

Saccharopinuria accompanied by hyperammonemia and hypercitrullinemia presented with elderly-onset epilepsy, progressive cognitive decline, and gait ataxia

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SUMMARY We report a case of saccharopinuria with hyperammonemia and hypercitrullinemia in a Japanese woman who presented with elderly-onset epilepsy, progressive cognitive decline, and gait ataxia. Blood amino acid analysis revealed an increase in citrulline, cystine, and lysine levels, and urine amino acid analysis showed increased citrulline and cystine levels. Urine metabolomics revealed an increased saccharopine level, leading to the definitive diagnosis of saccharopinuria. In western blots of liver biopsy samples, normal citrin levels were observed, suggesting that adult-onset citrullinemia type 2 (CTLN2) was not present. In addition, decreased argininosuccinate synthetase (ASS) levels were observed, and *ASS1* gene, a causative gene for citrullinemia type 1 (CTLN1), was analyzed, but no gene mutations were found. Because the causes of hypercitrullinemia were not clear, it might be secondary to saccharopinuria. Muscle biopsy findings of the biceps brachii revealed diminished cytochrome c oxidase (COX) activity, mitochondrial abnormalities on electron microscopy and p62-positive structures in immunohistochemical analyses. Saccharopinuria is generally considered a benign metabolic variant, but our case showed elevated lysine and saccharopine levels causing ornithine circuit damage, mitochondrial dysfunction, and autophagy disorders. This may lead to so far unknown neurological disorders.

Keywords saccharopinuria, hyperammonemia, hypercitrullinemia, metabolomics, elderly-onset neurological disorders

1. Introduction

Familial hyperlysinemia is an autosomal recessive disease caused by a defect in the bifunctional alpha-aminoacidic semialdehyde synthase (AASS) protein. AASS includes lysine-ketoglutarate reductase (LKR) and saccharopine dehydrogenase (SD) (1,2). A variant of familial hyperlysinemia, saccharopinuria (hyperlysinemia type II), has been described in which only SD activity was undetectable (3). While saccharopinuria is generally considered a benign metabolic variant, there are some case reports that describe that saccharopinuria exhibits neurological features such as epilepsy and intellectual impairment, and all of these cases were infant-adolescent cases as far as we know (4). On the other

hand, hypercitrullinemia is caused by citrullinemia type 1 (CTLN1) with argininosuccinate synthetase (ASS) deficiency and adult-onset citrullinemia type 2 (CTLN2) with citrin deficiency (5,6). CTLN1 and CTLN2 can cause neurological symptoms such as epilepsy and consciousness disturbance.

Herein, we report a rare case of saccharopinuria complicated with hyperammonemia and hypercitrullinemia presenting epilepsy, progressive cognitive decline, and gait ataxia.

2. Case Report

A 70-year-old woman was admitted to our hospital with a chief complaint of generalized convulsion (Figure 1).

Her medical history revealed that she had at least two episodes of emergency transport due to decreased level of consciousness for unknown reasons from around 60 years of age. In addition, she reported a history of progressive ataxic gait. Her past medical history described no particular illness. Her parents were not consanguineous and none of her family members had symptoms similar to hers. She had no specific food preferences.

She had been admitted to our hospital for the first time at 69 years of age to determine the cause of the intermittent decline in consciousness level. She was relatively short (height 134 cm) and clinically obese (weight 58.0 kg, body mass index 32.3). Neurological examination performed when her consciousness was clear revealed cognitive decline (Wechsler Adult Intelligence Scale-III, 68), ataxic gait, and deep sensory deficit in her lower limbs. Flapping tremor was not detected. In blood tests, hyperammonemia (75 $\mu\text{g/dL}$) and abnormal glucose tolerance (HbA1c, 6.5%) were

observed. Metabolic disorder was suspected owing to hyperammonemia. Further blood amino acid analysis showed increased levels of citrulline (206.2 nmol/mL, normal range 17.9-48 nmol/mL), cystine (188.5 nmol/mL, normal range 4.7-34.8 nmol/mL), and lysine (1,170.4 nmol/mL, normal range 125.7-281.9 nmol/mL). In addition, urine amino acid analysis showed increased levels of citrulline (396.5 $\mu\text{mol/g}\cdot\text{cre}$, normal range 2-41 $\mu\text{mol/g}\cdot\text{cre}$) and cystine (9,546.4 $\mu\text{mol/g}\cdot\text{cre}$, normal range 13-76 $\mu\text{mol/g}\cdot\text{cre}$). Brain T2-weighted magnetic resonance imaging (MRI) revealed high intensities in the bilateral middle cerebellar peduncles and bilateral precentral gyrus (Figure 2). Based on these results, CTLN2 was suspected, as were fragile X syndrome, mitochondrial disorders, and spinocerebellar ataxias (SCA).

