

# Catabolism of Branched-Chain Amino Acids in Heart Failure: Insights from Genetic Models

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**Abstract** Genetic defects in amino acid metabolism are major causes of newborn diseases that often lead to abnormal development and function of the central nervous system. Their direct impact on cardiac development and function has rarely been investigated. Recently, the authors have established that a mitochondrial targeted 2C-type ser/thr protein phosphatase, PP2Cm, is the endogenous phosphatase of the branched-chain alpha keto acid-dehydrogenase complex (BCKD) and functions as a key regulator in branched-chain amino acid catabolism and homeostasis. Genetic inactivation of PP2Cm in mice leads to significant elevation in plasma concentrations of branched-chain amino acids and branched-chain keto acids at levels similar to those associated with intermediate mild forms of maple syrup urine disease. In addition to neuronal tissues, PP2Cm is highly expressed in cardiac muscle, and its expression is diminished in a heart under pathologic stresses. Whereas phenotypic features of heart failure are seen in PP2Cm-deficient zebra fish embryos, cardiac function in PP2Cm-null mice is compromised at a young age and deteriorates faster by mechanical overload. These observations suggest that the catabolism of branched-chain amino acids also has physiologic significance in maintaining normal cardiac function. Defects in PP2Cm-mediated catabolism of branched-chain amino acids can be a potential novel mechanism not only for maple syrup urine disease but also for congenital heart diseases and heart failure.

**Keywords** Amino acid metabolism · Branched-chain amino acid catabolism · Branched-chain alpha keto acid-dehydrogenase complex · PP2Cm

## Amino Acid Metabolism and Genetic Disorders

Amino acids are key nutrient molecules essential for cell growth, survival, and normal function. In addition to providing building blocks for protein synthesis, many amino acids are an essential ingredient for biosynthesis of other molecules as the sources of nitrogen and carbon [8]. Some amino acids, including branched-chain amino acids, also have been shown to possess a potent signaling function to regulate global growth and metabolism [2, 13, 14, 17, 18, 22, 23]. Therefore, the impact of amino acid metabolism on embryonic development and human congenital diseases has long been recognized.

Maple syrup urine disease, one of the most common genetic disorders caused by defects in amino acid metabolism, is a target of mandatory newborn screening in most of the United States and throughout the world [5, 10, 20]. The clinical manifestations of classical maple syrup urine disease are mostly neuropathologic, including seizure and mental retardation [7]. However, recent studies from animal models of maple syrup urine disease raise concerns about the potential adverse impact of branched-chain amino acid metabolic defects on cardiac development and function. These concerns are the focus of the discussion in this review.

## Catabolism of Branched-Chain Amino Acids

Branched-chain amino acids (BCAAs) including leucine, isoleucine, and valine are essential amino acids that must

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be acquired from external food. In addition to their abundant presence in protein peptides as key hydrophobic building blocks, BCAAs also serve as significant sources for biosynthesis of sterol, keto bodies, and glucose [3]. Among the BCAAs, particularly leucine has potent signaling activity to promote protein synthesis, cellular metabolism, and cell growth in a mammalian target of rapamycin (mTOR)-dependent manner [4, 15, 19].

Although BCAAs are necessary for normal growth and function at cellular and organism levels, an excess amount of free BCAAs also can be pathologic. A high plasma level of BCAAs is the benchmark and cause of maple syrup urine disease, a potentially life-threatening disorder affecting 1 of 180,000 newborn babies on the average in the general population, with a much higher prevalence among certain Amish, Mennonite, and Jewish communities [5, 20].

Because BCAAs are essential amino acids with no biosynthesis pathways in animal cells, their homeostasis is determined largely by catabolic activities in a number of organs, particularly the liver [9]. The first step in the catabolism of BCAAs is carried out in brain, muscle, and many nonhepatic tissues by the branched-chain aminotransferase (BCAT) to convert BCAAs into branched-chain alpha keto acids (BCKAs) [9]. The BCKAs are decarboxylated by the branched-chain alpha keto acid dehydrogenase (BCKD) complex in the liver as well as other tissues and eventually degraded into acetyl-coenzyme A (CoA) or succinyl-CoA to fuel the TCA cycle. As BCKD mediates this rate-limiting step in BCAA catabolism, its activity dictates the steady-state levels of BCAA and BCKA. It thus is a target of multiple regulatory mechanisms, including cyclic adenosine monophosphate (cAMP)-mediated induction of RNA transcription and phosphorylation-mediated inhibition/activation of its enzymatic activity.

