Lessons from Genetic Disorders of Branched-Chain Amino Acid Metabolism

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ABSTRACT Genetic disorders of BCAA metabolism produce amino acidopathies and various forms of organic aciduria with severe clinical consequences. A metabolic block in the oxidative decarboxylation of BCAA caused by mutations in the mitochondrial branched-chain α-keto acid dehydrogenase complex (BCKDC) results in Maple Syrup Urine Disease (MSUD) or branched-chain ketoaciduria. There are presently five known clinical phenotypes for MSUD, i.e., classic, intermediate, intermittent, thiamin-responsive, and dihydrolipoamide dehydrogenase (E3)-deficient, based on severity of the disease, response to thiamin therapy, and the gene locus affected. Reduced glutamate, glutamine, and γ-aminobutyrate concentrations induced by the accumulation of branched-chain α-ketoacids in the brain cortex of affected children and neonatal polled Hereford calves are considered the cause of MSUD encephalopathies. The long-term restriction of BCAA intake in diets and orthotopic liver transplantation have proven effective in controlling plasma BCAA levels and mitigating some of the above neurological manifestations. To date, ~100 mutations have been identified in four (branched-chain α-ketoacid decarboxylase/dehydrogenase α [E1α], E1β, dihydrolipoyl transacysylase [E2], and E3) of the six genes that encode the human BCKDC catalytic machine. We have documented a strong correlation between the presence of mutant E2 proteins and the thiamin-responsive MSUD phenotype. We show that the normal E1 component possesses residual decarboxylase activity, which is augmented by the binding to a mutant E2 protein in the presence of the E1 cofactor thiamin diphosphate. Our results provide a biochemical model for the effectiveness of thiamin therapy to thiamin-responsive MSUD patients. J. Nutr. 136: 243S–249S, 2006.

KEY WORDS: branched-chain amino acid metabolism, Maple Syrup Urine Disease, E2 deficiency, thiamin-responsive MSUD, thiamin supplementation, branched-chain α-ketoacid dehydrogenase, thiamin diphosphate

Metabolic block in Maple Syrup Urine Disease

In 1954, John Menkes at Boston Children’s Hospital reported that four siblings from a single family died of progressive neurological degeneration characterized by brain edema, seizures, spasticity, and respiratory arrest. These infants showed an early onset of severe ketoacidosis in the first week of life. Notably there was a strong maple syrup odor to the urine and, hence, later the name of Maple Syrup Urine Disease (MSUD) (2). The presence of ketoacids in the patient’s urine provided the clue for Joe Dancis and associates to identify the metabolic block in MSUD (3). In this seminal experiment in 1960, white blood cells from normal infants and an MSUD patient were incubated with [1-14C]-labeled BCAAs leucine, isoleucine, and valine. Cells from both a normal infant and the MSUD patient were able to convert BCAAs to their corresponding branched-chain α-ketoacids (BCKAs). However, cells from the patient, unlike those from normal infants, were unable to decarboxylate BCKA as measured by the evolution of radioactive CO2. These results established, for the first time, that the metabolic block in MSUD is at the decarboxylation of BCKA and not in the transamination of BCAA.

The oxidative degradation of BCAA (Fig. 1) begins with the reversible transamination of leucine, isoleucine, and valine by a single branched-chain amino transferase, which exists in two
isoforms, one in the cytosol and the other in mitochondria, based on the elegant work from Susan Hutson's laboratory (4). The next step is the irreversible oxidative decarboxylation of the BCKA by an also single enzyme complex, i.e., the mitochondrial branched-chain α-ketoacid dehydrogenase complex (BCKDC), as demonstrated by Reed and associates (5). The steps after the BCKDC are diverse for the degradation of the three BCKAs. The end products succinyl-CoA and acetyl-CoA either enter the Krebs cycle or are precursors for lipogenesis. The metabolic block at the second reaction results in MSUD.

