[Frontiers in Bioscience 3, d517-524, June 3, 1998]

HUMAN MUTATIONS AFFECTING BRANCHED CHAIN α-KETOACID DEHYDROGENASE

Dean J. Danner and Christopher B. Doering

Emory University School of Medicine, Department of Genetics, 1462 Clifton Road NE, Atlanta, GA 30322

Received 5/13/98 Accepted 5/24/98

TABLE OF CONTENTS

1. ABSTRACT

Maple syrup urine disease results from defective function of the branched chain α-ketoacid dehydrogenase complex [BCKD] within the matrix of the mitochondria. This disorder in humans is inherited as an autosomal recessive trait with an incidence of 1 in 150,000 live-births in the general population and 1/176 for the Mennonite population. Over 50 different causal mutations are known to exist scattered among the three genes unique to the catalytic function of the enzyme complex. The defect was first described in 1954 and much has been learned about the genes and proteins involved in this rare human disorder. The enzyme is present in all mammalian cells that contain mitochondria, and the activity of BCKD is regulated by phosphorylation through a complex-specific kinase. Expression of the kinase is regulated by metabolic and hormonal components. Naturally occurring mutations are used to define the molecular mechanisms of transcription, translation, protein import into mitochondria and the assembly of the component proteins into a functional complex. The long-term pathophysiology of BCKD dysfunction remains to be explained. What began as a focused interest in BCKD due to the associated disease, has broadened into a quest to understand the role of BCKD in regulation of leucine levels and in turn controlling protein metabolism and hormone release.

2. HISTORY OF MAPLE SYRUP URINE DISEASE

Maple syrup urine disease [MSUD] derives its name from the sweet odor imparted to the body fluids when an individual lacks the ability to metabolize the branched chain α–ketoacid [BCKA] derived from their respective precursor branched chain amino acids [BCAA] leucine, isoleucine, and valine. This disorder was first described by Menkes in a 1954 article recounting a family with several affected children (1). Biochemists and physicians worked together to define this "inborn error of metabolism." Historically, these errors of nature have provided investigators with a genetic tool to define unknown metabolic pathways and MSUD served this role for BCAA catabolism. Another important advancement in technology was occurring at this time, the preparation of human cells for cell culture enabling extensive analysis. Especially important were preparations of white blood cells that were amenable to enzyme analysis (2). During the 1960's investigators devised methods to maintain human cells in culture and these became the basis for diagnostic studies as well as tools for investigating the pathophysiology of various diseases (3). Using these tools, it was learned that MSUD resulted from defective function of the mitochondrial multienzyme complex, branched chain α-ketoacid dehydrogenase [BCKD] (4, 5). The enzyme defect was detectable in cells from the affected child, but parents exhibited no phenotype and often had apparently normal enzyme activity (6). This inheritance pattern suggested an autosomal recessive transmission, indicative of a single gene trait. The multiprotein nature of BCKD has been used to explain the wide variance in phenotype and BCKD activity found in affected individuals (5, 7, 8).

Individuals with impaired BCKD function are born apparently healthy but rapidly develop neurologic problems, vomiting, seizures and often die in infancy (1, 9-11). BCAAs and their respective ketoacids accumulate in body fluids, but the mechanisms for the resulting pathology remain to be explained. The presence of ketoacidosis provided an early diagnostic tool. Urine from infants suspected to have MSUD was mixed with 2,4-dinitrophenylhyrazine [DNPH], which forms yellow crystals if ketoacids are present. Analysis of these crystals confirmed them to be derived from the BCKAs. Around this time, the Guthrie test for phenylketonuria was established based on the bacterial auxotroph that required phenylalanine for growth. A similar bacterial auxotroph for leucine dependence was engineered. Newborn screening programs were then instituted for early identification of neonates at risk for MSUD based on elevated leucine in their blood (12). Once identified and confirmed by quantitation of plasma BCAAs, corrective intervention followed.

Treatment was to limit the dietary intake of the BCAAs (1, 9, 10, 13, 14). Since BCAAs are essential in humans, they must be supplied in quantities sufficient for growth but below levels that result in ketoacidosis. This treatment enables individuals to follow near normal growth and weight curves and their intellectual development is minimally impacted (7). Special formulas are now available through various drug suppliers, and BCAA content of natural foods have been determined making the management more effective and the food selection more palatable (15). These protein-modified diets [PMD] must be adjusted for each individual with MSUD by careful monitoring of plasma amino acids involving a nutritional expert and a proficient biochemical laboratory. In this way, ketoacidosis can be avoided except when induced by infections or other crisis inducing events. In these situations, correction can be effected without severe consequences (16, 17). Even in the extreme conditions of ketoacidosis that result in brain edema, rapid and aggressive treatment to reduce the plasma BCAAs to normal values reverses the trauma within a few days (18). In isolated cases, additional complications are described in older children including ophthalmic, CNS

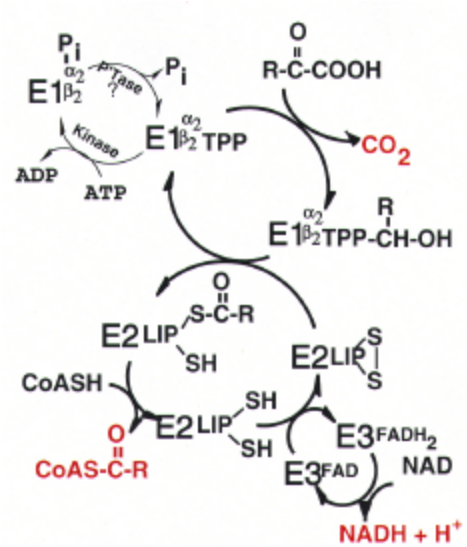

Figure 1. Reactions catalyzed by the enzymes of the branched chain α-ketoacid dehydrogenase complex.