The BCKD complex is genetically linked with the pyruvate dehydrogenase complex (PDH) because they share extensive homology in their subunit composition and regulation. Like PDH, BCKD holoenzyme contains 24 copies of catalytic subunits E2/E3 and an equal number of regulatory subunits E1 $\alpha$  and E1 $\beta$ . At low BCAA levels, E1 $\alpha$  is hyperphosphorylated by BCKD kinase, leading to lower BCKD activity and reduced loss of BCAA. At a high BCAA level, E1 $\alpha$  is dephosphorylated by BCKD phosphatase, leading to induced BCKD activity and the removal of excess BCAA. Therefore, BCKD phosphorylation/dephosphorylation is critical to BCAA homeostasis [9].

### Molecular Identification of BCKD Phosphatase

Based on the importance of BCKD phosphorylation in its regulation, BCKD phosphatase has been well established

as a key regulator in BCAA catabolism. However, its molecular identity has remained elusive for decades. Recently, through genome scanning, we discovered a mitochondrial targeted 2C-type ser/thr protein phosphatase that we named PP2C in mitochondria (PP2Cm) [11].

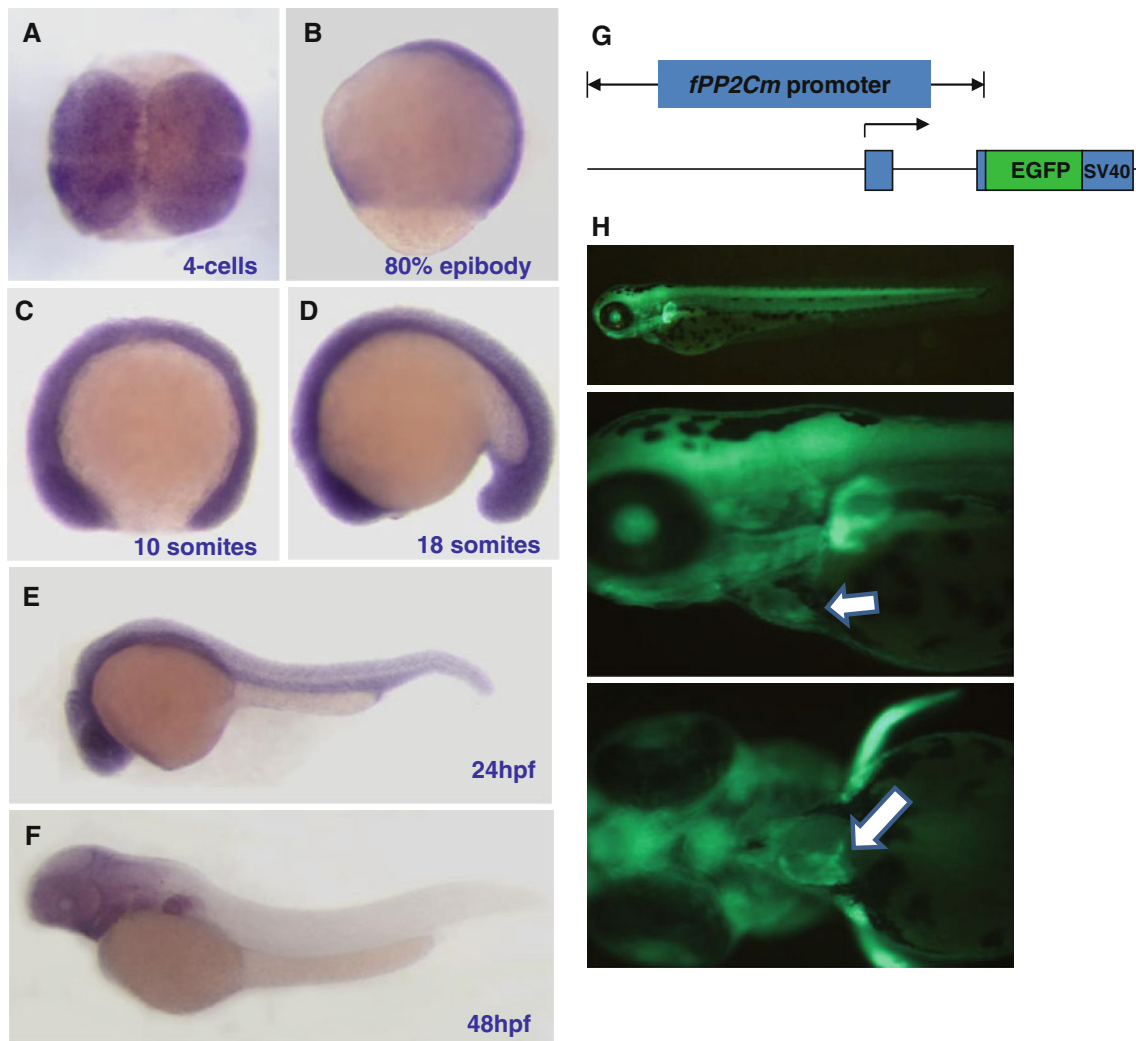
Through extensive proteomic and biochemical analysis, we established that PP2Cm is the missing BCKD phosphatase responsible for BCAA-induced dephosphorylation and activation of BCKD activity [12]. This conclusion is based on the following lines of evidence: (1) BCKD subunits, including E2 and E1 $\alpha$ , are specifically associated with PP2Cm *in vitro* and *in vivo*; (2) PP2Cm expression effectively dephosphorylates E1 $\alpha$  Ser-293 phosphorylation, a key residue in BCKD enzymatic regulation; (3) PP2Cm-deficient cells have elevated basal E1 $\alpha$  phosphorylation, which remains high at BCKA treatment; (4) PP2Cm-deficient mice have a significantly higher plasma level of BCAA and BCKA with impaired BCAA clearance after a high dose of BCAA ingestion; and (5) PP2Cm-deficient newborn mice have significantly higher mortality under a high-protein diet challenge.