The screening and diagnosis of MSUD

Today, the elevated plasma BCAA level in MSUD can be readily detected by state-of-the-art automated tandem mass spectrometry. In the fragmentation patterns provided by the New England Newborn Screening Program, the blood sample from an infant shows an elevated peak at mass-to-charge ratio of 188 that corresponds to protonated molecular ions of leucine, isoleucine, and alloisoleucine compared to the control (6). Levels of other amino acids, such as methionine, phenylalanine, and tyrosine, are not elevated in the infant. A deuterated internal standard is provided for each amino acid. The results identify the infant as a suspected MSUD patient, which would be confirmed by enzyme assays (7). The presence of L-alloisoleucine in MSUD patients but not the control was observed early on by Snyderman and associates and has become a diagnostic marker of MSUD (8). The separation of L-alloiso-leucine from its enantiomer L-isoleucine can be readily achieved by gas chromatography–mass spectrometry. The mechanism of conversion from L-isoleucine to L-alloisoleucine

![FIGURE 1](image1.png) Oxidative degradation of the BCAAs leucine, isoleucine, and valine. The transamination of BCAA is catalyzed by a single branched-chain aminotransferase (reaction 1) that exists as both the cytosolic and mitochondrial isoforms. The oxidative decarboxylation of BCKAs is catalyzed by the single mitochondrial branched-chain α-ketoacid dehydrogenase complex (reaction 2). The metabolic block at the second reaction results in MSUD.

![FIGURE 2](image2.png) Neurotransmitter amino acid concentrations in the fresh brain cortex of MSUD calves. Data are expressed as means ± SD. Values in affected Australian Polled Hereford calves as a percentage of those in control calves are indicated above the solid bars. gab, γ-aminobutyric acid. Reproduced with permission from Dodd et al. (15).
is through an enol-keto tautomerization as confirmed by N\textsuperscript{15}-isotopic studies of Matthews and associates (9). In a normal individual, one of the intermediates in the pathway, i.e., (+)-\alpha-keto-\beta-methyl-valeric acid, is a substrate for BCKDC, which prevents its accumulation, thereby thwarting the conversion of L-isoleucine to L-alloisoleucine.

The clinical phenotypes of MSUD

Since the original report by Menkes and associates, there have been variant forms of MSUD. Today, MSUD is classified into five distinct clinical phenotypes (6). The classic form described by Menkes is the most severe with 0–2% of normal BCKDC activity and an early onset of ketoacidosis. The surviving children often manifest mental retardation. The intermediate and intermittent types are milder in clinical presentations and are associated with higher residual BCKDC activity. The hallmark of intermittent type is its late onset and episodic nature. The thiamin-responsive MSUD first described by Scriver and associates (10) responded favorably to thiamin supplement in diet, which will be discussed later in terms of the possible biochemical mechanism. Dihydrolipoamide dehydrogenase (E3) is a common component of BCKDC, the pyruvate dehydrogenase complex, and the \( \alpha \)-ketoglutarate dehydrogenase complex (11). As first described by Robinson and associates, a dysfunctional E3 results in combined ketoacidosis.
dehydrogenase deficiencies and is the most severe in clinical manifestations among the five MSUD phenotypes (12).

The neuropathology of MSUD

There have been extensive studies on the neuropathology of MSUD. Brain edema during acute metabolic decompensation triggered by infection and stress is often the cause of death (13). However, the direct cytotoxic agents for brain edema are to date still uncertain. The spongy changes in white matter and demyelination are thought to be due to chronic exposure to high leucine concentrations but are not specific to MSUD (14). An analysis of the brain cortex from neonatal MSUD calves in Australia shows that the levels of neurotransmitter amino acids glutamate, alanine, aspartic acid, and g-aminobutyric acid are uniformly reduced; other nonexcitotary amino acids are not affected (Fig. 2) (15). The reduced level of neurotransmitters is likely the culprit for acute MSUD encephalopathies, such as coma. The decreased level of glutamate in the brain can be explained by the so-called leucine–glutamate cycle proposed by Yudkoff and associates (16). Leucine enters the brain astrocyte compartment through the capillary or the blood–brain barrier and through transamination donates its amino group to a-ketoglutarate to give rise to glutamate. Glutamate is then converted to glutamine and circulated to the neuron, where glutamine is reverted to glutamate and then to leucine by reverse transamination. Leucine is circulated back to astrocytes to complete the cycle. The astrocyte is the major compartment for glutamate production. The accumulated a-ketoisocaproate from leucine during the acute phase of MSUD enters the astrocyte compartment to promote reverse transamination, converting glutamate back to a-ketoglutarate. The perturbation of the glutamate homeostasis results in the reduced neurotransmitter in the brain and, therefore, acute encephalopathies in MSUD (17,18).