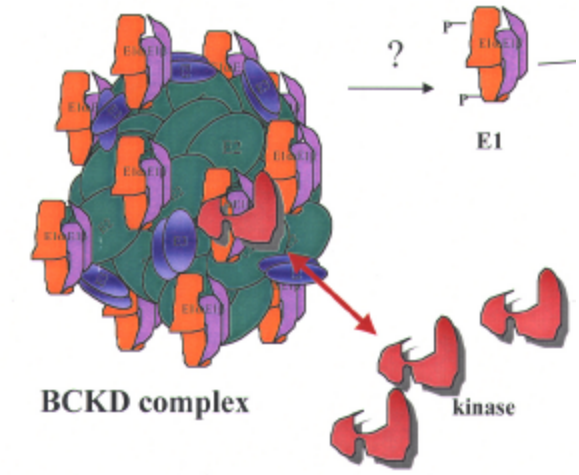

Figure 2. Diagramatic representation of the branched chain α-ketoacid dehydrogenase complex. The kinase can freely associate and dissociate. The loss of the E1 subunit after phosphorylation by the kinase followed by degradation is hypothesized.

pathologies, and skin and hair disorders (16, 19-22). Furthermore, in rare cases, even in the well-managed patient, crisis situations can lead to death (23, 24).

3. THE BCKD COMPLEX

Purification of the BCKD complex confirmed its multiprotein nature and supported the opinion that the complex resides on the matrix side of the mitochondrial inner membrane (25, 26). BCKD catalyzes the committed step in BCAA catabolism when it oxidatively decarboxylates the BCKAs. All three BCKAs are converted by BCKD with a relative preference of KIV>KIC≥KMV, the ketoacids of valine, leucine and isoleucine respectively (25, 26). Products of the reation are CO_2, the respective CoA thiol ester, and NADH with a 1:1:1 stoichiometry (figure 1) (25). Each acyl-CoA is further catabolized in a substrate-specific pathway to their ultimate fate (27).

Physically, the complex is imagined as being near 4 million daltons based on its proposed subunit association. This modeling is derived from electron micrographs of the isolated complex and not on *in vivo* analysis. Crystal structure is not yet available for the full complex nor for any of the component proteins. The hypothesized structure consists of 24 units of the branched chain dihydrolipoamide acyltransferase [E2], which form the core of the complex. Decorated around this core are 12 $\alpha_2\beta_2$ heterotetramers of the decarboxylase [E1] and six homodimers of the dihydrolipoamide dehydrogenase [E3], a flavoprotein also found as a component of the pyruvate and α-ketoglutarate dehydrogenase complexes and the glycine cleavage complex (28, 29). Identification of a substrate specific BCKD-kinase contributes additional weight to the complex although the amount of kinase found associated with BCKD varies as described below (30, 31). One report describes a BCKD-specific phosphatase in bovine tissue (32, 33) while substantiating reports of the phosphatase from other tissues or species have not appeared. A cartoon model for the complex is seen in figure 2.

BCKD is present in all mammalian cells that have mitochondria and one report describes this complex in *Saccharomyces cerevisiae* (34). The activity state of the complex varies with the tissue and is directly related to the expression of the kinase within that tissue (35-37). Changes in the activity state occur in response to diet, excercise, hormones and the concentration of the BCAA (36, 38-40). These metabolite induced changes alter the expression of the kinase as well as the expression of the other subunits (41-43). Therefore subunit association may also play a role in the amount of active complex present within a cell (44-49). If activity-state is determined only by a balance of phosphorylation-dephosphorylation, then, one might anticipate a constant presence and amount of all the components of the complex. This most certainly would include the kinase and phosphatase. As stated previously, the relative amounts of the component proteins as indicated by immunodetection vary with metabolic state, hormonal status, and tissue. Antibodies against the putative BCKD-phosphatase are not available so data on this subunit is lacking. It remains to be tested whether phosphorylation of the E1α subunit signals dissociation of the $\alpha_2\beta_2$ tetramer from the complex followed by degradation of the separated proteins. Reactivation could then be through incorporation of new, unphosphorylated E1 units to the complex. Kinase activity is greatly stimulated when bound to the lipoylated E2 subunit so it is unlikely that free E1 tetramers or E1α subunits are phosphorylated (50). The *in vivo* organization of BCKD remains to be elucidated. The minimal number of subunits needed to provide full or partial activity may not reflect the hypothetical arrangement proposed from studies of the isolated, purified complex.

4. MOLECULAR GENETICS OF BCKD

In 1985, the first cDNA clone for a component of the BCKD complex (human E2) was isolated and defined (51). At present cDNA and genomic clones are described for all the catalytic components of BCKD from a variety of species (52-67). Clones for the regulating BCKD-kinase are also described, again from a variety of species (37, 68), and references therein). As yet, no clones for the putative BCKD-phosphatase have been reported, further adding to the confusion over its existence.

Table 1. Human BCKD genes and their products

Gene	chromosome	mRNA(nt)	preprotein (#AA)**	mature (#AA)**	Reference
E1α	19	1743	50,455 (445)	45,513 (400)	56
E1β	6	1349	42,959 (392)	37,830 (342)	59
E2	1	3545	53,487 (482)	46,369 (421)	63
E3	7	2064	54,214 (509)	50,216 (474)	66
kinase	16	1848	46,408 (412)	43,402 (392)	GB AF026548

*nt=nucleotides, **#AA= number of amino acids in the protein

As seen in table 1, all the genes encoding BCKD are located in the nucleus on autosomal chromosomes. Products of the mitochondrial genome do not appear to play a role in the production, assembly, or function of this complex (69). Nuclear encoded mitochondrial proteins are made on cytosolic polysomes and targeted to the mitochondria by amino terminal peptides [MTS]. After import the MTS is proteolytically removed and a protein is further processed into its functional form by the aid of mitochondrial chaperones (70, 71). BCKD components follow this pathway (72). Other alterations may be needed to fully activate a protein such as the covalent addition of lipoic acid to lysine 44 of the E2 component. It is not yet known when after entry into the matrix this event occurs. Further, thiamin pyrophosphate [TPP] needs to be associated with the E1 component for it to be catalytically active.

