Supplemental Materials

Methods

Background of the citrin-deficient children

Fifty-five citrin deficient children were diagnosed by genetic analysis of the SLC25A13 gene (Yasuda et al 2000) in several hospitals in Japan from 2009 to 2014 as followed: Saiseikai Yokohamashi Tobu Hospital (Yokohama, Kanagawa), Kobe University Graduate School of Medicine (Kobe, Hyogo), Tsuyama-Chuo Hospital (Tsuyama, Okayama), and Osaka City General Hospital (Miyakojima, Osaka). In the 55 citrin deficient children, 20 affected children were selected on the conditions that blood could be collected in fasting before lunch, information of usual meals could be confirmed, and informed consent could be obtained from their parent.

Twenty citrin deficient children aged from 5 years 5 months to 13 years 3 months (affected children) and 35 age-matched healthy children aged 5-13 years as healthy controls were enrolled for blood amino acid profile evaluation. Blood samples were collected from the children before lunch after a 4-5 h fast. Total protein, albumin, aspartate transaminase (AST), alanine transaminase (ALT), γ-glutamyl transpeptidase (γ-GTP), total bile acids (TBA), glucose, hemoglobin A_{1c} (HbA_{1c}), insulin, pyruvate, and ammonia levels in the plasma were examined as routine clinical blood biochemical analyses (Nagasaka et al 2017), and there were not other clinical observations because the affected children in this period as well as the healthy children were considered as healthy. Total daily energy intake was similar between the affected and control children, although the nutrients-to-daily total energy ratios were 17-21% (as a reference, Japanese normal subjects, 14-15%) for protein, 40-47% (25-30%) for fat, and 33-40% (54-58%) for carbohydrate as previously described (Saheki et al 2008; Saheki et al 2012).

Of the 20 affected children, 14 had metabolic abnormalities (hypergalactosemia, n=9; hyperphenylalaninemia, n=4; hypermethionemia, n=2) detected during a neonatal mass screening performed at the age of 5 days. A few months later, they developed considerable liver dysfunction along with cholestasis, hyperbilirubinemia, hypoproteinemia, and a prolonged blood coagulation time. The remaining 6 children had jaundice observed by their parents, and were diagnosed as having citrullinemia after admission to local hospitals between the ages of 1-6 months. Amino acids concentrations in plasma were analyzed as clinical examination at local hospitals by Bio Medical Laboratories, Inc. (Tokyo, Japan). Amino acids profile in the NICCD period of the 20 affected children was shown in Supplemental Table S1. All of the affected children were finally diagnosed as the citrin deficiency from the age of 3 weeks to 4 years 1 month by the genetic analysis. Blood AST, ALT, γ-GTP, TBA, and total bilirubin as well as other blood liver function markers were improved to the normal ranges in the citrin-deficient infants at ages ranging from 7 to 18 months.

Serum amino acid analyses

Serum concentrations of α-amino acids and other related molecules (β-alanine, GABA, taurine [Tau], and 3-aminoisobutyrate [3AIB]) were quantified by a derivatization method with 3-aminopyridyl-N-hydroxysuccinimidyl carbamate (APDS) using the LC-ESI-MS/MS system (Shimbo et al 2009).

The LC-ESI-MS/MS system consisted of a TSQ Vantage triple stage quadruple mass spectrometer (Thermo Fisher Scientific, Waltham, MA) equipped with an HESI-II probe and a Prominence ultra-fast liquid chromatography system (Shimadzu, Kyoto, Japan). Fifty μL of serum was mixed with 50 μL of APDSTAG® Wako Amino Acids Internal Standard Mixture solution (Wako Pure Chemical Industries, Osaka, Japan) and 100 μL of acetonitrile and centrifuged at 20,000×*g* for 10 min. Then, 20 μL of APDS-acetonitrile solution (20 $mg \cdot mL^{-1}$; Wako Pure Chemical Industries) and 60 μL of 0.2 M sodium borate buffer, at *pH* 8.8, were mixed with 20 μL of the supernate, and incubated at 55 °C for 10 min. Thereafter, the reaction mixture was added to 100 μL of 0.1% formic acid solution, and 5 μL was injected into the LC-P-ESI-MS/MS system. Amino Acids Mixture Standard solutions of Type B and AN-2 (Wako Pure Chemical Industries) were used for quantification. The APDS-derivatized amino acids were separated with a 100 × 2.0 mm i.d. Wakosil-II $^3C_8$-100HG column (particle size 3 μm) for the analytical column and a 10 × 1.5 mm i.d. Wakosil-II $^3C_8$-100HG column for the guard column (particle size 3 μm) (Wako Pure Chemical Industries Ltd), 0.3 mL/min at 40°C by gradient flow. The general HPLC and

MS/MS conditions were carried out using Shimbo's method (Shimbo et al 2009).

Serum 3-hydroxyisobutyrate (3HIB) analysis

The serum 3HIB concentration was determined by HPLC-ESI-MS/MS with the quantification method of serum 3-hydroxybutyrate (3HB) reported previously (Nagasaka et al 2017). In brief, 5 μL of serum was mixed with 100 ng of sodium DL-[$^{13}C_4$]3HB acetonitrile solution (100 μL) as an internal standard. After centrifugation at 2,000×*g* for 1 min, the liquid phase was collected and evaporated to dryness at 55 °C under a nitrogen stream. The residue was redissolved in 70 μL of 0.1% formic acid solution, and an aliquot (5 μL) was injected into the HPLC-MS/MS system. The general HPLC and MS/MS conditions were similar to that in our previous study (Nagasaka et al 2017), and the SRM and collision energy for 3HIB and [$^{13}C_4$]3HB were **m/z** 103 → **m/z** 73 (15 V) and *m/z* 107→*m/z* 61 (15 V), respectively.