Effective therapies for MSUD

Currently, there are three effective therapies that are in use for MSUD. The dietary therapy, which involves feeding patients with a synthetic diet containing reduced BCAA contents, was originally instituted by Snyderman in 1964 (8). When a classic MSUD patient was placed on this dietary regimen for 3 months, the plasma BCAA level was decreased to the normal range, including the disappearance of the alloisoleucine marker for MSUD. Commercial medical diets have since been developed for MSUD patients based on Snyderman’s synthetic formula. Orthotopic (normal) liver transplantations (OLTs)

### TABLE 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Allele</th>
<th>Residual intact-cell activity %</th>
<th>Residual E2 protein</th>
<th>Clinical phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>H.C.</td>
<td>H391R (homozygous)</td>
<td>11</td>
<td>Yes</td>
<td>Thiamin-responsive</td>
</tr>
<tr>
<td>H.S.</td>
<td>H391R (homozygous)</td>
<td>11</td>
<td>Yes</td>
<td>Thiamin-responsive</td>
</tr>
<tr>
<td>WG-34</td>
<td>F215C/IVS4del[−3.2 kb::−15]</td>
<td>30–40</td>
<td>Yes</td>
<td>Thiamin-responsive</td>
</tr>
<tr>
<td>MGL-290</td>
<td>R240C/H281T</td>
<td>30</td>
<td>Yes</td>
<td>Thiamin-responsive</td>
</tr>
<tr>
<td>RD1</td>
<td>P73R/G292R</td>
<td>16</td>
<td>Yes</td>
<td>Thiamin-responsive</td>
</tr>
<tr>
<td>MGF-497</td>
<td>R230G/IVS4del[−3.2 kb::−15]</td>
<td>14</td>
<td>Yes</td>
<td>Thiamin-responsive</td>
</tr>
<tr>
<td>C.R.</td>
<td>S133stop (homozygous)</td>
<td>0</td>
<td>No</td>
<td>Classic</td>
</tr>
<tr>
<td>T.G.</td>
<td>S133stop (homozygous)</td>
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<td>No</td>
<td>Classic</td>
</tr>
<tr>
<td>S.G.</td>
<td>IVS4del[−3.2 kb::−15] (homozygous)</td>
<td>0</td>
<td>No</td>
<td>Classic</td>
</tr>
<tr>
<td>S.B.</td>
<td>IVS4del[−3.2 kb::−15]/IVS4del[−15::−4]</td>
<td>0</td>
<td>No</td>
<td>Classic</td>
</tr>
<tr>
<td>A.L.</td>
<td>Δ27E/I77T</td>
<td>0</td>
<td>No</td>
<td>Classic</td>
</tr>
<tr>
<td>DaRa</td>
<td>354 del 7 (homozygous)</td>
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<td>No</td>
<td>Classic</td>
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<td>W.J.</td>
<td>IVS10del[−9::+1] (homozygous)</td>
<td>ND²</td>
<td>No</td>
<td>Classic</td>
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<tr>
<td>C.E.</td>
<td>90delAT/IVS5del[−1::−1]</td>
<td>0</td>
<td>No</td>
<td>Classic</td>
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<td>IVS9–7A→G (homozygous)</td>
<td>1</td>
<td>No</td>
<td>Classic</td>
</tr>
</tbody>
</table>

1 The allele name conforms to the recommendations of the Ad Hoc Committee on Mutations Nomenclature for the journal Human Mutation.
2 Not determined.

FIGURE 6 Regulation of E1 activity by reversible phosphorylation/d Dephosphorylation. Phosphorylation of Ser-292 (major site) and Ser-302 (minor site) in the E1-α-subunit results in the inactivation of E1. Removal of the phosphoryl group from E1 reactivates E1. Inhibitors (−) and activators (+) for the kinase and the phosphatase are shown. CIC, a-chloroisocaproate; ArDP, aryldenepyruvate; NDP, nucleoside diphosphate; NTP, nucleoside triphosphate. Reproduced with permission from Chuang and Shih (6).
have been performed on four MSUD patients for other medical reasons (6,19). This procedure turned out to be effective in controlling plasma BCAA concentrations. There was a dramatic drop of plasma leucine concentrations in a classic MSUD patient to the nearly normal level after the liver transplantation while the patient was on a normal diet (Fig. 3A) (20). This brings up an interesting question in terms of interorgan relationships of BCAA catabolism. The prevailing concept has been that the skeletal muscle is the major site for the decarboxylation of BCAA due to its large mass and the presence of branched-chain amino transferase transaminase activity in the human muscle (21), as shown in controls (Fig. 3B, upper panel). The fact that the plasma leucine level returns to normal after the liver transplantation indicates that the transplanted liver alone is capable of degrading >90% of BCAA (Fig. 3B, lower panel). Other organs, including the skeletal muscle, are silent for BCAA decarboxylation due to genetic defects in the BCKDC.