Subunit association and/or their availability within the matrix are likely to play a role in the amount of complex able to catalyze the oxidative decarboxylation reaction. Available data suggest that the import of E1β can alter the amount of E1 subunits within a mitochondrion (46, 72, 73). Likewise, insulin appears to influence the production of E1α which could help to determine the amount of E1 present at any one time and glucocorticoids have been shown to alter E2 and E1β concentration (74). Limiting E1 tetramer formation and/or amount of E1 present would provide an additional means of regulating BCKD activity state along with phosphorylation (47).

Since MSUD can result from mutations in any of three genes (E1α or E1β, and E2), it is difficult to know which gene is altered simply from the clinical presentation of elevated plasma BCAAs. Even enzyme activity measurements will not identify the affected subunit. Western blots determine the antigenic presence of the proteins and an absence of E2 indicates mutations in that gene (75). Mutations in either E1α orE1β can decrease the presence of both since these two proteins must assume their tetrameric structure to stabilize each against degradation (76, 77). Under these circumstances, both genes must be analyzed. The antigenic presence of all protein components suggests that a missense mutation is present in one of the alleles and results in a pathogenic amino acid substitution. This mutation could affect the catalytic properties or assembly of the complex. Given this scenario, nucleic acid sequence analysis of all three cDNAs is required to uncover the nucleotide substitution and inferred amino acid change. Since all cells with mitochondria express BCKD, any cell of this type can be used; most often transformed lymphocytes. Analysis of DNA from the parents confirms inheritance and identifies the parental origin of each mutant allele (75, 78). Only one mutation is found with an increased frequency, the 1325T→A transversion that results in a Y393N substitution in E1α. This is the founder mutation responsible for the high incidence of MSUD (1/176) in the Mennonite population (76, 79, 80). It has been suggested that this mutation may also be found with a high frequency in the general population (81). Outside of the Mennonite community, no other population has a single mutant gene frequency with an incidence high enough to merit newborn screening by DNA analysis. In most families, the proband is a compound heterozygote with each parent contributing a different mutant allele at one locus. Figure 3 depicts the currently known mutations.

5. LESSONS LEARNED FROM FUNCTIONAL ANALYSIS OF MSUD MUTATIONS

Equating nucleotide changes with a dysfunctional gene product provides a challenge for any inherited disorder. The multiprotein nature of BCKD adds another dimension to this challenge. An ideal test situation would be to have a host cell that does not endogenously express the genes for BCKD. However, since the proteins of the complex work in concert, the cells would need to be supplied with wild-type copies of the subunits not involved in the mutation. Human cells lacking only one of the components could be used, but few of the characterized cell lines are truly null at any of the gene loci. In most cases, there is a nonfunctional protein produced or at least an mRNA from the gene of interest. The presence of a non-functional mutant gene product could exert a dominant negative effect, further complicating the analysis, although this state has never been described. Second, human fibroblasts have limited life span in culture and lymphoblasts are not easily transfected minimizing the effective use of either cell type. Since all mammalian cells with mitochondria express BCKD, a BCKD-null background cell line is not available from any species.

It was thought that the lower eukaryote, *Saccharomyces cerevisaeia* might serve as a recipient cell with a null background. However, Sinclair *et al.* reported the isolation of a BCKD complex from this organism (34). The proteins and genes providing this activity in yeast remain to be discovered. Antibodies to the mammalian proteins do not recognize any proteins in yeast. Searches of the published yeast genome database using sequences for either the amino acids or nucleotides of the mammalian or prokaryote counterparts have failed to identify yeast homologs for the BCKD genes. Still the possibility exists that yeast can be used for testing functional consequences of amino acid substitutions in the BCKD subunits. An E3-null strain of yeast was constructed and used to test human mutations in this gene (82).

Some success with a bacterial expression system for evaluating the interaction of the E1 subunits has been reported (83). Using this system, investigators have identified several amino acid substitutions within the E1α subunit that reduce the interaction of the α and β subunits.

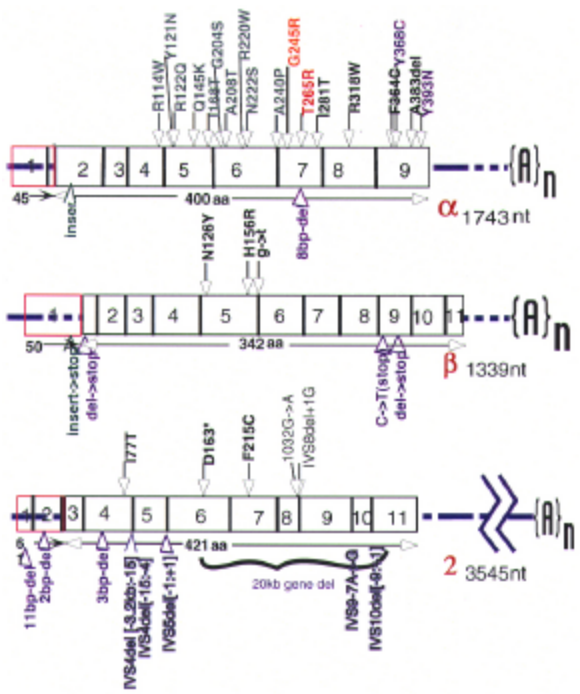

Figure 3. Causative mutations described in the genes for the human branched chain α-ketoacid dehydrogenase complex.

