Branched-chain amino acids enhance cyst development in autosomal dominant polycystic kidney disease

Junya Yamamoto[1], Saori Nishio[1], Fumihiko Hattanda[1], Daigo Nakazawa[1], Toru Kimura[3], Michio Sata[2], Minoru Makita[1], Yasunobu Ishikawa[1] and Tatsuya Atsumi[1]

[1]Division of Rheumatology, Endocrinology and Nephrology, Hokkaido University Graduate School of Medicine, Sapporo, Japan; [2]Liver Cancer Research Division, Kurume University, Kurume, Japan; and [3]Department of Pharmacology and Toxicology, Kyorin University School of Medicine, Tokyo, Japan

Autosomal dominant polycystic kidney disease (ADPKD) is characterized by the progressive development of kidney and liver cysts. The mammalian target of rapamycin (mTOR) cascade is one of the important pathways regulating cyst growth in ADPKD. Branched-chain amino acids (BCAAs), including leucine, play a crucial role to activate mTOR pathway. Therefore, we administered BCAA dissolved in the drinking water to *Pkd1*$^{flox/flox}$:*Mx1-Cre* (cystic) mice from four to 22 weeks of age after polyinosinic-polycytidylic acid–induced conditional *Pkd1* knockout at two weeks of age. The BCAA group showed significantly greater kidney/body weight ratio and higher cystic index in both the kidney and liver compared to the placebo-treated mice. We found that the L-type amino acid transporter 1 that facilitates BCAA entry into cells is strongly expressed in cells lining the cysts. We also found increased cyst-lining cell proliferation and upregulation of mTOR and mitogen-activated protein kinase/extracellular signal–regulated kinase (MAPK/ERK) pathways in the BCAA group. *In vitro*, we cultured renal epithelial cell lines from *Pkd1* null mice with or without leucine. Leucine was found to stimulate cell proliferation, as well as activate mTOR and MAPK/ERK pathways in these cells. Thus, BCAA accelerated disease progression by mTOR and MAPK/ERK pathways. Hence, BCAA may be harmful to patients with ADPKD.

Kidney International (2017) **92,** 377–387; http://dx.doi.org/10.1016/j.kint.2017.01.021

KEYWORDS: ADPKD; BCAA; LAT-1; mTOR

Correspondence: *Saori Nishio, Hokkaido University School of Medicine, Kita-15 Nishi-7, Kita-ku, Sapporo, Hokkaido 060-8638, Japan. E-mail: saorin@med.hokudai.ac.jp*

Received 27 April 2016; revised 3 January 2017; accepted 5 January 2017; published online 22 March 2017

Autosomal dominant polycystic kidney disease (ADPKD) is a human genetic disorder characterized by cyst formation in the kidneys that leads to progressive bilateral renal enlargement and fibrosis in the renal parenchyma.[1,2] ADPKD patients develop progressive chronic kidney disease (CKD) that can reach to end-stage kidney failure.

The main pathogenic features of ADPKD are enhanced tubular-cell proliferation, fluid secretion, and cyst formation along all segments of the nephron.[3,4] The cyclic adenosine monophosphate (cAMP),[5–7] mitogen-activated protein kinase/extracellular signal–regulated kinase (MAPK/ERK)[8,9], and mammalian target of rapamycin (mTOR) cascades are major signaling pathways implicated in cyst development. mTOR is aberrantly activated in human kidney cyst-lining cells.[10] mTOR has 2 distinct complexes: mTOR complex 1 (mTORC1) and mTOR complex 2. mTORC1 modulates cell growth, tumor development, and autophagy and is influenced by growth factors, stress, and nutrition status.[11,12] Sirolimus and everolimus inhibit mTORC1 and attenuate cyst development in human ADPKD[13,14] and PKD models[15–20]; however, the effects of mTOR inhibitors in human ADPKD remain controversial.[21,22]

Branched-chain amino acids (BCAAs) play a crucial role to activate mTORC1 and exert physiological effects by activating the mTOR cascade.[23,24] The physiological effects of BCAAs have been verified in multiple organs.[25–29] Although the precise mechanism by which BCAAs activate mTORC1 remains unknown, there are several candidate molecules that mediate BCAA-mTORC1 signaling.[30–32] BCAAs are frequently utilized in life, but their role in ADPKD has not been investigated. We hypothesized that BCAA supplementation in ADPKD stimulates cell proliferation through mTOR activation and thus accelerates cyst development.

To elucidate the effects of BCAAs in ADPKD, we established *Pkd1*$^{flox/flox}$:*Mx1-Cre* mice as an orthologous model for human ADPKD.[33,34] BCAAs were administered to mice via drinking water, and the effects on cyst progression and signaling pathways were analyzed and compared with placebo-treated mice. In addition, we utilized *Pkd1* heterozygous (*Pkd1*$^{+/-}$) and *Pkd1* null (*Pkd1*$^{-/-}$) mouse renal epithelial cell lines that are derived from a single *Pkd1*$^{flox/-}$

clone[33,35] to examine the effects of leucine on *Pkd1*$^{-/-}$ cells. In this study, we elucidated that BCAAs accelerated cyst development via the mTOR and MAPK/ERK pathways in ADPKD.

