

# Hypervalinemia and hyperleucine-isoleucinemia caused by mutations in the branched-chain-amino-acid aminotransferase gene

X. L. Wang · C. J. Li · Y. Xing · Y. H. Yang · J. P. Jia

Received: 28 July 2014 / Revised: 9 January 2015 / Accepted: 12 January 2015  
© SSIEM 2015

**Abstract** Valine, leucine, and isoleucine are essential branched chain amino acids (BCAAs). When BCAA metabolism is genetically impaired in human, serum levels of BCAA and/or their metabolites rise considerably, causing severe neurological dysfunction. The first step in BCAA catabolism is catalyzed by branched chain aminotransferase (BCAT). Hypervalinemia and hyperleucine-isoleucinemia caused by *BCAT* gene mutation in human have not been reported previously. A 25-year-old man presented with headache complaints and mild memory impairment for about six years. Brain MRI showed symmetric white matter abnormal signals. Metabolic studies revealed remarkably elevated plasma valine and leucine concentrations. Maple syrup urine disease (MSUD) diagnosis was not supported since all genes for the branched-chain  $\alpha$ -keto acid dehydrogenase complex (BCKD) gene were normal. Interestingly, two heterogeneous *BCAT2* gene mutations were found in the patient, including c.509G>A (p.Arg170Gln) and c.790G>A (p.Glu264Lys). In addition, c.509G>A (p.Arg170Gln) and c.790G>A (p.Glu264Lys) were found in his father and mother, respectively, suggesting an autosomal recessive disorder. *BCAT2* functional studies demonstrated that the two *BCAT2* gene mutations resulted in decreased *BCAT2* enzyme activity. After treatment with vitamin B6, the levels of BCAA, especially valine were remarkably decreased and brain MRI lesions were improved. These findings suggest a new type of branched

chain amino acid metabolism disorder. This rare case provides great insight into the further understanding of BCAA metabolism and its defect in human. *BCAT2* gene mutations can cause hypervalinemia and hyperleucine-isoleucinemia, which are associated with brain white matter lesions.

## Introduction

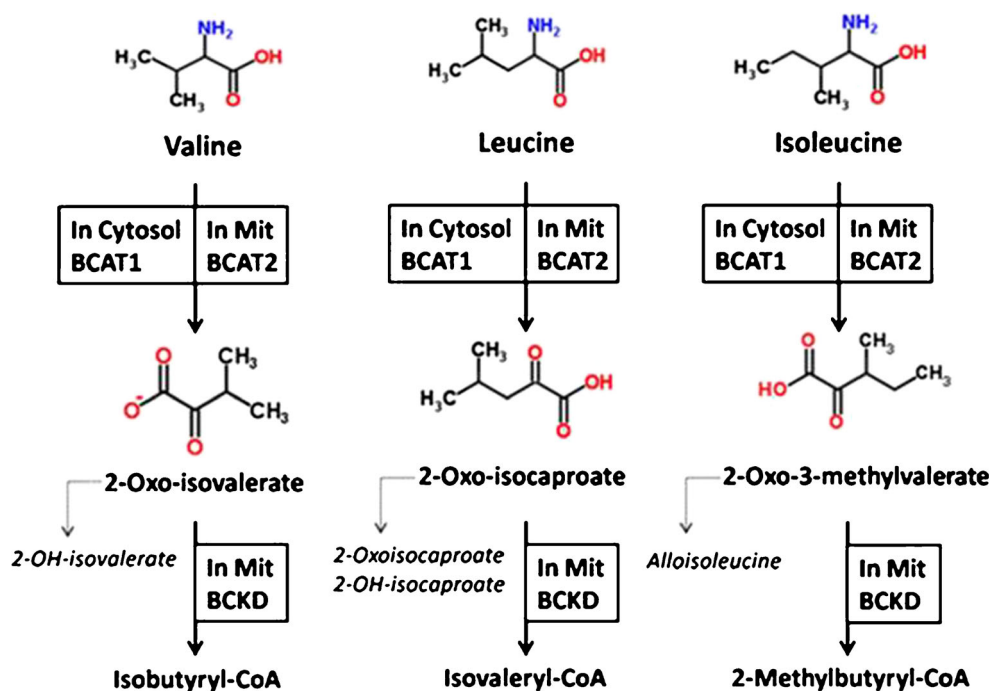
The branched chain amino acids (BCAAs) leucine, isoleucine, and valine, are classified as indispensable amino acids because they cannot be synthesized *de novo* in mammals (Tom and Nair 2006). BCAAs account for 35 % of all essential amino acids in muscle proteins and 40 % of preformed amino acids required by mammals (Lu et al 2013). In addition, inborn errors of BCAA metabolism have shown that dysregulation of the BCAA catabolic pathways that results in excess BCAAs or their derivatives is toxic to the central nervous system (Hutson et al 2005). BCAA metabolism (Fig. 1) in human is not completely understood. The first two steps in BCAA catabolism are common to all three amino acids, and involve BCAA aminotransferase (BCAT) and branched-chain  $\alpha$ -keto acid dehydrogenase (BCKD) (Brosnan and Brosnan 2006). In the first step, BCAT catalyzes the transamination of the branched chain amino acids to their respective  $\alpha$ -keto acids. There are two main human isoforms of BCAT, namely BCAT1 (cytoplasm) and BCAT2 (mitochondria). The mitochondrial isoform, BCAT2, is expressed in most tissues (Sweatt et al 2004). BCKD catalyzes the oxidative decarboxylation of the branched-chain  $\alpha$ -keto acids. Severe deficiency in BCKD activity leads to maple syrup urine disease (MSUD), an autosomal recessive metabolic disorder (Strauss and Morton 2003). Theoretically, genetic alteration of the BCAT enzyme could cause hypervalinemia and hyperleucine-isoleucinemia. However, no human BCAT mutation has been