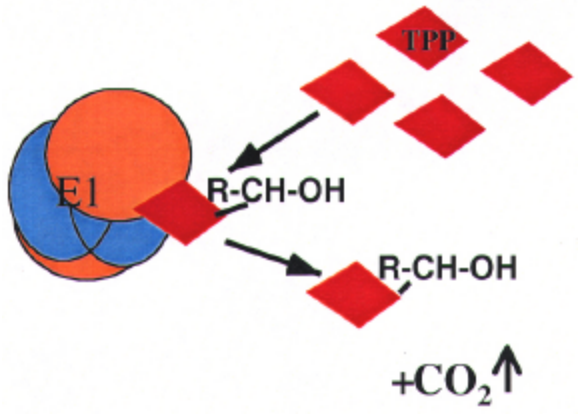

Figure 4. Potential mechanism for the decarboxylation of the branched chain α-ketoacids in the thiamin responsive individuals with maple syrup urine disease who lack the acyltransferase (E2) component of the complex. When excess thiamin pyrophosphate (TPP) is available the TPP-acyl intermediate is released form the enzyme after decarboxylation. New TPP replaces the lost cofactor and the enzyme can again function.

Loss of this interaction leads to the apparent proteolytic loss of both proteins. One of these residues is the tyrosine 393 asparagine substitution in the E1α subunit. This is the identified common mutation within the Mennonite population. Other amino acid substitutions in the C-terminal end of the E1α protein have also been implicated in the binding of E1β. Amino acid substitutions in the N-terminal region of the protein do not appear to affect the interaction of these two proteins. These results suggest that only the C-terminal end of E1α is important for binding with the β component.

As yet, critical residues within the E1β subunit for binding to the α subunit have not been identified. The number of mutations identified in E1β are far less than those known for E1α. Functional analysis of two E1β mutations have been reported (78). One mutation results in a substitution of tyrosine for asparagine126 and the second truncates the protein at arginine274, shortening the subunit by 68 amino acids. The truncated protein can be made *in vitro* and retains its antigenic properties while being imported and processed by the mitochondria. Western blots of mitochondrial proteins from a cell line harboring this mutation do not contain this truncated antigenic protein. The implication is that the C-terminal end of E1β is involved in the interaction with E1α. Confirmation of this hypothesis remains to be accomplished.

The other mutant allele product with a single amino acid substitution does interact with E1α to form the BCKD complex, yet catalytic activity is absent (78). Based on computer alignment of the $\alpha_2\beta_2$ structure with the crystal structure of transketolase, it has be speculated that this amino acid substitution interferes with TPP binding. Transketolase also utilizes TPP as a cofactor in a chemically similar reaction as performed by the BCKD –E1 component.

From studies unrelated to mutant allele analysis we showed that over-expression of the E1β subunit can increase the total amount of BCKD complex within the mitochondrion. Over-expression of neither of the other two subunits affected the amount of complex. Thus, E1β may be a regulating subunit for the amount of complex found within a mitochondrion. Regions of E1β important for interacting with E2 have been described as well (84).

Mutations in the E2 subunit do not seem to affect the amount of E1 formation. Indeed E2 cells show full expression of the E1subunits by western blot analysis (85). What has emerged is that the so-called "thiamin-responsive" MSUD patient is strongly associated with the E2 phenotype (86). These findings suggest that E1, in the presence of pharmacologic excess of TPP, can decarboxylate sufficient amounts of the potentially toxic ketoacids to minimize the dire consequences. A potential mechanism for this is shown in figure 4. Speculation on mechanisms for this thiamin-responsive condition is described in the literature without firm conclusions or understanding (87-89).

6. PERSPECTIVES/ FUTURE GOALS

At the molecular genetic and biochemical level there is much to learn about the regulated expression of all the BCKD gene products. This could be by gene transcription, mRNA translation, protein import into mitochondria, and/or subunit assembly and turnover. It is likely that several of these mechanisms are in effect, different tissues may respond to a host of stimuli and the mechanisms could vary with tissue and stimuli Crystallographic analysis of the individual proteins and potentially the entire complex would help in understanding the functional changes resulting from amino acid substitutions within a protein.

The ultimate goal is to provide better treatment and even a cure for MSUD. The PMDs currently used have greatly improved life expectancies, and minimized consequences of crisis induced ketoacidosis. Yet, we should be able to further improve the quality of life for MSUD patients with additional discoveries leading to newer therapies. These diets have resulted in a new dilemma, maternal MSUD. Females with MSUD are

fertile and can reproduce. Being affected with MSUD, the mother is intolerant of high protein diets, yet, the fetus requires large amounts of protein for proper growth. A successful pregnancy for this situation has been reported (90), but the newborn was small for gestational age. Development of animal models for MSUD could shed light on additional means of therapy under this delicate situation. Understanding the protein structures would provide information on the tertiary structure and critical residues within the complex. Drugs could then be designed to bind mutant proteins altering their conformation to a catalytically more favorable shape. Studies on the regulation of gene expression within different tissues and the means these tissues use to carry out this regulation will help to elucidate the important tissues in BCAA metabolism. These studies will also indicate the essential tissues for targeted gene replacement therapy. Several reports have indicated the feasibility of gene therapy on inborn errors of metabolism (91-93). Recently, it was shown that a liver transplant could eliminate the need for a PMD in an MSUD proband (94). This is not to suggest that organ transplantion be used as a primary means of treatment but does indicate the liver as a primary site for gene therapy. A great deal has been learned since 1954 with even greater accomplishments still on the horizon.

7. ACKNOWLEDGEMENT

This work was supported in part by research grants from the Emory University Research Committee and the NIH DK38320 to DJD. Lymphocyte transformations were done in the Clinical Research Center, Emory University supported by NIH MO1-RR00039. CBD was supported in part by an NIH training grant 5T32GM08490.