All-exon sequencing of the *SLC25A13* gene, which is a causative gene for CTLN2, was performed, but no gene mutations were found. Genetic tests for fragile X syndrome (*FMRI*), mitochondrial disorders (A1555G,

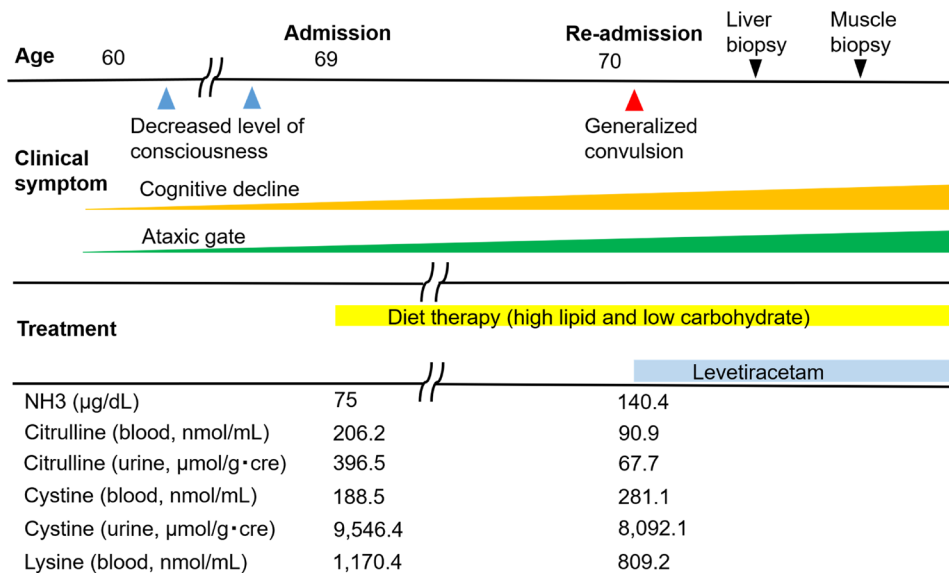


Figure 1. Clinical course of an elderly woman with saccharopinuria accompanied by hyperammonemia and hypercitrullinemia. Since around 60 years of age, the patient had at least two episodes of emergency transport owing to an unexplained decreased level of consciousness. At 69 years of age, she was admitted to our hospital for the first time to identify the cause of the unexplained decreased level of consciousness. At 70 years of age, she was readmitted due to seizures. She was diagnosed with saccharopinuria by metabolomics.

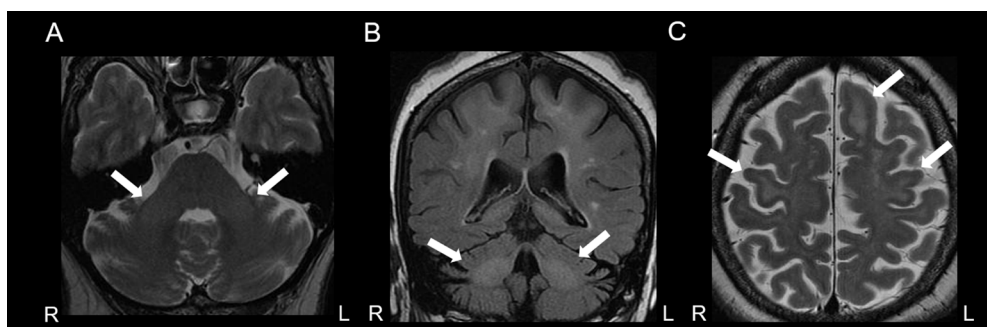


Figure 2. Brain MRI findings for an elderly woman with saccharopinuria accompanied by hyperammonemia and hypercitrullinemia. (A-C) Brain T2-weighted MRI and fluid-attenuated inversion recovery imaging show high-intensity lesions in the bilateral middle cerebellar peduncles and bilateral precentral gyrus (white arrows). MRI, magnetic resonance imaging.