Communicated by: Jerry Vockley

X. L. Wang · C. J. Li · Y. Xing · J. P. Jia (✉)  
Department of Neurology, Xuanwu Hospital, Capital Medical University, 45 Changchun Street, Beijing 100053, People's Republic of China  
e-mail: jjp@ccmu.edu.cn

Y. H. Yang  
Department of Radiology, Xuanwu Hospital, Capital Medical University, Beijing, China

**Fig. 1** First two steps of the BCAA metabolism pathway. Mit: mitochondria



described to date. In this work, we present a case report of a patient with hypervalinemia and hyperleucine-isoleucinemia that appears to be caused by *BCAT2* gene mutations. The clinical data of this case and gene analysis are presented as well. The treatment with vitamin B6 reversed the phenotype as observed with decreased valine levels. Overall, this rare case of *BCAT2* gene mutations provides new insight in the understanding of BCAA metabolism and its defect in humans.

## Methods

### Clinical data, neuroimaging, and treatment

Upon patient admission to the hospital, brain MRI and MRS were performed. MRI was carried out on a 1.5 T units Magnetom Sonata (Siemens, Erlangen, Germany). In addition, routine serum tests, EEG and EMG were carried out. Furthermore, urinary organic acid analysis was performed by gas chromatography/mass spectrometry on a Shimadzu QP 2010 instrument (Shimadzu, Kyoto, Japan); blood amino acid was determined on an LC/MS-MS ABI 3200 (Applied Biosystems, Foster City, CA, USA) from blood specimens dried on filter paper, based on tandem mass spectrometry. Psychological parameters were assessed and the patient was followed up for three years. Thereafter, another brain MRI was performed. At this time, *BCAT2* gene mutations were detected and the patient was administered vitamin B6 at 200 mg/day by intravenous injection. After 5 days of parenteral treatment, the patient was submitted to a daily oral treatment with 200 mg vitamin B6 and the changes in BCAA

serum levels assessed at 3 months. Blood amino acid analysis and brain MRI were also carried out in the patient's parents.

### *BCKD* gene analysis

Genomic DNA was extracted using standard methods from peripheral blood leukocytes. The exonic regions were specifically enriched using a biotinylated capture probe (MyGenostics, Baltimore, MD, USA). Then, bound DNA was eluted and amplified by a 15 cycle PCR. The enrichment libraries were sequenced on Illumina HiSeq 2000 sequencer (Illumina, Inc., USA) for 100 bp paired end reads. Short read mapping and alignment were performed using the BWA (Burrows Wheeler Aligner) software. SNPs and indels were detected using the SOAPsn software and GATK Indel Genotyper (<http://www.broadinstitute.org/gsa/wiki/index.php>, The Genome Analysis Toolkit), respectively. All reference sequences were based on the NCBI37/hg19 assembly of the human genome.