8. REFERENCES

1. Menkes, J.H., P.L. Hurst, and J.M. Craig, A new syndrome: Progressive familial infantile cerebral dysfunction associated with an unusual urinary substance. *Pediatrics*, 14:462-467 (1954)

2. Dancis, J., J. Hutzler, and M. Levitz, Metabolism of the white blood cells in maple syrup urine disease. *Biochim. Biophys. Acta*, 43:342-343 (1960)

3. Priest, J.H., *Human cell culture in diagnosis of disease*. American lectures in living chemistry, ed. I.N. Kugelmass. Springfield, IL: Charles C. Thomas. 295 (1971)

4. Dancis, J., J. Hutzler, and R.P. Cox, Enzyme defect in skin fibroblasts in intermittent branched chain ketonuria and in maple syrup urine disease. *Biochem. Med.*, 2:407-411 (1969)

5. Dancis, J., J. Hutzler, S.E. Snyderman, and R.P. Cox, Enzyme activity in classical and variant forms of maple syrup urine disease. *J. Pediatr.*, 81:312-320 (1972)

6. Wendel, U., W. Wöhler, H.W. Goedde, U. Langenbeck, E. Passarge, and H.W. Rüdiger, Rapid diagnosis of maple syrup urine disease (Branched chain ketoaciduria) by micro-enzyme assay in leukocytes and fibroblasts. *Clin. Chim. Acta*, 45:433-440 (1973)

7. Peinemann, F. and D.J. Danner, Maple syrup urine disease 1954 to 1993. *J. Inherit. Metab. Dis.*, 17:3-15 (1994)

8. Chuang, D.T. and V.E. Shih, Disorders of branched chain amino acid and keto acid metabolism, in *The metabolic basis of inherited disease*, C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle, Editors., McGraw-Hill: New York. 1239-1277 (1995)

9. Menkes, J.H., Maple syrup disease. Isolation and identification of organic acids in the urine. *Pediatrics,* 23:348-353 (1959)

10. Dancis, J., M. Levitz, S. Miller, and R.G. Westall, Maple syrup urine disease. *Brit. Med .J.* 1:91-93 (1959)

11. Mikati, M.A., G.E. Dudin, V.M. DerKaloustian, P.F. Benson, and A.H. Fensom, Maple syrup urine disease with increased intracranial pressure. *Am. J. Dis. Child.*, 136:642-643 (1982)

12. Naylor, E.W. and R. Guthrie, Newborn screening for maple syrup urine disease. *Pediatr.*, 61:262-266 (1978)

13., Snyderman, S.E., P.M. Norton, E. Roitman, and L.E. Holt, Maple syrup urine disease, with particular reference to dietotherapy. *Pediatr.*, 34:454-472 (1964)

14. Snyderman, S.E., The therapy of maple syrup urine disease. *Am. J. Dis. Child.*, 113:68-73 (1967)

15. Elsas, L.J. and P.E. Acosta, *Nutritional management of inherited metabolic disorders*. 7 ed. Modern nutrition in health and disease, ed. M.E. Shils and V. Young., Philadelphia: Lea and Febiger. Chapter 66 (1987)

16. Treacy, E., C.L. Clow, T.R. Reade, D. Chitayat, O.A. Mamer, and C.R. Scriver, Maple syrup urine disease: Interrelationships between branched chain amino-, oxo-, and hydroxyacids; implications for treatmnet; asscoiation with CNS dysmyelination. *J. Inherit. Metab. Dis.*,15:121-135 (1992)

17. Felber, S.R., W. Sperl, A. Chemelli, C. Murr, and U. Wendel, Maple syrup urine disease: metabolic decompensation monitored by proton magnetic resonance imaging and spectroscopy. *Ann. Neurol.*, 33:396-401 (1993)

18. Brismar, J., A. Aqeel, G. Brismar, R. Coates, G. Gascon, and P. Ozand, Maple syrup urine disease: Findings on CT and MR scans of the brain in 10 infants. *AJNR Am. J. Neororadiol.*, 11:1219-1228 (1990)

19. Burke, J.P., M. O'Keefe, R. Bowell, and E.R. Naughten, Ophthalmic findings in maple syrup urine disease. *Metab. Pediatr. Syst. Ophthalmol.*, 14:12-15 (1991)

20. Jones, L.N., D.J. Peet, D.M. Danks, A.P. Negri, and D.E. Rivett, Hairs from patients with maple syrup urine disease show a structural defect in the fiber cuticle. *J. Invest. Dermatol.*, 106:461-464 (1996)

21.Koch, S.E., S. Packman, T.K. Koch, and M.L. Williams, Dermatitis in treated maple syrup urine disease. *J. Am. Acad. Dermatol.*, 28:289-292 (1993)

22.Koch, S.E., T.K. Kock, and M.L. Williams, A dermatitis secondary to amino-acid deficiency in treated maple syrup urine disease. *Arch. Pediatr. Adolesc. Med.*, 148:993-994 (1994)

23. Berry, G.T., Branched-chain amino acid-free parenteral nutrition in the treatment of acute metabolic decompensation in patients with maple syrup urine disease. *N. Eng. J. Med.*, 324:175-179 (1991)

24. Riviello, J.J., I. Rezvani, A.M. DiGeorge, and C.M. Foley, Cerebral edema causing death in children with maple syrup urine disease. *J. Pediatr.*, 119:42-45 (1991)

25. Danner, D.J., S.K. Lemmon, J.C. Besharse, and L.J. Elsas, Purification and characterization of branched chain α-ketoacid dehydrogenase from bovine liver mitochondria. *J. Biol. Chem.*, 254:5522-5526 (1979)

26. Pettit, F.H., S.J. Yeaman, and L.J. Reed, Purification and characterization of branched chain α-ketoacid dehydrogenase complex of bovine kidney. *Proc. Natl. Acad . Sci.USA*, 75:4881-4886 (1978)

27. Danner, D.J. and L.J. Elsas, Disorders of branched chain amino acid and keto acid metabolism, in *The Metabolic Basis of Inherited Disease*, C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle, Editors., McGraw-Hill Book Company: New York. 671-692 (1989)

28. Reed, L.J. and M.L. Hackert, Structure-function relationships in dihydrolipoamide acyltransferases. *J .Biol. Chem.*, 265:8971-8974 (1990)

