

Electronic letter

A Chinese adult onset type II citrullinaemia patient with 851del4/1638ins23 mutations in the *SLC25A13* gene

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EDITOR—Classical citrullinaemia (CTLN1) is a rare metabolic disease caused by the deficiency of argininosuccinate synthetase (ASS, E.C.6.3.4.5), which usually has its onset in neonates or young infants.¹ These patients may present with acute onset of disturbance of consciousness and hyperammonaemia. ASS activity is very low in all tissues tested and over 30 mutations of the ASS gene have been identified in about 50 CTLN1 patients.² However, in Japan, many cases with adult onset type II citrullinaemia (CTLN2) have been reported.³ A previous study in CTLN2 showed a specific deficiency of ASS protein in the liver, but no ASS gene mutation could be found.⁴ Recently, the gene responsible for CTLN2 was identified by homozygosity mapping.⁵ This gene, *SLC25A13*, encodes a putative calcium dependent mitochondrial carrier protein (called citrin). A loss of organisation caused by the absence of functional citrin has been proposed to lead to the reduction of ASS protein in the liver.

In this report, we present a Chinese patient with CTLN2. She was well until 40 years of age, when she underwent surgery for chronic otitis media. Chronic renal insufficiency was discovered during her stay in hospital and renal parenchymal disease was suspected. Two months later, an acute alteration of consciousness occurred with a blood ammonia level up to 269 $\mu\text{mol/l}$. Subsequent disturbances of consciousness of variable severity, ranging from coma to drowsiness, occurred once or twice a month thereafter. Some of the attacks were associated with large meals, but her condition did not improve after protein restriction. She had a peculiar fondness for eggs from early childhood.

A plasma amino acid analysis showed a raised citrulline level (672.5 $\mu\text{mol/l}$, normal 25–75 $\mu\text{mol/l}$), but normal to mildly raised arginine levels (up to 390 $\mu\text{mol/l}$, normal 45–176 $\mu\text{mol/l}$). Subsequently, plasma citrulline varied greatly from 225 to 1375 $\mu\text{mol/l}$. A diagnosis of lysinuric protein intolerance was excluded by normal urinary excretion of lysine. Skin and liver biopsies were performed after informed consent. ASS activity in her skin fibroblasts was normal. Analyses of her liver

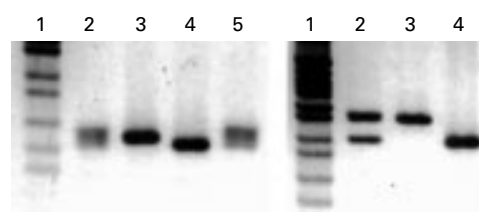


Figure 1 Mutation analysis of the *SLC25A13* gene. Left panel: 851del4 mutation. The sizes of PCR products are 82 bp for wild type and 78 bp for mutant. Lanes 1–5 are DNA size marker, heterozygote control, normal, homozygote control, and the patient, respectively. The heterozygotes may show three bands because of the formation of a heteroduplex. Right panel: 1638ins23 mutation. The sizes are 123 bp for wild type and 146 bp for mutant. Lanes 1–4 are DNA size marker, the patient, homozygote control, and normal, respectively.

biopsy specimen showed normal carbamoyl-phosphate synthetase, ornithine carbamoyl-transferase, argininosuccinate lyase, and arginase activities. However, ASS activity, measured by the method of Su *et al.*,⁶ was very low in her liver (0.156 U/g liver, normal 2.59±1.13 U/g liver). She had a very high serum pancreatic secretory trypsin inhibitor level (631 ng/ml, normal 5.9–22.7 ng/ml), but both abdominal echo and tumour markers, including αFP and CEA, were normal. Genomic DNA was extracted from her peripheral blood leucocytes and mutation analysis of the *SLC25A13* gene was performed as described previously.⁵ The results showed that she was a compound heterozygote for 851del4 and 1638ins23 mutations (fig 1). Both mutations detected in this patient have been reported in Japan and 851del4 is actually the most common mutation in Japanese patients.^{5,7} CTLN2 appears to be almost exclusively found in Japan, but a suspected case has been reported in a Chinese.⁸ Because Japanese and Chinese are ethnically related, it is not surprising to find a case in Taiwan. However, it is important to note that the mutations may be as common in Chinese as in Japanese if the mutations have originated from the common ancestor for Japanese and Chinese.