All these data establish that PP2Cm is the endogenous BCKD phosphatase with an essential function in BCAA catabolism. The analysis with PP2Cm KO mice indicates that a defect in PP2Cm is a potential novel mechanism for the intermediate/inducible forms of maple syrup urine disease.

### PP2Cm in Cardiac Regulation

The discovery of PP2Cm as the BCKD phosphatase and a key regulator in BCAA metabolism as well as the establishment of PP2Cm-deficient zebra fish and mouse models offers an opportunity to investigate the impact of BCAA regulation in intact animals. In both zebra fish embryos and adult mice, PP2Cm is highly expressed in both cardiac muscle and the central nervous system (Figs. 1, 2). Its expression in the heart is dynamically regulated by stress, as measured by both mRNA and protein levels, with significantly reduced levels in hypertrophic and failing hearts [11].

It is not clear whether loss of PP2Cm is a contributing factor to cardiac pathology or simply a phenomenon associated with the diseased hearts. To investigate this question, we analyzed the cardiac performance in PP2Cm-deficient zebra fish and mice. Using morpholinos specifically targeted to zPP2Cm translation initiation codon (ATG-MO), we demonstrated that PP2Cm-deficient fish embryos displayed a dose-dependent loss of cardiac contractility and that most of them did not survive beyond the early embryonic stage [11]. Accelerated heart failure developed in PP2Cm-deficient mice after mechanical overload induced by trans-aortic constriction (Fig. 3).



**Fig. 1** Expression pattern of PP2C in mitochondria (PP2Cm) of developing zebra fish embryos. **a–f** *In situ* hybridization signal of fPP2Cm mRNA in developing zebra fish embryos at a specific stage as indicated (hpf: hour postfertilization). **g** Transgene construct of

fPP2Cm promoter driving GFP expression. **h** GFP signal observed under ultraviolet illumination in transgenic zebrafish at 72 hpf stage under different magnifications. *Arrows* indicate heart tube

These evidences suggest that loss of PP2Cm is not a mere consequence but rather a significant contributor to the pathogenesis of heart failure. These studies for the first time implicated BCAA catabolism also as an important aspect of cardiac pathophysiology and showed that defects in BCAA homeostasis can have a significant adverse impact on cardiac function and disease progression.

