and inactivates the enzyme complex. PPM1K (protein phosphatase, pp2c domain containing, 1k) dephosphorylates and inactivates the BCKDC.

Low BCAA and neurocognitive phenotypes

Mutations in the genes encoding components of the BCKDC or PPM1K result in increased BCAA levels and MSUD. In contrast, mutations in the BCKDK, which phosphorylates and activates the BCKDC, have been recently associated with decreased BCAA levels and a phenotype of autism with seizures (86). The affected patients had lower plasma BCAA levels despite normal protein consumption (86). In a separate report, inactivating mutations in BCKDK in two unrelated children were found to cause developmental delay, microcephaly and neurobehavioral abnormalities (87). Normalizing BCAA levels in one of these patients required supplementation with high-protein diet and frequent BCAA supplement dosing throughout the day (87). The authors state that normalization of plasma BCAAs was associated with improvements in attention span, hyperactivity, communication and gross motor skills. Corroborating these findings, a mouse model of BCKDK deficiency exhibits similar features as the patients including seizures, tremors, lower plasma and brain levels of BCAAs and elevations in other large neutral amino acids (86).

ABNORMALITIES OF BCAA AND COMMON DISORDERS

Cancer

Until recently, no known human diseases had been associated with mutations in *BCAT*. However, recently, transcriptional studies in glioblastomas revealed a high level of expression of the *BCAT1* in primary glioblastomas compared with secondary glioblastomas, diffuse astrocytomas or anaplastic astrocytoma (88). Furthermore, *BCAT1* expression was higher in gliomas that have wild-type IDH1 when compared with gliomas with mutant forms of IDH1 or normal brain tissue (88). Mutations in *IDH1* and *IDH2* have been identified in 70% of astrocytomas, oligodendrogliomas and glioblastomas that developed from gliomas (89) and are associated with improved outcome compared with those without mutations (89). Knockdown of *BCAT1* in glioblastoma cell cultures resulted in reduced cell proliferation, whereas overexpression of *BCAT1* resulted in increased cell proliferation (88). When *BCAT1* knockdown cells were implanted into mice, they resulted in smaller tumors than control cells (88). Thus, BCAT1 has been hypothesized to be a useful diagnostic marker but also a possible promising therapeutic target for glioblastoma. Studies in other forms of cancer must be performed to evaluate whether BCAA metabolism plays a role in other cancers.

Insulin resistance

A positive correlation between insulin resistance and BCAA levels was first recognized in the late 1960s (90,91), and interest on the topic has been recently stimulated by a series of studies that have refined this correlation with newer methods. Using liquid

chromatography–tandem mass spectrometry metabolomics and principal component analysis, Newgard *et al.* noted that obese and insulin-resistant individuals had higher serum BCAAs and related metabolites than their lean and insulin-sensitive counterparts (92). These findings were confirmed using different study designs, methodologies and populations in independent studies (93–95). Importantly, the elevated BCAAs can be used to predict long-term insulin resistance (96,97) and response to treatment (98). While the correlation is clear, whether elevated BCAAs are a cause or an effect of insulin resistance remains unclear. Leucine does lead to increased insulin secretion (99) and can activate mTOR, the master regulator of cell growth and metabolism. In rats fed a high-fat, high-BCAA diet, the diet led to insulin resistance and this effect could be curtailed by the mTOR inhibitor rapamycin (92). However, other studies have shown that supplementation with BCAAs alone does not lead to insulin resistance and that in some cases, they can be beneficial for metabolic health, a topic that has been extensively reviewed elsewhere (100).

Hepatic disease

As liver is a central organ involved in amino acid metabolism, it is not surprising that patients with chronic liver disease (CLD) have abnormalities in BCAA metabolism. However, there is a growing body of evidence that suggests low plasma levels of BCAA in patients with CLD is more than a mere epiphenomenon and may have a role in progression of disease. Patients with CLD have decreased ratio of BCAA to aromatic amino acids (Fischer ratio) that has been associated with decreased albumin synthesis and progression of CLD (101). Whereas the mechanisms by which low BCAA contribute to the overall outcome in CLD are not known, many controlled clinical studies have shown that supplementation with BCAA can lead to increase in serum albumin, decrease in hepatic failure, improvements in manifestations associated with hepatic encephalopathy, and better quality of life (102–106). Whereas not all studies have shown beneficial effects of BCAA supplementation in CLD, they are now considered in treatment of subsets of patients with CLD, a topic reviewed elsewhere (107).

CONCLUSIONS

Recent advances in the understanding of BCAA metabolism have shown that these amino acids are not mere constituents for protein synthesis but have a central role in regulation of pivotal pathways. Dysregulation of BCAA metabolism not only results in well-characterized Mendelian disorders but also contributes to pathogenesis of more common disorders. Thus, lessons learned from therapeutic modulation of BCAA metabolism in inborn errors of metabolism could have significant impact on the treatment of common multifactorial diseases. At the same time, treatment approaches whether cell, gene or small molecule based, may offer improved outcomes for Mendelian disorders of BCAA metabolism but also may impact common diseases such as cancer, diabetes and liver failure. An increasingly in depth appreciation of how apparently simple Mendelian diseases can inform pathogenesis in complex disease will be important in the development of mechanism-based therapies.

Conflict of Interest statement. None declared.

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