T3271C, A8344G, T8363A, T8993G/C, T9176C, G11778A, G13513A, and A3243G), and common SCA in the Japanese population (SCA1, SCA2, SCA3, SCA4, SCA6, SCA7, SCA8, SCA12, SCA17, SCA31 and dentatorubral-pallidoluysian atrophy) also yielded negative results. Without a definitive diagnosis, the only clinical support that could be provided was moderate dietary guidance (high lipid and low carbohydrate).

At 70 years of age, she was readmitted owing to the development of generalized convulsions. Her cognitive decline had progressed since the last hospitalization. As with the first hospitalization, blood tests showed hyperammonemia (140.4 µg/dL), and blood amino acid

analysis showed increased levels of citrulline (90.9 nmol/mL), cystine (281.1 nmol/mL), and lysine (809.2 nmol/mL). In addition, urine amino acid analysis showed increased levels of citrulline (67.7 µmol/g-cre) and cystine (8,092.1 µmol/g-cre). Urine organic acid analysis showed no increase in orotic acid level and uracil excretion, and blood amino acid analysis showed that arginine was within the normal limits. Further testing to determine the cause of her hyperammonemia was conducted, but no indication of gastrointestinal diseases, such as portal venous circulation shunts and cirrhosis, was discovered. Although a liver biopsy was performed, citrin was detected, which suggested that CTLN2 was not present. However, we observed a decrease in ASS (Figure 3). All-exon sequencing of the *ASS1* gene, which is a causative gene for CTLN1, was performed, but no gene mutations were found.

A muscle biopsy sample of the biceps brachii was taken, and structures positive for periodic acid Schiff (PAS) staining were found in the muscle fiber cytoplasm. The nicotinamide adenine dinucleotide dehydrogenase-tetrazolium reductase (NADH-TR) stained sections showed defective areas with a moth-eaten appearance, as well as an irregular intramyofibrillar network. The cytochrome c oxidase (COX)-stained sections showed diminished COX activity. Structures positive for p62 staining were found in the muscle fiber cytoplasm. Electron microscopy showed marked mitochondrial proliferation of different sizes (Figure 4). Filter paper blood screening for Pompe disease yielded normal acid alpha-glucosidase (GAA) activity.

Blood and urine amino acid analysis had been performed three times in total, and reproducibility of the tests was confirmed. For the first and second tests, blood

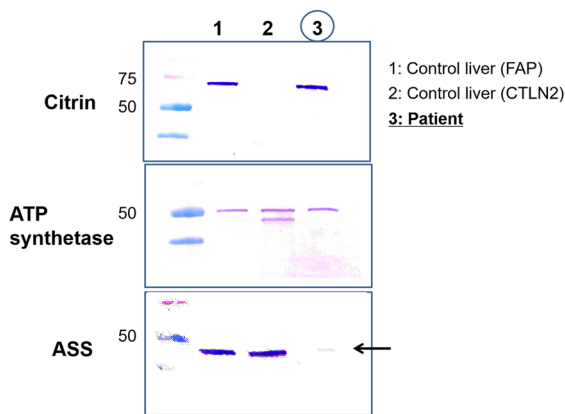


Figure 3. Western blot of liver biopsy. 1 = control liver (FAP), 2 = control liver (CTLN2), 3 = our patient. In our patient, citrin was detected, suggesting the absence of CTLN2. However, a decrease in ASS was observed (a black arrow). FAP, familial amyloid polyneuropathy; CTLN2, adult-onset citrullinemia type 2; ASS, argininosuccinate synthetase; ATP, adenosine triphosphate.