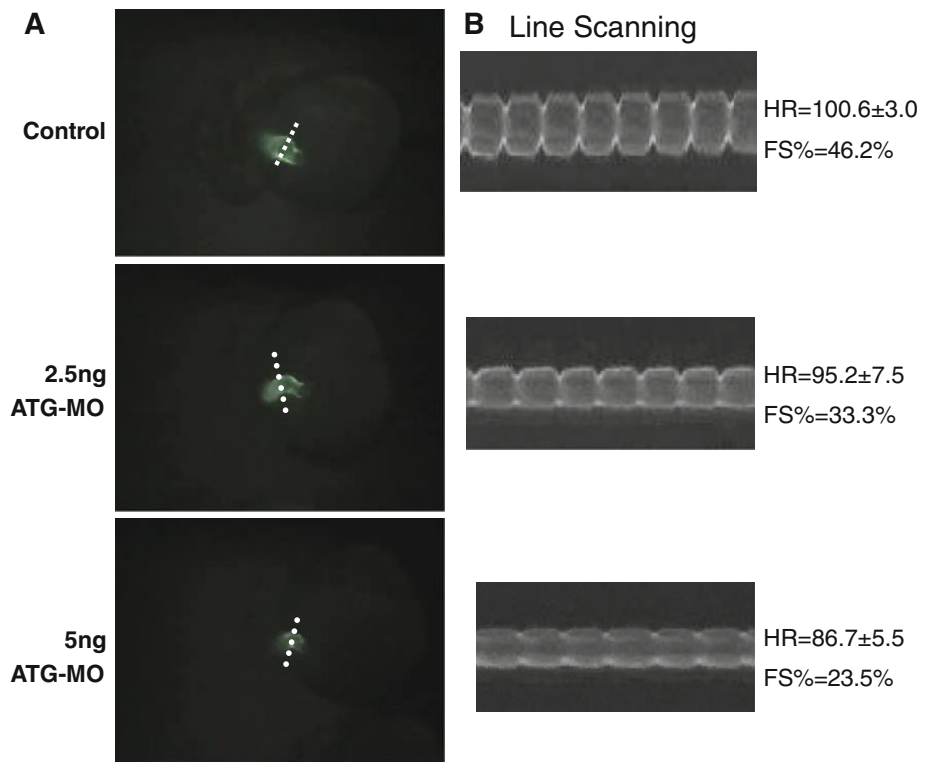
The underlying mechanisms for the clinical manifestations of maple syrup urine disease still are not well established, and the adverse impact on glutamine transport and the induction of reactive-oxygen species (ROS) have been implicated [1, 6, 24]. In our own studies with cardiac tissue or cultured cardiomyocytes, we also explored a number of these possible mechanisms.

1. We examined the cytotoxic effect of the accumulated BCAA/BCKA on cardiomyocyte survival, demonstrating

that PP2Cm-deficient mice have a significantly elevated ROS level and are more susceptible to calcium-induced permeability transition pore opening in mitochondria [11]. Whether and how this effect is caused by accumulated BCAA or BCKA remains to be investigated further. However, a clear implication of the observed cytotoxicity is more cell death. Indeed, we observed that PP2Cm deficiency can lead to more apoptotic cell death in both developing embryos and cultured myocytes.

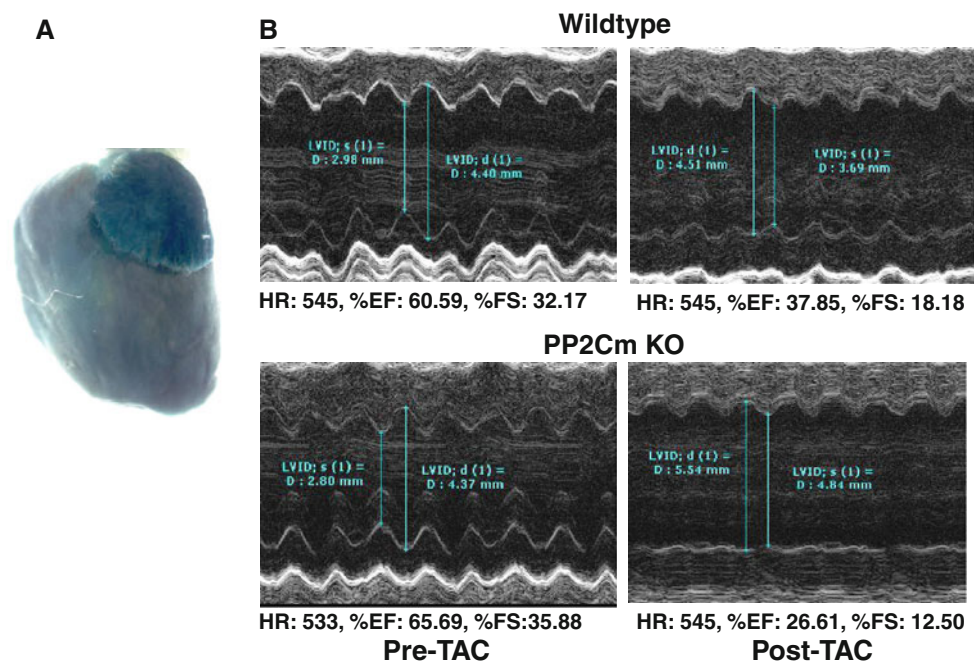
2. Metabolic effects of accumulated BCAA/BCKA can be pathologic in the heart. It is known that BCAA/BCKA can inhibit pyruvate and fatty acid transport and utilization [16, 19]. Because cardiac tissue has a constant high demand for pyruvate and fatty acid as its main fuel source, chronically elevated BCAA/BCKA can potentially block normal bioenergetic homeostasis