### *BCAT* gene analysis

Two main human isoforms of the *BCAT* gene have been described, including *BCAT1* (cytoplasm) and *BCAT2* (mitochondria). Human *BCAT2* is the predominant form, expressed in most tissues. Both *BCAT1* and *BCAT2* genes were analyzed: genomic DNA was extracted from 300  $\mu$ l fresh whole blood sample by routine methods. All exons of candidate genes (*BCAT1* and *BCAT2*) were amplified by PCR and sequenced. Primers were designed by the Primer3 software and listed in Table 1. For all primer sets, PCR was run in a total volume of

**Table 1** Primers used for *BCAT2* (NM\_001190) amplification

Exons	Forward primer	Reverse primer	Product length (base pair)
1	CGGAACGTCTCTCTCCG	CAGGCCAGTGGTCTTCCC	157
2-3	GAAACTGGGACAGAACAGGC	GACACAGCAAGGAGGAGGAG	737
4-6	TCTTGGCCTCCATCTTTCAG	CTGGTCCCTGTGTCTCCAAC	802
7-9	GGGAGACGGGAGAGAGTAGG	AGGTTCTGGGACAGAAGGTG	931
10	TGTACAAAGACAGGGTGAGGC	AGGAGGCTGGCTGTAGTCC	255
11	GGGCCTACTGGGAGTTGTAG	ATTGGTAGGGAGGCGAGTG	170

50  $\mu$ l containing 500 ng genomic DNA, 0.2 M each primer, 5  $\mu$ l of 10 $\times$  PCR buffer, 0.2 mM deoxyribonucleoside triphosphate (dNTP), and 5 U Taq polymerase. *BCAT2* sequences were analyzed for the patient and his parents.

#### *BCAT2* gene cloning and mutagenesis, recombinant protein purification, and enzyme activity assays

Total RNA was isolated from HeLa cells, and 2  $\mu$ g subjected to reverse transcription using SuperScript III Reverse Transcriptase (Life Technologies) to obtain cDNA. For amplification of the *BCAT2* open reading frame, the following primers were used: Forward, 5'-GGTGGTCATATGGCCGCAGCCGCTCTGG-3' (NdeI site underlined); Reverse, 5'-GGTGGTACTAGTGCATCTCCCGTGATGCACACCGGG AACATCCACTCGTGGG-3' (SpeI site underlined). PCR products were cloned into pMXB10 (NEB) using NdeI and SpeI restriction sites.

The cloned cDNA was inserted into pET-28a, and site specific mutagenesis was utilized to obtain the R170Q and E264K variants (QuikChange kit, Stratagene, USA) with the following primers:

R170Q-F: ACCAGCCTCTATGTGCAGCCTGTGCT CATTGGG;

R170Q-R: CCCAATGAGCACAGGCTGCACATAGA GGCTGGT;

E264K-F: GACCACCAGCTCACCAAGGTGGGAAC CATGAAC;

E264K-R: GTTCATGGTCCACCTTGGTGAGCT GGTGGTC.

Both wild type and mutated *BCAT2* genes were verified by DNA sequencing.

Purification of wild type and mutant *BCAT2* proteins was performed as described previously (Conway et al 2002), with minor modifications. Briefly, His-tagged proteins were purified by nickel affinity chromatography followed by anion exchange chromatography using a HiTrap Q HP strong anion column. The bound proteins were eluted with a 0–500 mM

sodium chloride gradient in 100 mM potassium phosphate buffer (pH 8.0) at a flow rate of 1 mL min<sup>-1</sup> for 20 min. The purified *BCAT2* proteins were then dialyzed at 4 °C overnight against the storage buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 5 mM glucose, 1 mM EDTA, 1 mM KIC, and 5 mM DTT), and protein concentration was determined using the BCA kit (Pierce, USA). Protein samples (10  $\mu$ g) were separated by SDS-PAGE and Coomassie Blue staining was used to verify purity and molecular weights.

*BCAT2* activity assays were performed at 37 °C by assessing the formation of [1-<sup>14</sup>C] valine from  $\alpha$ -keto [1-<sup>14</sup>C] isovalerate as described previously (Davoodi et al 1998). The standard assay solution (0.5 mL) contained 25 mM potassium phosphate buffer (pH 7.8), 5 mM DTT, 1 mM  $\alpha$ -keto [1-<sup>14</sup>C] isovalerate, and 12 mM isoleucine at 37 °C. A unit of enzyme activity was defined as 1  $\mu$ mol of <sup>14</sup>C-valine formed per minute under standard conditions. All assays were performed in triplicate.