Initial treatment of the patient consisted of a low protein diet plus sodium phenylbutyrate (Buphenyl), but she tolerated sodium phenylbutyrate poorly. Oral citrulline 6 g/day was given before the establishment of the diagnosis

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because of the suspicion of lysinuric protein intolerance.⁹ When the correct diagnosis was made, plans were made to discontinue the citrulline; however, the patient insisted on maintaining the supplement. After two months of treatment with oral citrulline, the frequency of her episodes began to decrease and she has had no attack in the past year on a maintenance dose of 3 g per day of citrulline (around 60 mg/kg/day). Her plasma citrulline stabilised at a level below 500 $\mu\text{mol/l}$ (362.5–432.5 $\mu\text{mol/l}$).

In CTLN2, deficiency of ASS is limited to the liver. Renal ASS can still convert citrulline and aspartate into argininosuccinate, which can then be excreted in the urine.¹⁰ We are not sure if the citrulline supplement did help this patient. However, if progressive renal parenchymal loss were a trigger factor in this patient, then her prognosis may not be good.

During the preparation of this manuscript, her renal condition deteriorated further with serum creatinine increased from 2.6 mg/dl to 6.48 mg/dl. Severe anaemia occurred which required repeated transfusion. Episodes with hyperammonaemia and disturbance of consciousness increased in frequency. She is now under evaluation for combined liver-kidney transplantation. We believe that the presentation of this case will increase our understanding of this interesting disease.

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- 1 Brusilow SW, Horwich AL. Urea cycle enzymes. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic and molecular bases of inherited disease*. 7th ed. Vol 1. New York: McGraw-Hill, 1995:1187-232.
- 2 Kakinoki H, Kobayashi K, Terazono H, Nagata Y, Saheki T. Mutations and DNA diagnosis of classical citrullinemia. *Hum Mutat* 1997;9:250-9.
- 3 Kobayashi K, Horiuchi M, Saheki T. Pancreatic secretory trypsin inhibitor as a diagnostic marker for adult-onset type II citrullinemia. *Hepatology* 1997;25:116-15.
- 4 Kobayashi K, Shaheen N, Kumashiro R, Tanikawa K, O'Brien WE, Beaudet AL, Saheki T. A search for the primary abnormality in adult-onset type II citrullinemia. *Am J Hum Genet* 1993;53:1024-30.
- 5 Kobayashi K, Sinasac DS, Iijima M, Boright AP, Begum L, Lee JR, Yasuda T, Ikeda S, Hirano R, Terazono H, Crackower MA, Kondo I, Tsui LC, Scherer SW, Saheki T. The gene mutated in adult-onset type II citrullinemia encodes a putative mitochondrial carrier protein. *Nat Genet* 1999;22:159-63.
- 6 Su TS, Beaudet AL, O'Brien WE. Increased translatable messenger ribonucleic acid for argininosuccinate synthetase in canavanine-resistant human cells. *Biochemistry* 1981;20:2956-60.
- 7 Yasuda T, Yamaguchi N, Kobayashi K, Nishi I, Horinouchi H, Jalil MA, Li MX, Ushikai M, Iijima M, Kondo I, Saheki T. Identification of two novel mutations in the SLC25A13 gene and detection of seven mutations in 102 patients with adult-onset type II citrullinemia. *Hum Genet* 2000;107:537-45.
- 8 Chow WC, Hg HS, Tan IK, Thum TY. Case report: recurrent hyperammonemic encephalopathy due to citrullinemia in a 52 year-old man. *J Gastroenterol Hepatol* 1996;11:621-5.
- 9 Rajantie J, Simell O, Rapola J, Perheentupa J. Lysinuric protein intolerance: a two-year trial of dietary supplementation therapy with citrulline and lysine. *J Pediatr* 1980;97:927-32.
- 10 Saheki T, Kobayashi K, Matuo S, Hagihara S, Noda T. Increased urinary excretion of argininosuccinate in type II citrullinemia. *Clin Chim Acta* 1987;170:297-304.