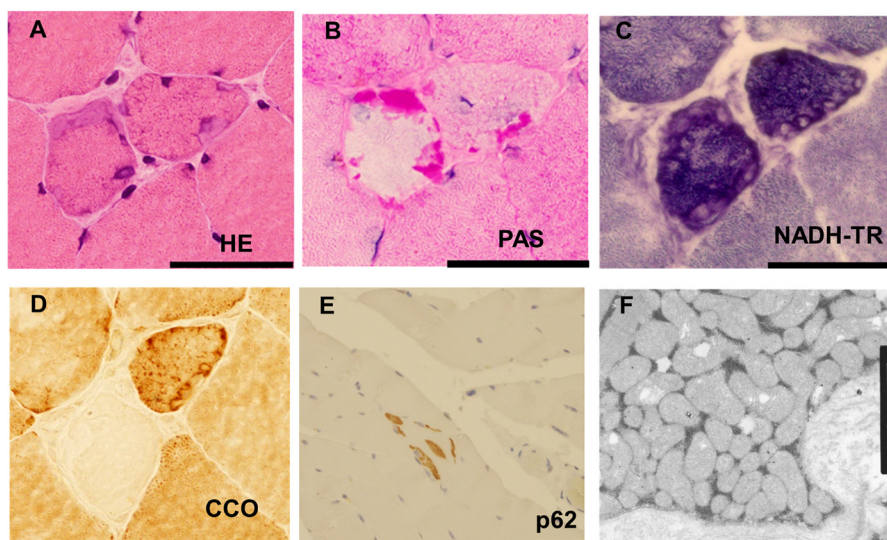


Figure 4. A muscle biopsy of the biceps brachii. (A) HE stain. (B) Structures positive for PAS staining were found in the muscle fiber cytoplasm. (C) The NADH-TR-stained sections showed defective areas with a moth-eaten appearance, as well as an irregular intramyofibrillar network. (D) The COX-stained sections showed diminished COX activity. (E) Structures positive for p62 staining were found in the muscle fiber cytoplasm. (F) Electron microscopy showed marked mitochondrial proliferation of different sizes. Scale bar 50 µm in (A)-(E). HE, hematoxylin and eosin; PAS, periodic acid Schiff; NADH-TR, nicotinamide adenine dinucleotide dehydrogenase-tetrazolium reductase; COX, cytochrome c oxidase.

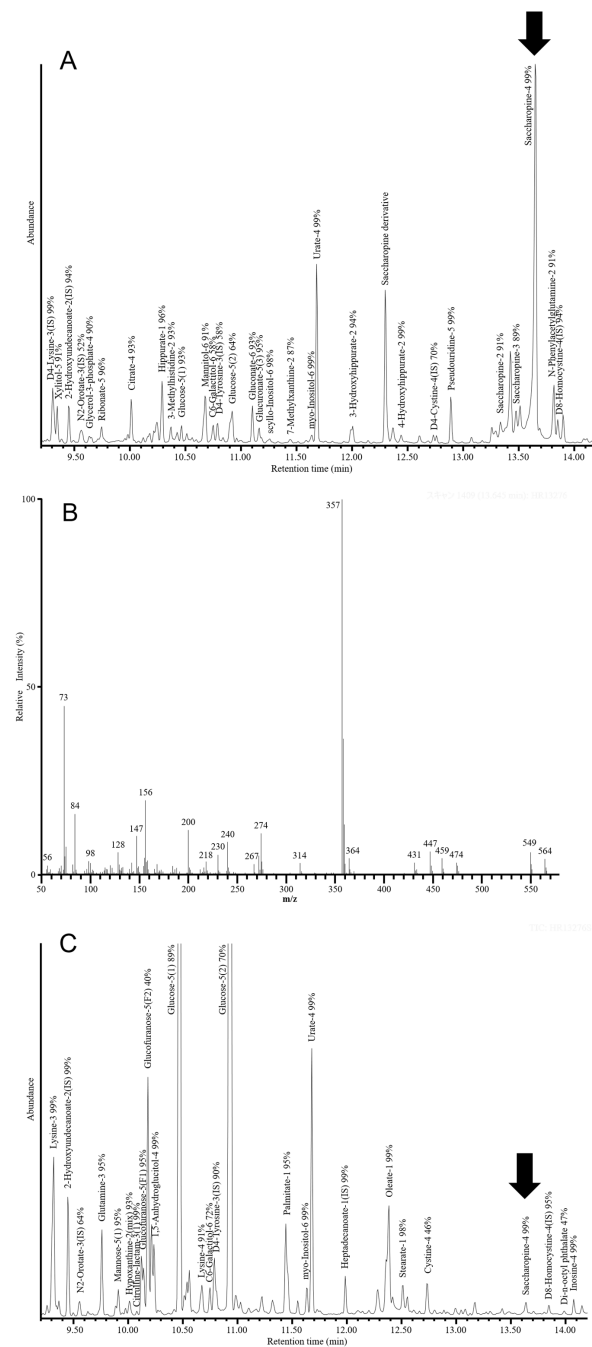


Figure 5. The total ion current chromatogram obtained by GC/MS-based metabolomics of spot urine sample from a 70-year-old female patient. (A) A huge peak was detected at retention time of 13.65 min and the component was identified as saccharopine from the patient sample (a black arrow). (B) The mass spectrum of the prominent peak at 13.65 min. (C) The total ion current chromatogram obtained by GC/MS-based metabolomics of serum from the same patient. A peak was also detected at retention time of 13.65 min and the mass spectrum of the component was identical to saccharopine (a black arrow). Hyperlysinemia was clearly shown from the peak at 9.314 min (lysine 3-trimethylsilyl derivative). GC, gas chromatography; MS, mass spectrometry.

and urine were analyzed simultaneously. In all cases, citrulline, cystine, and lysine levels were increased in the blood, and citrulline and cystine levels were increased in the urine.