## Results

### Clinical data

A 25-year-old man presented with a headache that had lasted for about six years. The patient declared that the disease began with a mild and tolerable paroxysm occipital headache without any apparent cause. Meanwhile, he found his memory ability mildly impaired, although ordinary work and life duties were not affected. Prior to this, he was healthy without any mental retardation. The parents were healthy and not consanguineous. There was no sibling. No maple sugar like odor of urine, regarded as a characteristic for maple sugar urine disease, was detected. Upon admission, the patient was alert and cooperative. He had normal muscular power, sensory and coordination. The tendon reflex was active for the four limbs. The Hoffman sign of left upper limb was positive, and the bilateral Babinski sign was negative. Examination revealed normal fundus oculi and detailed psychological tests showed

a mild memory decline (Wechsler Adult Intelligence Scale [WAIS]: VIQ 83, PIQ93, FIQ86 and MQ 79).

#### Laboratory data

Routine blood and urine analysis showed no abnormalities. However, abdominal ultrasound showed steatohepatitis. The hepatic function was mildly impaired with 50 IU/L ALT (normal range, 10–40 IU/L). Serum triglyceride levels were slightly elevated: 3.5 mmol/L (0.45–2.2). Fasting blood-glucose (5.03 mmol/L; normal range, 3.9–6.1), plasma very-long-chain fatty acid and arylsulfatase A levels were normal. In addition, no abnormalities were found in renal and thyroid functions, and vitamin B12 and folate levels. Lumbar puncture revealed normal pressure (170 mmH<sub>2</sub>O) for the cerebrospinal fluid, normal cell count, normal levels of glucose (57 mg/dL) and chloride (120 mmol/L), and mildly elevated protein content (57 mg/dL; normal range, 15–45). CSF anti-virus antibodies against rubella virus, herpes simplex virus type-1 (HSV-1), herpes simplex virus type-2 (HSV-2), and cytomegalovirus were all negative. Kidney ultrasound examination showed normal bilateral kidneys and adrenal glands. However, a moderate abnormality was found in EEG (moderate amplitude 5–6C/S chronic waves present in all leads). Laboratory analyses revealed no heavy metal poisoning in plasma or urine samples.

Interestingly, plasma valine and leu/Ile levels were remarkably elevated with 1754  $\mu$ mol/L (normal range, 60–280) and 646  $\mu$ mol/L (normal range, 50–150), respectively (Chace et al 1995). During the follow up period of three years, BCAA levels were assessed every 6 months and the valine contents ranged from 1450 to 1754  $\mu$ mol/L without any diet control.

#### Brain MRI and MRS

Brain MRI showed symmetric abnormal signals in bilateral frontal lobes, occipital lobes, periventricular white matter, and callosum, with low signals in T1 and high signals in T2, FLAIR, and DWI. No overt lesions were found in brain stem and cerebellum (Fig. 2). The lesions were well demarcated and not enhanced after injection of Gd-DTPA. Proton NMR spectroscopy of the periventricular white matter revealed decreased N-acetyl aspartate (NAA) peak with respect to choline and creatine. Lactic acid peak was not evident (Fig. 2).

#### BCKD gene analysis

The branched-chain  $\alpha$ -keto acid dehydrogenase (BCKD) complex is composed of three subunits (E1–E3), encoded by the nuclear genes *BCKDHA*, *BCKDHB*, *DBT*, and *DLD*. All four genes were analyzed in this patient and no mutations were found.

#### BCAT gene analysis

*BCAT* sequencing showed no mutation in the cytoplasmic isoform (*BCAT1*) but revealed mutations in *BCAT2*, the mitochondrial isoform. The *BCAT2* gene encodes a branched chain aminotransferase found in mitochondria that forms a dimer and catalyzes the first step in the metabolism of the BCAAs leucine, isoleucine, and valine. Two heterogeneous mutations, including c.509G>A (p.Arg170Gln) and c.790G>A (p.Glu264Lys), shown in Fig. 3a and b, respectively, were found in this patient. Interestingly, c.509G>A (p.Arg170Gln) was found in his father while c.790G>A (p.Glu264Lys) was found in his mother.

#### Functional analysis of mutant BCAT2 proteins

Wild type and two mutant forms of BCAT2 were expressed as His-tagged recombinant proteins. All three proteins were of high purity and similar molecular weights (Fig. 3c). As shown in Fig. 3d, BCAT2 enzyme activity was reduced in mutant proteins.