**Thiamin supplementation for thiamin-responsive MSUD**

The third and final approach to MSUD involves thiamin (vitamin B-1) therapy for a subclass of patients with the thiamin-responsive phenotype; this, in fact, will be the topic of discussion for the remainder of this presentation. Thiamin supplements (10–1000 mg/d) to MSUD diets, irrespective of the clinical phenotype, have been a common practice adopted by pediatricians, since excess vitamin B-1 poses no harm and is excreted in the urine. The first thiamin-responsive patient WG-34, a French Canadian female infant, was reported in 1971 by Scriver and associates (10). She was admitted to Montreal Children’s Hospital with moderately elevated plasma BCAA concentrations. An oral supplement of thiamin hydrochloride at the dosage of 10 mg/d dramatically reduced her plasma BCAA concentrations to the normal levels without the restriction of the BCAA intake (Fig. 4). The withdrawal of the vitamin supplement caused a fast return of plasma BCAA concentrations to the prethiamin levels. This was followed by another sharp decline of BCAA concentrations upon reinstition of the thiamin supplement. More significantly, the MSUD phenotype was corrected completely without recourse to the dietary restriction of BCAA.

**The thiamin-responsive phenotype is linked to the presence of mutant E2 proteins**

The biochemical mechanism underlying thiamin-responsive MSUD has since been a subject of intense interests and controversies. To approach this problem, one has to understand the enzymology of the BCKDC multienzyme complex. The BCKDC is a macromolecular machine of four million daltons in size (6). It consists of three catalytic components, i.e., branched-chain α-ketoacid decarboxylase/dehydrogenase (E1) (EC 1.2.4.4.), dihydrolipoyl transacyslase (E2) transacyslase (EC 2.3.1.168.), and E3 (EC 1.8.1.4.), and two regulatory enzymes, the BCKD kinase and the BCKD phosphatase that regulate the activity of the complex by reversible phosphorylation. Each E2 subunit consists of three independently folded domains: the lipoic acid–bearing or lipoyl domain that carries a lipoylmetabolite prosthetic group for mediating the transfer of the acyl moiety, the subunit-binding domain, and the core or inner-core domain, with the three domains tethered by flexible linker regions. The core domains of E2 subunit form a 24-meric scaffold, which is decorated by multiple copies of E1 and E3 attached through the subunit-binding domain. We have determined the crystal structure of the E1 decarboxylase component of human BCKDC in collaboration with Wim Hol’s group (22). The structure shows a tetrahedral arrangement of the two α- and two β-subunits. The cofactor thiamin diphosphate (ThDP), a phosphorylated derivative of thiamin that mediates the decarboxylation of BCKA, binds to the two active sites formed between each of the two α- and β-subunits. Since ThDP is a cofactor of E1, it was speculated that the mutations in thiamin-responsive patients involved either the α- or the β-subunit. Therefore, it was a big surprise when we found that the E2 subunit in the cell extract from Scriver’s thiamin-responsive patient WG-34 was much reduced compared to the control samples (23) (Fig. 5). The levels of E1α- and E1β-subunits were normal in WG-34. This was confirmed by a report from Robert Harris’s laboratory, which showed that the cDNA sequence of the E1α unit of WG-34 was normal (24). We subsequently identified the two mutant E2 alleles in WG-34, one contained the F215C (Phe-215 to Cys) missense mutation (25) and the other harbored a 3.2-kb...
deletion in intron 4 of the E2 gene (26) (Table 1). The 3.2-kb intronic deletion resulted in a null E2 mRNA containing a 17-bp frameshift insertion. The F215C allele produced a functional but unstable E2 protein, which accounts for the relatively high 30–40% residual BCKDC activity in cultured fibroblasts. Studies of additional thiamin-responsive patients revealed that they invariably contained at least one missense E2 allele that produces a full-length mutant E2 protein. The latest are two Israeli thiamin-responsive patients who are homozygous for the H391R substitution in the E2 subunit (27). This study was carried out in collaboration with Hanna Mendel of Haifa, Israel. His-391 is the key catalytic residue serving as a base in the BCKA decarboxylation by E1 due to thiamin (27), and the H391R substitution is a hallmark of the Israeli thiamin-responsive patients that are homozygous for the 3.2-kb intronic deletion null allele. The other MSUD patients that carry either two null E2 alleles or mutations that render the E2 protein unstable, resulting in its absence in patient’s cells (A.L.), uniformly show the classic phenotype with inability to decarboxylate BCKA. Therefore, there appears to be a tight linkage between the thiamin-responsive phenotype and the presence of at least one allele producing the mutant E2 protein.