Considering the possibility of hyperlysinemia, metabolomics was performed. Sample preparation and gas chromatography (GC)/ mass spectrometry (MS) measurement were performed as described previously (7,8). In Figure 5, the total ion current chromatogram obtained by GC/MS-based metabolomics of spot urine sample from the patient is shown. A huge peak was detected at retention time of 13.65 min (Figure 5A) which is not seen in control subjects or patients with other inborn errors of metabolism. The mass spectrum of this peak is shown in Figure 5B. This component was identified as saccharopine 4-trimethylsilyl derivative because the retention time and the mass spectrum of this peak were the same as those of authentic saccharopine. In Figure 5C, the total ion current chromatogram obtained by GC/MS-based metabolomics of serum from the same patient is shown. Although the component of the peak at 13.65 min had the same mass spectrum of saccharopine 4-trimethylsilyl derivative (as shown in Figure 5B), it was clear that the urine is superior to the serum for the detection of saccharopinuria. Serum lysine was markedly high (+8 standard deviation) suggesting that she had severe hyperlysinemia. Therefore, we diagnosed the patient with saccharopinuria.

3. Discussion

We derived two important findings from the present case. First, elderly-onset epilepsy and progressive cognitive decline and gait ataxia can occur with saccharopinuria accompanied by hyperammonemia and hypercitrullinemia. Second, metabolomics is particularly useful for the diagnosis of saccharopinuria.

Previously, saccharopinuria was thought to present no neurological symptoms, but this issue is now controversial. There are some reports that describe that saccharopinuria may cause neurological symptoms (4), which are now corroborated by this report. The patient's complicating hypercitrullinemia might also have affected her symptoms. CTLN2 is an adult-onset hypercitrullinemia, but it was ruled out diagnostically in this case, as citrin was detected and the genetic test yielded negative results. An enzyme deficiency in ASS, the cause of CTLN1, was recognized in our patient. CTLN1 includes an acute neonatal form and a milder late-onset form (9). In view of the possibility of milder late-onset CTLN1, genetic analysis of *ASS1* was performed and was negative. In contrast, saccharopinuria has been reported with and without hypercitrullinemia (10,11). There are reports that saccharopine and lysine may inhibit normal urea cycle function due to inhibition of ASS and argininosuccinate lyase (ASL), and cause hypercitrullinemia (12), as was also the case with our patient. Urea cycle dysfunction may also have led to hyperammonemia because no other cause was identified.

In addition, it had been reported that elevation of saccharopine caused abnormalities in mitochondrial

function (13,14), and it had been demonstrated that lysine and saccharopine suppress autophagic-proteolysis through the Akt pathway (15). In this case, muscle biopsy findings revealed diminished COX activity, mitochondrial abnormalities on electron microscopy and p62-positive structures in immunohistochemical analyses. It is known that autophagy disorders cause p62 accumulation (16). Mitochondrial dysfunction and autophagic failure may have occurred due to saccharopinuria and may be associated with neurological symptoms.

In this case, metabolomics was particularly useful for the diagnosis of saccharopinuria. The patient's epilepsy, cognitive decline, and gait ataxia were accompanied by hyperammonemia, and metabolic disorders were suspected. Amino acid analysis, a liver biopsy and muscle biopsy did not lead to a diagnosis, but the diagnosis of saccharopinuria was soon made by metabolomics. It is important to note, regarding the results of the amino acid analysis, that cystine and saccharopine are indistinguishable chromatographically. Our case also showed elevated levels of cystine in blood and urine, which may have reflected elevated saccharopine levels. For this reason, metabolomics is extremely useful and should be performed at an early stage.

In conclusion, our case findings show that saccharopinuria with hyperammonemia and hypercitrullinemia causes elderly-onset epilepsy, progressive cognitive decline, and gait ataxia, which may be due to ornithine circuit damage, mitochondrial dysfunction, and autophagy disorders.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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