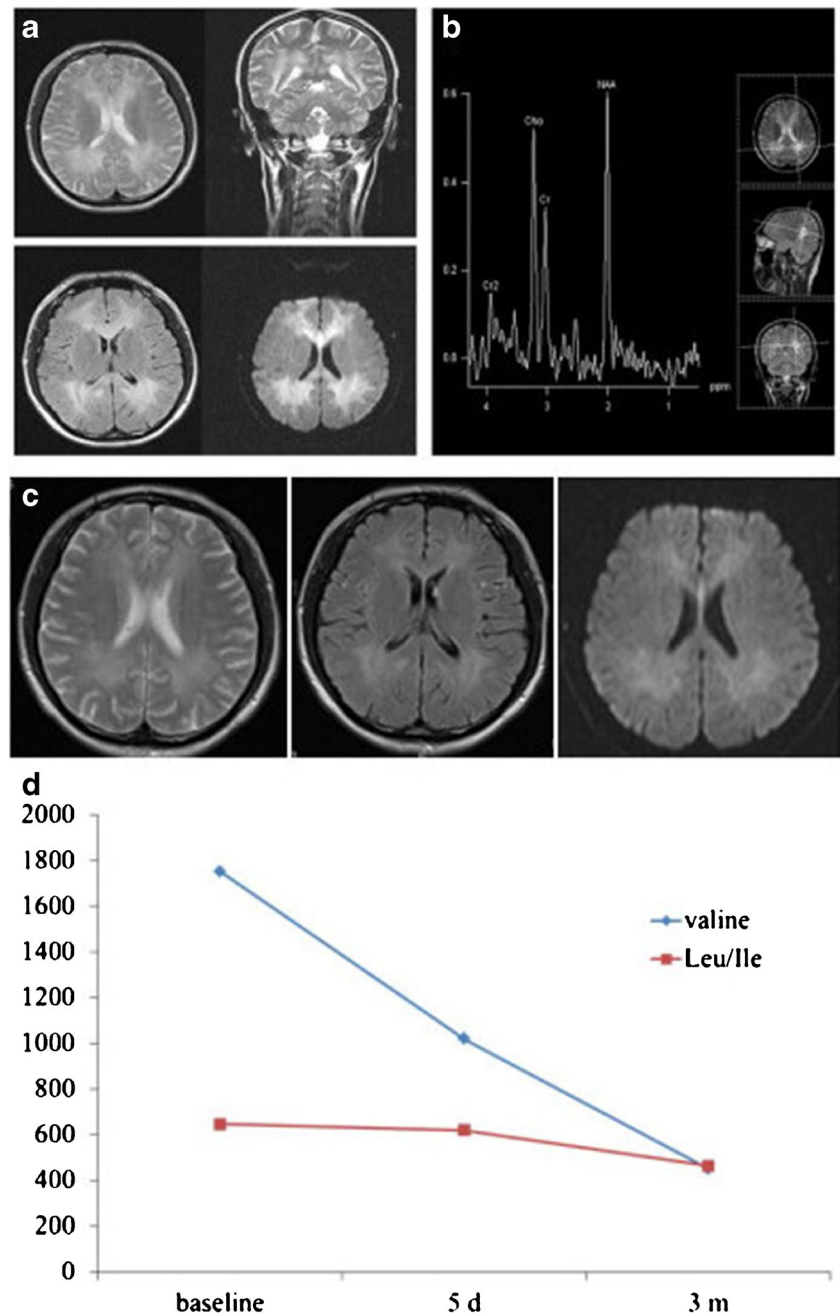
#### Treatment and prognosis

After first admission, the patient was prescribed oral vitamin B1 (60 mg/d) and no diet control. However, symptoms, signs, and brain MRI did not change considerably during the follow up period of three years. Valine levels ranged from 1450 to 1754  $\mu$ mol/L. The treatment with B1 was discontinued at second admission when *BCAT2* mutations were detected. The patient was then treated with vitamin B6, a coenzyme of BCAT2, intravenously (100 mg/d) for five days. Interestingly, this resulted in a moderate decrease of valine level and a mild decrease of Leu/Ile level: at admission, valine and Leu/Ile were detected at 1754 and 646  $\mu$ mol/L, respectively; after 5 days treatment, these levels were reduced to 1020 and 620  $\mu$ mol/L, respectively, for valine and Leu/Ile. When this treatment was followed by oral vitamin B6 at 200 mg/d, blood Val and Leu/Ile continued to decrease, and 451 and 463  $\mu$ mol/L were obtained for valine and Leu/Ile after 3 months treatment, respectively (Fig. 2d). Clinical, neuropsychological and neuroimaging re-evaluation were carried out at follow up for 10 months, and brain MRI properties have been remarkably improved, with decreased size distribution of white matter lesions and reduced high signals. The memory impairment has been improved as well, as evidenced by the WAIS parameters: VIQ 88, PIQ118, and FIQ101.