The biochemical model for thiamin-responsive MSUD

These findings pose a paradox in which thiamin supplements exert their effect on E1, which binds ThDP, but the MSUD mutations are on E2. To approach this problem, one has to be reminded that the E1 component catalyzes two half-reactions: the ThDP-mediated decarboxylation of BCKAs (step 1) and the reductive acylation of the lipoamide prosthetic group on the lipoyl domain of E2 (step 2). The sum of the sequential steps catalyzed by E1, E2, and E3 results in the overall reaction of the BCKDC.

![FIGURE 8](https://example.com/figure8.png)

**FIGURE 8** A biochemical model for thiamin-responsive MSUD. The residual normal E1 activity in thiamin-responsive patients is activated by both the ThDP-mediated dephosphorylation of E1 and the binding of E1 to the SBD of the mutant E2 harboring the H391R substitution. The thick curved arrow depicts the increased rate of BCKA decarboxylation by E1 due to thiamin supplements. Reproduced with permission from Chuang et al. (27).
The results support the thesis that thiamin supplements render the normal E1 component fully active by locking the decarboxylase in the dephosphorylated state.

The second and pivotal question is why the presence of mutant E2 protein is required for the thiamin response. In an in vitro experiment, activity of the normal E1 component is measured in the presence of a wild-type or a mutant E2 protein harboring the H391R mutation of the homozygous thiamin-responsive Israeli MSUD patient. The result (Fig. 7) shows that E1 activity is augmented by 4-fold over E1 alone. When E1 is incubated with the subunit-binding domain (SBD) or a lipoylated di-domain containing both the lipoylated lipoyl-bearing domain and SBD, E1 activity is also enhanced, although to a lesser degree than the lipoylated full-length E2 (27). The lipoylated lipoyl-bearing domain itself does not bind E1 and is therefore without effect on E1 activity. These in vitro data show that the binding of E1 to the SBD domain of E2 is required for robust decarboxylation of BCKA in thiamin-responsive patients. To date, missense mutations in the inner core domain, which produce full-length mutant E2, are present in all thiamin-responsive patients studied in this laboratory (27). Naturally occurring di-domains due to non-sense mutations are not stable and therefore are not present in cells from E2-deficient MSUD patients.

Based on the above data, we propose the following biochemical model for thiamin-responsive MSUD (27). In patients that are homozygous or compound heterozygous for E2 null mutations, the BCKD kinase is inactive, and E1 is therefore completely dephosphorylated. However, basal E1 activity is too low to clear BCKA in vivo; this is consistent with the classic phenotype in the E2 null patients. By contrast, when a full-length mutant E2 is present, for example, the one containing the H391R substitution in the inner-core domain, normal E1 binds to this mutant E2 protein and is phosphorylated by the BCKD kinase (Fig. 8). Thiamin supplements increase mitochondrial ThDP concentrations that inhibit the BCKD kinase, resulting in dephosphorylation and the activation of E1. In the presence of a full-length mutant E2 protein, E1 activity is further augmented by the binding of E1 to the SBD. These combined positive effects lead to the significant decarboxylation of BCKA and, therefore, the improved clinical outcome. Our data illustrate the intricate cross talks between different enzyme components of the BCKDC catalytic machine, which we have learned from genetic defects that produce the thiamin-responsive phenotype in MSUD.

LITERATURE CITED