29. Yeaman, S.J., The 2-oxo acid dehydrogenase complexes: recent advances. *Biochem. J.*, 257:625-632 (1989)

30. Odessey, R., Direct evidence for the inactivation of branched chain oxo-acid dehydrogenase by enzyme phosphorylation. *FEBS Lett.*, 121:306-308 (1980)

31. Cook, K.G., R. Lawson, and S.J. Yeaman, Multi-site phosphorylation of bovine kidney branched chain 2-oxoacid dehydrogenase complex. *FEBS Lett.*, 157:59-62 (1983)

32. Damuni, Z., M.L. Merryfield, J.S. Humphreys, and L.J. Reed, Purification and properties of branched-chain α-ketoacid dehyrogenase phosphatase from bovine kidney. *Proc. Natl .Acad. Sci.USA*, 81:4335-4338 (1984)

33. Damuni, Z. and L.J. Reed, Purification and properties of the catalytic subunit of the branched-chain α-ketoacid dehydrogenase phosphatase from bovine kidney mitochondria. *J .Biol .Chem.*, 262:5129-5132 (1987)

34. Sinclair, D.A., I.W. Dawes, and J.R. Dickinson, Purification and characterization of the branched-chain α-ketoacid dehydrogenase complex from *Saccharomyces cerevisiae. Biochem. Mol. Biol. Internatl.*, 31:911-922 (1993)

35. Wagenmakers, A.J.M., J.T.G. Schepens, J.A.M. Veldhuizen, and J.H. Veerkamp, The activity state of the branched-chain 2-oxoacid dehydrogenase complex in rat tissues. *Biochem. J.*, 220:273-281 (1984)

36. Popov, K.M., Dietary control and tissue specific expression of branched-chain α-ketoacid dehydrogenase kinase. *Arch. Biochem. Biophys.*, 316:148-154 (1995)

37. Huang, Y.-S. and D.T. Chuang, Structural organization of the rat branched -chain 2-oxo acid dehydrogenase kinase gene and partial characterization of the promoter-regulatory region. *Biochem. J.*, 313:603-609 (1996)

38. Block, K.P., R.P. Aftring, and M.G. Buse, Regulation of rat liver branched-chain α-ketoacid dehydrogenase activity by meal frequency and dietary protein. *J. Nutr.*, 120:793-799 (1990)

39. Kasperek, G.J. and R.D. Snider, Effect of exercise intensity and starvation on activiation of branched-chain keto acid dehydrogenase by exercise. *Am. J. Physiol.*, 252:E33-E37 (1987)

40. Kobayashi, R., Gender difference in regulation of branched-chain amino acid catabolism. *Biochem. J.*, 327:449-453 (1997)

41. Frick, G.P. and H.M. Goodman, Insulin regulation of the activity and phosphorylation of branched-chain 2-oxo acid dehydrogenase in adipose tissue. *Biochem. J.*, 258:229-235 (1989)

42. England, B.K., M. Grewal, J.L. Bailey, and S.R. Price, Acidosis and glucocorticoids induce branched chain amino acid catabolism. *Miner. Electrolyte Metab.*, 22:69-71 (1996)

43. Lombardo, Y.B., M. Thamotharan, S.Z. Bawani, H.S. Paul, and S.A. Adibi, Post-translational alterations in protein masses of hepatic branched chain keto acid dehydrogenase and its associated kinase in diabetes. *Proc. Assoc. Am. Physicians*, 110:40-49 (1998)

44. Shimomura, Y., H. Fujii, M. Suzuki, T. Murakami, N. Fujitsuka, and N. Nakai, Branched-chain α-ketoacid dehydrogenase complex in rat skeletal muscle: regulation of the activity and gene expression by nutrition and physical exercise. *J. Nutr.*, 125:1762S-1765S (1995)

45. Miller, R.H., R.S. Eisenstein, and A.E. Harper, Effects of dietary protein intake on branched-chain keto acid dehydrogenase activity of the rat. Immunochemical analysis of the enzyme complex. *J .Biol .Chem.*, 263:3454-3461 (1988)

46. McConnell, B.B., *Formation of the branched chain* a*-ketoacid dehydrogenase complex and characterization of the E1*b *subunit*, ., Dissertation, Emory University. (1996)

47. Harris, R.A., Studies on the regulation of the mitochondrial α-ketoacid dehydrogenase complexes and their kinases. *Advan. Enzyme Regul.*, 17:271-293 (1997)

48. Fujii, H., Regulation by physical training of enzyme activity and gene expression of branched-chain 2-oxo acid dehydrogenase complex in rat skeletal muscle. *Biochim. Biophys. Acta*, 1243:277-281 (1995)

49. Zhao, Y., Effect of dietary protein on the liver content and subunit composition of the branched-chain α-ketoacid dehydrogenase complex. *Arch. Biochem. Biophys.*, 308:446-453 (1994)

50. Davie, J.R., R.M. Wynn, M. Meng, Y.-S. Huang, G. Aalund, D.T. Chuang, and K.S. Lau, Expression and characterization of branched-chain α-ketoacid dehydrogenase kinase from the rat. Is it a histidine-protein kinase? *J. Biol. Chem.*, 270:19861-19867 (1995)

51. Litwer, S. and D.J. Danner, Identification of a cDNA clone in λgt11 for the transacylase component of branched chain ketoacid dehydrogenase. *Biochem.Biophys. Res. Comm*, 131:961-967 (1985)

52. Hu, C.-W.C., K.S. Lau, T.A. Griffin, J.L. Chuang, C.W. Fisher, R.P. Cox, and D.T. Chuang, Isolation and sequencing of a cDNA encoding the decarboxylase (E1) a precursor of bovine branched chain α-keto acid dehydrogenase complex. Expression of E1α mRNA and subunit in maple syrup urine diesease and 3T3-L1 cells. *J. Biol. Chem.*, 263:9007-9014 (1988)