#### Discussion

Hypervalinemia and hyperleucine-isoleucinemia were found in a patient with remarkable brain white matter lesions.

**Fig. 2** Brain MRI showing symmetric abnormal signals in bilateral frontal lobes, occipital lobes, periventricular white matter and callosum, with low signals in T1 and high signals in T2, FLAIR, and DWI. The lesions had a well demarcated margin and were not enhanced after injection of Gd-DTPA. Proton NMR spectroscopy of the periventricular white matter lesions revealed decreased N-acetyl aspartate (NAA) peak with respect to choline and creatine. Lactic acid peak was not evident (b). The brain MRI at follow-up (10 months) showed decreased size distribution of white matter lesions and reduced high signals (c). After treatment with vitamin B6, the BCAA levels were continuously decreased after 5 days and 3 months (d)

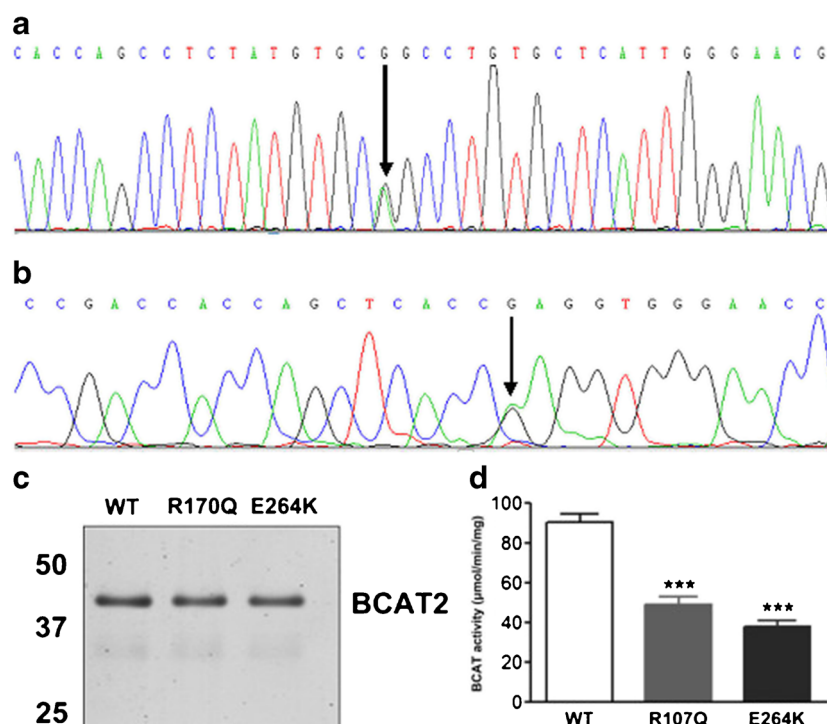


Conditions with elevated BCAA blood levels include starvation, diabetes mellitus, and obesity, which were ruled out for this patient. High valine and leucine-isoleucine levels were observed during the three years follow-up. Therefore, hypervalinemia and hyperleucine-isoleucinemia were likely due to inborn errors of metabolism. MSUD, an inborn error of metabolism caused by severe deficiency in BCKD activity (Strauss and Morton 2003), was the proposed diagnosis. As a consequence of BCKD blockage, BCAAs (leucine, isoleucine, valine, and alloisoleucine) and their corresponding transaminated branched-chain  $\alpha$ -keto acids (BCKAs) accumulate in tissues and body fluids. However, BCKA levels

were not elevated in urine samples collected from this patient. Moreover, no mutation was found in all four *BCKD* genes, and the diagnosis of MSUD was not supported.