RESULTS

BCAA administration induces progressive cystic disease in *Pkd1*$^{flox/flox}$:*Mx1-Cre* mice

We evaluated the cystic burden in both the kidney and liver (Figure 1a) using a cystic index and the ratios of the combined kidney weight to the total body weight (2KW/BW) and the liver weight to the total body weight (LW/BW). The 2KW/BW ratio was higher in *Pkd1*$^{flox/flox}$:*Mx1-Cre* mice (cystic mice) treated with BCAAs than in cystic mice treated with placebo (Figure 1b). The cystic index for the kidney and liver was higher in BCAA-treated cystic mice (BCAA group) than in placebo-treated cystic mice (placebo group) (Figure 1c). We measured daily water intake in cystic mice during BCAA or placebo administration. Water intake was higher in the BCAA group than in the placebo group (Supplementary Figure S1). The average daily protein intake was 885 mg in the placebo group and 977 mg in the BCAA group. The

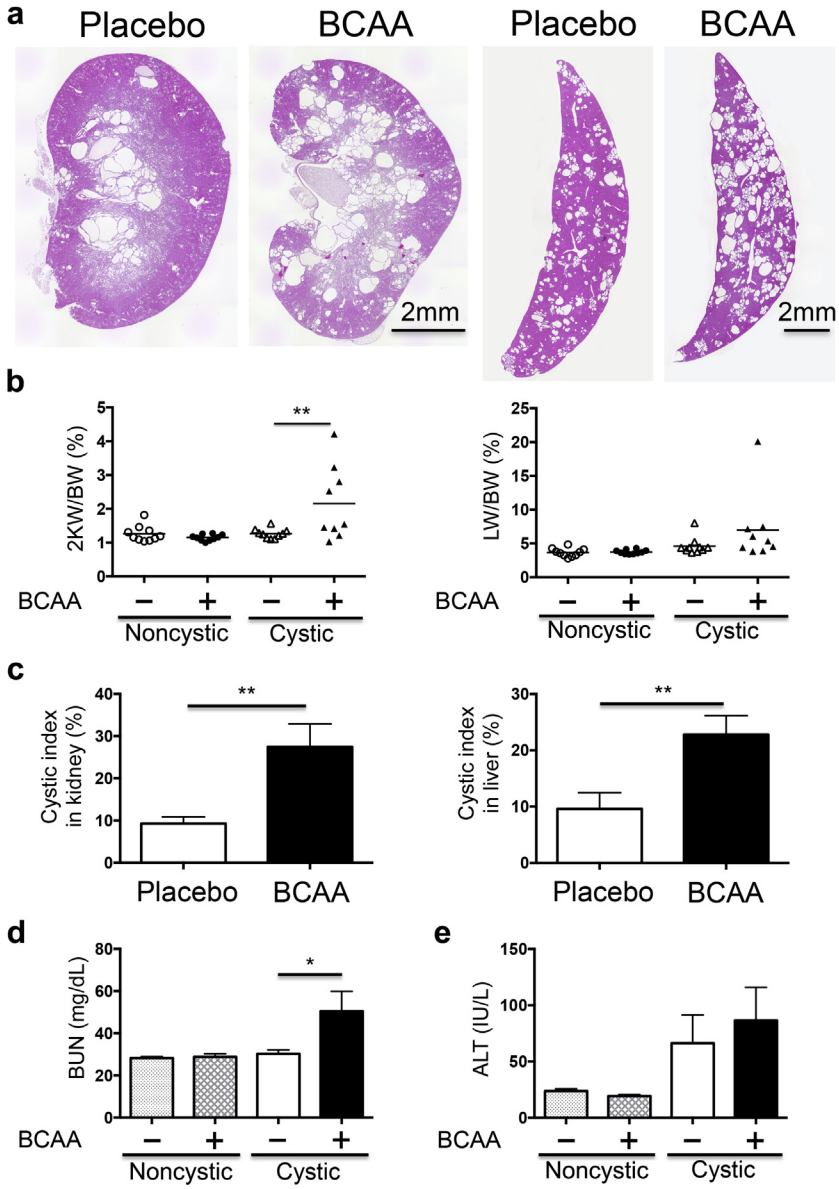

Figure 1 | Phenotype of *Pkd1*$^{flox/flox}$:*Mx1-Cre* mice (cystic) and *Pkd1*$^{flox/flox}$ mice (noncystic) at 22 weeks of age. (**a**) Representative hematoxylin and eosin–stained kidney and liver sections of *Pkd1*$^{flox/flox}$:*Mx1-Cre* mice. The branched-chained amino acid (BCAA) group showed more developed cysts than the placebo group. (**b**) The ratio of the combined kidney weight to the total body weight (2KW/BW) and that of the liver weight to the total body weight (LW/BW). The 2KW/BW ratio was higher in BCAA-treated cystic mice (2.16%, N = 9) than in placebo-treated cystic mice (1.27%, N = 10). (**c**) The cystic index of the kidney and liver in *Pkd1*$^{flox/flox}$:*Mx1-Cre* mice. The cystic indices of the kidney and liver were higher in the BCAA group than in the placebo group (kidney: 27.5% ± 5.42% vs. 9.29% ± 1.61%, liver: 22.8% ± 3.38% vs. 9.60% ± 2.87%). (**d,e**) Serum blood urea nitrogen (BUN) and alanine aminotransferase (ALT) levels at 22 weeks of age. N = 9-10, *$P < 0.05$, **$P < 0.01$.
To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.