53. Zhang, B., M.J. Kuntz, G.W. Goodwin, R.A. Harris, and D.W. Crabb, Molecular cloning of a cDNA for the E1α subunit of rat liver branched chain α-ketoacid dehydrogenase. *J. Biol. Chem.*, 262:15220-15224 (1987)

54. Zhang, B., D.W. Crabb, and R.A. Harris, Nucleotide and deduced amino acid sequence of the E1α subunit of human liver branced-chain α-ketoacid dehydrogenase. *Gene*, 69:159-164 (1988)

55. Dariush, N., C.W. Fisher, R.P. Cox, and D.T. Chuang, Structure of the gene encoding the entire mature E1α subunit of human branched chain α-keto acid dehydrogenase complex. *FEBS Lett.*, 291:376-377 (1991)

56. Chuang, J.L., R.P. Cox, and D.T. Chuang, Characterization of the promoter-regulatory region and structural organization of E1α (BCKDHA) of human branched-chain α-keto acid dehydrogenase complex. *J.Biol.Chem.*, 268:8309-8316 (1993)

57. Nobukuni, Y., H. Mitsubuchi, F. Endo, J. Asaka, R. Oyama, K. Titani, and I. Matsuda, Isolation and characterization of a complementary DNA clone coding for the E1β subunit of the bovine branched-chain α-ketoacid dehydrogenase complex: Complete amino acid sequence of the precursor protein and its proteolytic processing. *Biochemistry*, 29:1154-1160 (1990)

58. Chinsky, J.M. and P.A. Costeas, Molecular cloning and analysis of the expression of the E1β subunit of branched chain α-ketoacid dehydrogenase in mice. *Biochim. Biophys. Acta*, 1216:499-503 (1993)

59. Mitsubuchi, H., Y. Nobukuni, F. Endo, and I. Matsuda, Structural organization and chromosomal localization of the gene for the E1β subunit of human branched chain α-keto acid dehydrogenase. *J. Biol. Chem.*, 266:14686-14691 (1991)

60. Zhao, Y., M.J. Kuntz, R.A. Harris, and D.W. Crabb, Molecular cloning of the E1β subunit of the rat branched chain α-ketoacid dehydrogenase. *Biochim. Biophys. Acta*, 1132:207-210 (1992)

61. Chuang, J.L., R.P. Cox, and D.T. Chuang, Molecular cloning of the mature E1b subunit of human branched-chain α-ketoacid dehydrogenase complex. *FEBS Lett.*, 262:305-309 (1990)

62. Danner, D.J., S. Litwer, W.J. Herring, and J. Pruckler, Construction and nucleotide sequence of a cDNA encoding the full-length preprotein for human branched chain acyltransferase. *J. Biol. Chem.*, 264:7742-7746 (1989)

63. Lau, K.S., W.J. Herring, J.L. Chuang, M. McKean, D.J. Danner, R.P. Cox, and D.T. Chuang, Structure of the gene encoding dihydrolipoyl transacylase (E2) component of human branched chain α-keto acid dehydrogenase complex and characterization of an E2 pseudogene. *J. Biol. Chem.*, 267:24090-24096 (1992)

64. Lau, K.S., J.L. Chuang, W.J. Herring, D.J. Danner, R.P. Cox, and D.T. Chuang, The complete cDNA sequence for dihydrolipoyl transacylase (E2) of human branched chain α-keto acid dehydrogenase complex. *Biochim. Biophys. Acta*, 1132:319-321 (1992)

65. Costeas, P.A., L.A. Tonelli, and J.M. Chinsky, Molecular cloning of the murine branched chain α-ketoacid dehydrogenase E2 subunit: presence of 3' B1 repeat elements. *Biochim. Biophys. Acta*, 1305:25-28 (1996)

66. Pons, G., Cloning and cDNA sequence of the dihydrolipoamide dehydrogenase component of human alpha-ketoacid dehydrogenase complexes. *Proc. Natl. Acad. Sci.USA*, 85:1422-1426 (1988)

67. Feigenbaum, A.S. and B.H. Robinson, The structure of the human dihydrolipoamide dehydrogenase gene (DLD) and its upstream elements. *Genomics*, 17:376-381 (1993)

68. Doering, C.B., C. Coursey, W.E. Spangler, and D.J. Danner, Murine branched chain α-ketoacid dehydrogenase kinase: cDNA cloning, tissue distribution, and temporal expression during embryonic development. *Gene* 212:213-219 (1998)

69. Shoffner, J.M. and D.C. Wallace, Oxidative Phosphorylation Diseases, in *The metabolic basis of inherited disease*, C.R. Scriver, A.L. Beaudet, W.S. Sly, D Valle., Editors., McGraw-Hill, Inc.: New York 1535-1609 (1995)

70. Schatz, G., The protein import system of mitochondria. *J. Biol. Chem.*, 271:31763-31766 (1996)

71. Schatz, G. and B. Dobberstein, Common principles of protein translocation across membranes. *Science*, 271:1519-1526 (1996)

72. Sitler, T.L., M.C. McKean, F. Peinemann, E. Jackson, and D.J. Danner, Import rate of the E1β subunit of human branched chain α-ketoacid dehydrogenase is a limiting factor in the amount of complex formed in the mitochondria. *Biochim. Biophys. Acta.*, 1998 (In Press).