**Fig. 2** Loss of fPP2Cm expression leads to heart failure in zebra fish embryo. **a** Video frames of *cmhc*-GFP transgenic fish embryo recorded under ultraviolet illumination after treatment with different concentrations of PP2Cm morpholigo. **b** M-mode image of zebra fish hearts from line-scanning analysis as shown in A as well as heart rate (HR) and percentile fractional shortening (FS%) measurements. Adapted from Lu et al. [11] with permission



**Fig. 3** Expression and function of PP2Cm in the heart.

**a** Expression of PP2Cm in the heart illustrated by positive Lac-Z staining in a PP2Cm<sup>+lacZ</sup> heart. **b** Representative echocardiogram of a wild type and a PP2Cm KO heart before and after 8 weeks of pressure overload induced by TA



in the heart, leading to contractile dysfunction and accelerated heart failure under increased mechanical load or aging. Although we observed significant changes in a number of metabolic genes in PP2Cm-deficient hearts (Lu et al., unpublished results), a direct analysis of cardiac metabolic status and energy consumption is needed to support this hypothesis.

3. Our findings showed that PP2Cm mediated direct modification of mitochondrial function in cellular bioenergetics and survival. Although BCKD is the only substrate identified for PP2Cm to date, it is possible that other uncharacterized substrates in mitochondria contribute to PP2Cm-mediated signaling. This is supported by our observation that the isolated

PP2Cm-deficient mitochondria have abnormal sensitivity to calcium-induced permeability transition pore opening even in the absence of BCAA or BCKA treatment [11]. The molecular basis of this observation remains unclear, but it may imply that branched-amino acid metabolism is functionally coupled with mitochondrial inner membrane permeability, which has a key role in mitochondrial calcium, respiration coupling, ROS, and cellular viability.

## Perspectives

Branched-chain amino acids are important nutrient molecules with a potent signaling effect. Free BCAAs and their catabolic intermediates, BCKAs, are tightly maintained in animals by a highly regulated catabolic pathway. Defects in catabolism of BCAAs cause maple syrup urine disease, one of the most common metabolic disorders in the human population.

Although most clinical features of maple syrup urine disease are neurologic, our recent findings from cellular studies and animal models suggest that missing a key regulator in the catabolism of BCAAs also can cause a significant impairment in cardiac function (Fig. 4). The implication of this observation remains to be further established. However, a recent study based on metabolic profiling of peripheral blood has demonstrated a link between abnormal metabolism of BCAAs and coronary

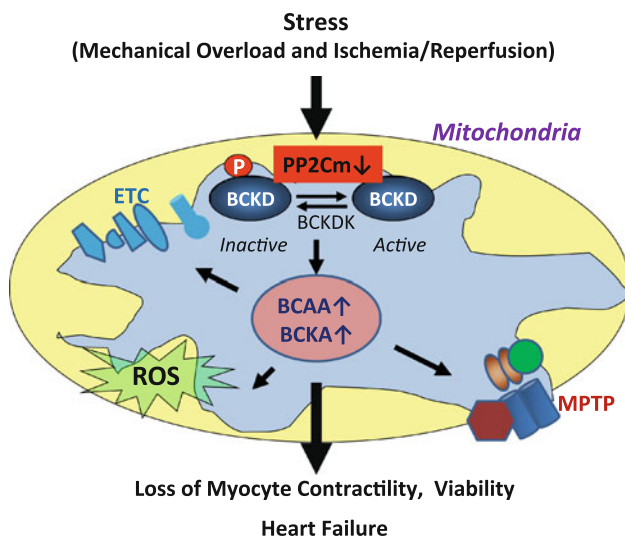
diseases [21]. As the complete molecular components of BCAA catabolic pathways are being discovered, better diagnosis will be available to identify patients with non-classic, intermediate, or inducible maple syrup urine disease based on sequencing evidence. The insights learned from our study argue strongly for a better understanding of the role that the catabolism of BCAAs plays in the heart and its potential impact on cardiac pathology in addition to its damage to the central nervous system.

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**Fig. 4** Potential role played by catabolism of branched-chain amino acids (BCAA)/branched-chain alpha keto acids (BCKA) in heart failure. Schematic illustration showing the potential negative impact of an elevated BCAA/BCKA level on cardiac function via its regulation of mitochondrial electrophone transfer chain (ETC), reactive oxygen species (ROS), and mitochondrial permeability transition pore (mPTP)

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