Hypervalinemia and hyperleucine-isoleucinemia without elevated BCKA levels raised the possibility that BCAT was genetically altered in this patient. Indeed, hereditary diseases, including hypervalinemia and hyperleucine-isoleucinemia, may result in principle from genetic alterations of the BCAT enzyme protein. In animal studies, ENU mutagenesis was used to identify marked elevation of BCAAs in mice with mitochondrial *BCAT* gene deficiency (Wu et al 2004). These findings have not been confirmed in human by gene mutation

**Fig. 3** *BACT2* gene mutations (a and b), Coomassie blue staining of purified recombinant wt, R170Q and E264K human BCAT2 protein (c); enzyme activity of wt, R170Q and E264K BCAT2 (d, \*\*\*  $P < 0.01$  compared to WT, One-way-ANOVA,  $n = 3$ )



analysis. The present work is the first report of *BCAT2* gene mutations in human. We identified two mutations in the *BCAT2* gene that cause enzyme activity deficiency. Elevated BCAA levels and similar brain white matter lesions were not found in the patient's parents, suggesting an autosomal recessive trait.

Inborn errors of BCAA metabolism, for example MUSD, are more common in children and infants, and adult-onset is rather scarce. Although clinical symptoms were mild in the case reported here, brain MRI data showed serious white matter lesions similar to those observed in MSUD patients (Jan et al 2003; Schonberger et al 2004). In adolescent and young adult MSUD patients, MRI abnormalities appear as increased signals in T2-weighted images of several areas, mainly mesencephalon and brain stem as well as thalamus and globus pallidus (Schonberger et al 2004). In this patient, mainly supratentorial lesions were found without overt abnormalities in brain stem and cerebellum. MRS revealed decreased NAA peak with respect to choline and creatine, demonstrating neuronal damage due to prolonged high brain BCAA concentrations.

This case has provided new insight into the study of BCAA metabolism and its defect in human, since most previous data were obtained in microorganisms. BCAA metabolism in human is not completely understood. It is still a debate whether all three BCAAs are transaminated by a common aminotransferase. In a previous report, a 2 month old baby was described with idiopathic hypervalinemia but without increase in serum leucine and isoleucine levels (Wada et al 1963). A follow-up study demonstrated two years later that hypervalinemia in this

baby was caused by a defect in valine transamination (Wada 1965). These findings indicated the presence of a specific transaminase for valine in humans. However, this has not been confirmed by gene mutation. Leucine levels are usually highest among all BCAAs in patients with MUSD. In the patient presented here, BCAA levels increased, with valine showing the highest content. After treatment with vitamin B6, valine levels decreased more sharply compared with those of leucine and isoleucine. Higher valine levels than leucine or isoleucine were reported in *BCAT2*-deficient mice (Wu et al 2004). Therefore, *BCAT2* might have a higher affinity for valine than leucine and isoleucine.

The mechanisms underlying the brain damage in this patient are not well established. BCAAs cross the blood-brain-barrier more readily than other amino acids. In patients with MSUD, increased leucine plasma concentrations have been associated with the appearance of neurological symptoms and are considered the main neurotoxic metabolites (Yudkoff 1997). In addition, several *in vitro* and *in vivo* studies have indicated that leucine and the BCKAs cause excitotoxicity, energy deficit, and oxidative stress (Barschak et al 2009; Funchal et al 2006; Sgaravatti et al 2003). Another possible mechanism in this patient is an altered glutamate metabolism. *BCAT* catalyzes the transfer of an amino group from a BCAA to  $\alpha$ -ketoglutarate, forming glutamate. Glutamate is the major excitatory neurotransmitter and precursor of the major inhibitory neurotransmitter  $\gamma$ -aminobutyric acid (GABA). *BCATs* are thought to participate in an  $\alpha$ -ketoacid nitrogen shuttle that provides nitrogen for synthesis of glutamate from  $\alpha$ -ketoglutarate (Hull et al 2012). Therefore,

*BCAT* gene mutation could result in abnormal glutamate metabolism. The pathophysiology of hypervalinemia and hyperleucine-isoleucinemia brain damage should be further explored.

*BCAT* utilizes pyridoxal 5'-phosphate (PLP) as a cofactor (Conway et al 2003). Short-term treatment of the patient studied here with vitamin B6 was effective. The levels of BCAAs, especially valine, were decreased remarkably. Further assessment of long term effects of vitamin B6 on BCAA levels and brain white matter lesions is under way in our laboratory.

Overall, our data indicate a new type of branched chain amino acid metabolism disorder caused by mutations of the *BCAT2* gene in an autosomal recessive mode. The treatment with vitamin B6 as coenzyme to *BCAT2* was effective. More clinical and laboratory studies are needed as this was only a single case report. Analysis of *BCAT2* gene is indicated if *BCKD* gene sequencing is normal in sequels with elevated branched chain amino acids.

**Acknowledgments** This work was supported by the Specialized Research Fund for the Doctoral Program of Higher Education (20131107120002). We acknowledge Professor Yanling Yang and Peng Yu for providing metabolite analyses.