73. McConnell, B.B., M.C. McKean, and D.J. Danner, Influence of subunit transcript and protein levels on formation of a mitochondrial multienzyme complex. *J. Cell. Biochem.*, 61:118-126 (1996)

74. Costeas, P.A. and J.M. Chinsky, Effects of insulin on the regulation of branched-chain α-ketoacid dehydorgenase E1α subunit gene expression. *Biochem. J.*, 318:85-92 (1996)

75. Herring, W.J., M. McKean, N. Dracopoli, and D.J. Danner, Branched chain acyltransferase absence due to an ALU-based genomic deletion allele and an exon skipping allele in a compound heterozygote proband expressing maple syrup urine disease. *Biochim. Biophys. Acta*, 1138:236-242 (1992)

76. Fisher, C.R., J.L. Chuang, R.P. Cox, C.W. Fisher, R.A. Star, and D.T. Chuang, Maple Syrup Urine Disease in Mennonites Evidence that the Y393N mutatiion in E1α impedes asssembly of the E1 component of branched-chain α-ketoacid dehydrogenase complex. *J. Clin. Invest.*, 88:1034-1037 (1991)

77. Chuang, J.L., C.R. Fisher, R.P. Cox, and D.T. Chuang, Molecular basis of maple syrup urine disease: Novel mutations at the E1α locus that impair E1($\alpha_2\beta_2$) assembly of decrease steady-state E1α mRNA levels of brancehd chain α-ketoacid dehydrogenase complex. *Am. J. Hum. Genet.*, 55:297-304 (1994)

78. McConnell, B.B., B. Burkholder, and D.J. Danner, Two new mutations in the human E1β subunit of branched chain α-ketoacid dehydrogenase associated with maple syrup urine disease. *Biochim. Biophys. Acta*, 1361:263-271 (1997)

79. Matsuda, I., Y. Nobukuni, H. Mitsubuchi, Y. Indo, F. Endo, J. Asaka, and A. Harada, A T-to A substitution in the E1α subunit gene of the branched chain α-ketoacid dehydrogenase complex in two cell lines derived from Mennonite maple syrup urine disease patients. *Biochem. Biophys. Res. Comm.*, 172:646-651 (1990)

80. Mitsubuchi, H., Gene analysis of Mennonite maple syrup urine disease kindred using primer-specified restriction map modification. *J. Inher. Metab. Dis.*, 15:181-187 (1992)

81. Phillips, C.L., J.A. Dyer, M.M. McLain, J.F. Grasela, and R.E. Hillman, High frequency of the Y393N mutation in the E1α subunit of the branched chain α-ketoacid dehydrogenase complex in non-Mennonnite maple syrup urine disease patients. *Am. J. Hum. Genet.*, 61:A259 (1997)

82. Lanterman, M.M., *Functional analysis of mutations in human dihyrolipoamide dehydrogenase in a yeast model system*, Dissertation, Emory University, (1995)

83. Davie, J.R., R.M. Wynn, R.P. Cox, and D.T. Chuang, Expression and assembly of a functional E1 component ($\alpha_2\beta_2$) of mammalian branched chain α-ketoacid dehydrogenase complex in *Escherichia coli. J. Biol. Chem.*, 267:16601-16606 (1992)

84. Wynn, R.M., J.L. Chuang, J.R. Davie, C.W. Fisher, M.A. Hale, R.P. Cox, and D.T. Chuang, Cloning and expression in Escherichia coli of mature E1β subunit of bovine mitochondrial branched-chain α-keto acid dehydrogenase complex. Mapping of the E1β-binding region on E2. *J. Biol. Chem.*, 267:1881-1887 (1992)

85. Danner, D.J., N. Armstrong, S.C. Heffelfinger, E.T. Sewell, J.H. Priest, and L.J. Elsas, Absence of branched chain acyl-transferase as a cause of maple syrup urine disease. *J. Clin. Invest.*, 75:858-860 (1985)

86. Ellerine, N.P., W.J. Herring, L.J. Elsas, M.C. McKean, P.D. Klein, and D.J. Danner, Thiamin-responsive maple syrup urine disease in a patient antigenically missing dihydrolipoamide acyltransferase. *Biochem. Med. Metab. Biol.*, 49:363-374 (1993)

87. Chuang, D.T., L.S. Ku, and R.P. Cox, Thiamin-responsive maple-syrup-urine disease: Decreased affinity of the mutant branched-chain α-keto acid dehydrogenase for α-ketoisovalerate and thiamin pyrophosphate. *Proc. Natl. Acad. Sci. USA*, 79:3300-3304 (1982)

88. Duran, M. and S.K. Wadman, Thaimine-responsive inborn errors of metabolism. *J. Inherit. Metab. Dis.*, 8:70-75 (1985)

89. Fernhoff, P.M., Thiamine responsive maple syrup urine disease. *Pediat. Res.*, 19:1011-1016 (1985)

90. Van Calcar, S.C., C.O. Harding, S.R. Davidson, L.A. Barness, and J.A. Wolff, Case reports of successful pregnancy in women with maple syrup urine disease and propionic acidemia. *Am. J. Med. Genet.*, 44:641-646 (1992)

91. Litwer, S., W.J. Herring, and D.J. Danner, Reversion of maple syrup urine disease phenotype of impaired branched chain α-ketoacid dehydrogenase complex activity in fibroblasts from an affected child. *J. Biol. Chem.*, 264:14597-14600 (1989)

92. Koyata, H., R.P. Cox, and D.T. Chuang, Stable correction of maple syrup urine disease in cells from a Mennonite patient by retroviral-mediated gene transfer. *Biochem. J.*, 295:635-639 (1993)

93. Mueller, G.M., L.R. McKenzie, G.E. Homanics, S.C. Watkins, P.D. Robbins, and H.S. Paul, Complementation of defective leucine decarboxylation in fibroblasts from a maple syrup urine disease patient by retrovirus-mediated gene transfer. *Gene Ther.*, 2:461-468 (1995)

94. Kaplan, P., A. Mazur, R. Smith, K. Olthoff, E. Maller, M. Palmieri, and G.T. Berry, Liver transplantation for maple syrup urine disease (MSUD) and methylmalonic acidopathy (MMA). *Am. J. Hum. Genet.*, 61:A254 (1997)

Key Word: Maple Syrup Urine Disease, Branched Chain α-Ketoacid Dehydrogenase, Human Mutations

---

Send correspondence to: Dean J. Danner, Ph.D., Dept. of Genetics, Emory University School of Medicine, 1462 Clifton Rd, Atlanta GA 30322, Tel: (404)-727-5784 Fax:(404)-727-3949, E-mail: ddanner@emory.edu