#### Compliance with Ethics Guidelines

**Conflict of interest** None.

**Human and animal rights and informed consent** All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (the Institutional Review Board of Xuan Wu Hospital, Capital Medical University, Beijing, China) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Written informed consent was obtained from the patient.

#### References

Barschak AG, Sitta A, Deon M et al (2009) Amino acids levels and lipid peroxidation in maple syrup urine disease patients. *Clin Biochem* 42:462–466

Brosnan JT, Brosnan ME (2006) Branched-chain amino acids: enzyme and substrate regulation. *J Nutr* 136:207S–211S

Chace DH, Hillman SL, Millington DS, Kahler SG, Roe CR, Naylor EW (1995) Rapid diagnosis of maple syrup urine disease in blood spots from newborns by tandem mass spectrometry. *Clin Chem* 41:62–68

Conway ME, Yennawar N, Wallin R, Poole LB, Hutson SM (2002) Identification of a peroxide-sensitive redox switch at the CXXC motif in the human mitochondrial branched chain aminotransferase. *Biochemistry* 41:9070–9078

Conway ME, Yennawar N, Wallin R, Poole LB, Hutson SM (2003) Human mitochondrial branched chain aminotransferase: structural basis for substrate specificity and role of redox active cysteines. *Biochim Biophys Acta* 1647:61–65

Davoodi J, Drown PM, Bledsoe RK, Wallin R, Reinhart GD, Hutson SM (1998) Overexpression and characterization of the human mitochondrial and cytosolic branched-chain aminotransferases. *J Biol Chem* 273:4982–4989

Funchal C, Latini A, Jacques-Silva MC et al (2006) Morphological alterations and induction of oxidative stress in glial cells caused by the branched-chain alpha-keto acids accumulating in maple syrup urine disease. *Neurochem Int* 49:640–650

Hull J, Hindy ME, Kehoe PG, Chalmers K, Love S, Conway ME (2012) Distribution of the branched chain aminotransferase proteins in the human brain and their role in glutamate regulation. *J Neurochem* 123:997–1009

Hutson SM, Sweatt AJ, Lanoue KF (2005) Branched-chain [corrected] amino acid metabolism: implications for establishing safe intakes. *J Nutr* 135:1557S–1564S

Jan W, Zimmerman RA, Wang ZJ, Berry GT, Kaplan PB, Kaye EM (2003) MR diffusion imaging and MR spectroscopy of maple syrup urine disease during acute metabolic decompensation. *Neuroradiology* 45:393–399

Lu J, Xie G, Jia W (2013) Insulin resistance and the metabolism of branched-chain amino acids. *Front Med* 7:53–59

Schonberger S, Schweiger B, Schwahn B, Schwarz M, Wendel U (2004) Dysmyelination in the brain of adolescents and young adults with maple syrup urine disease. *Mol Genet Metab* 82:69–75

Sgaravatti AM, Rosa RB, Schuck PF et al (2003) Inhibition of brain energy metabolism by the alpha-keto acids accumulating in maple syrup urine disease. *Biochim Biophys Acta* 1639:232–238

Strauss KA, Morton DH (2003) Branched-chain ketoacyl dehydrogenase deficiency: maple syrup disease. *Curr Treat Options Neurol* 5:329–341

Sweatt AJ, Wood M, Suryawan A, Wallin R, Willingham MC, Hutson SM (2004) Branched-chain amino acid catabolism: unique segregation of pathway enzymes in organ systems and peripheral nerves. *Am J Physiol Endocrinol Metab* 286:E64–E76

Tom A, Nair KS (2006) Assessment of branched-chain amino acid status and potential for biomarkers. *J Nutr* 136:324S–330S

Wada Y (1965) Idiopathic hypervalinemia: valine and alpha keto-acids in blood following an oral dose of valine. *Tohoku J Exp Med* 87:322–331

Wada Y, Tada K, Minagawa A, Yoshida T, Morikawa T, Okamura T (1963) Idiopathic hypervalinemia: probably a new entity of inborn error of valine metabolism. *Tohoku J Exp Med* 81:46–55

Wu JY, Kao HJ, Li SC et al (2004) ENU mutagenesis identifies mice with mitochondrial branched-chain aminotransferase deficiency resembling human maple syrup urine disease. *J Clin Invest* 113:434–440

Yudkoff M (1997) Brain metabolism of branched-chain amino acids. *Glia* 21:92–98