CASE REPORT

Hyperammonaemic encephalopathy in an adult patient with citrin deficiency associated with a novel mutation

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We report on an adult patient with citrin deficiency in Hong Kong, in whom a novel mutation was identified. The patient presented with recurrent hyperammonaemic encephalopathy due to impairment of the liver urea cycle enzyme argininosuccinate synthetase. This autosomal recessive condition is also characterised by interesting food preferences, notably aversion to carbohydrates and craving for protein-rich and/or lipid-rich foods, as well as neuropsychiatric symptoms. Plasma amino acid analysis is very useful in revealing urea cycle disorders, and mutational analysis of the *SLC25A13* gene can confirm the diagnosis.

Case report

In July 2009, a 42-year-old man with a history of chronic hepatitis C, heroin and hypnotic abuse and a 'stormy' life history was admitted for mental confusion. He was a non-drinker. Prior to this admission, he had been repeatedly admitted to various hospitals for fluctuating conscious states, drug overdoses, accidents, and suicides; and had crossed paths with the police and the legal system a number of times. Physical examination revealed a mentally slow and disorientated man, with no focal neurological signs. His body mass index was 17 kg/m^2. He had no fever and no stigmata of chronic liver disease. Computed tomographic brain showed a suspicious hypodense lesion in the left frontal lobe, later demonstrated by magnetic resonance imaging to be residual changes from previous insults.

Investigation results showed serum levels as follows: ammonia 380 μmol/L (reference range [RR], 14.7-55.3 μmol/L), albumin 41 g/L (RR, 35-52 g/L), globulin 35 g/L (RR, 22-36 g/L), bilirubin 18 μmol/L (RR, 5-27 μmol/L), alkaline phosphatase 113 IU/L (RR, 39-97 IU/L), alanine aminotransferase 53 IU/L (RR, 13-53 IU/L), alpha-fetoprotein 5 IU/mL (reference level, <6 IU/mL). Moreover his international normalised ratio was <1.0. Ultrasonography revealed liver echogenicity to be coarse, suggesting parenchymal liver disease. The spleen was not enlarged, and there was no ascites. Fibroscan revealed a mild degree of liver fibrosis only. Electrolytes, glucose, and renal function were normal. Fasting triglyceride was 1.4 mmol/L, total cholesterol was 3.6 mmol/L. Blood counts and morphology were normal apart from a marginally low platelet count of 99 x 10^9 /L (RR, 145-370 x 10^9 /L). Urine drug screen, septic workup, and echocardiogram were negative.

The patient was treated with anti-hepatic encephalopathy regimens as well as broad-spectrum antibiotics. After regaining orientation, he revealed his interesting dietary habits since childhood, with strong preferences for peanuts, beans, preserved bean curds, and cheese rings. Unlike others, even as a child he had an aversion to sweets. When he ate instant noodles, he consumed only the seasoning packs, discarding the noodle. He was born at full term of non-consanguineous parents, and had no history of neonatal jaundice. His father suffered from a hepatoma and chronic hepatitis B infection. None of his six siblings shared his dietary fads or medical history.

Plasma amino acid analysis (Table) showed markedly elevated citrulline and arginine levels. The ratio of threonine-to-serine was also high. The ratio of branched-chain amino acids (valine+leucine+isoleucine) to aromatic amino acids (tyrosine+phenylalanine) was low. The amino acid pattern was characteristic of citrin deficiency (type II citrullinaemia). The diagnosis was confirmed by mutational analysis of the *SLC25A13* gene, which showed that the patient was compound heterozygous for two mutations: c.851_854delGTAT and c.1231G>A, the latter changing codon 411 from GTG to ATG, that is, p.V411M (Fig). His father was heterozygous for c.851_854delGTAT and his mother was heterozygous for c.1231G>A.

His mental state waxed and waned. Neither the mental confusion nor ammonia level responded to a trial of oral arginine therapy, at a starting dose of 7.2 g daily (150 mg/

Key words
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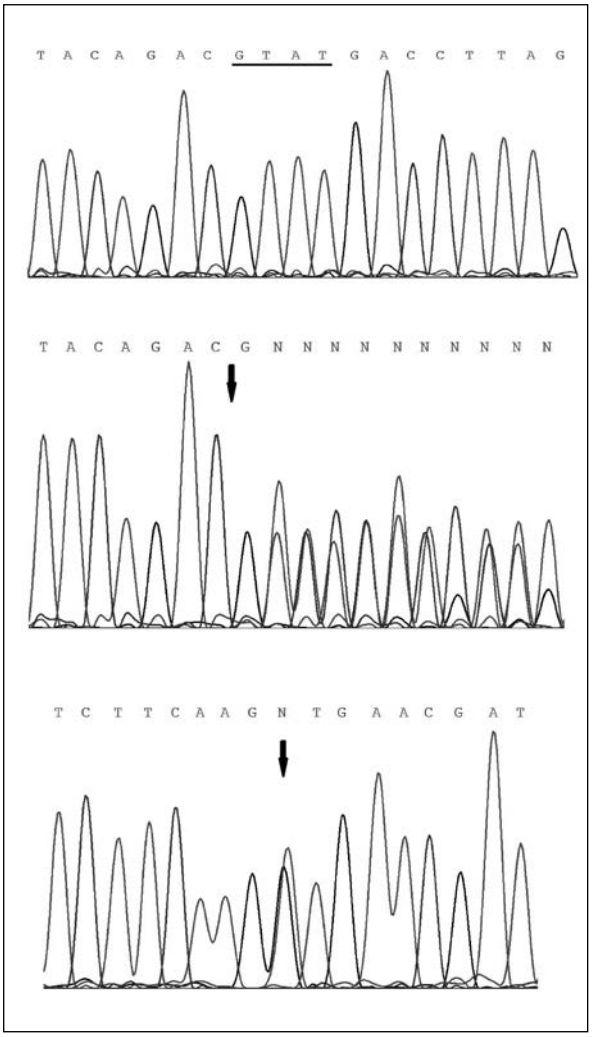

FIG. Electropherograms of segments of the *SLC25A13* gene showing the mutation sites in the patient. The letter N indicates the heterozygous sites and the arrows indicate the mutation sites. All are in sense direction

Upper panel: wild-type sequence for segment of exon 9; the four nucleotides deleted in the patient are underlined. Middle panel: heterozygous deletion of c.851_854; the arrow indicates the breakpoint. Lower panel: heterozygous p.V411M (GTG→ATG)

一名與新型基因突變有關的citrin蛋白缺乏症成年患者的高血氨腦病

本文報告在一名citrin蛋白缺乏症香港成年患者身上發現一種新型的基因突變。病人因肝尿素循環精氨酸代琥珀酸合成酶受到破壞而出現復發性高血氨腦病。這種常染色體隱性遺傳病的特徵是患者對於高蛋白及高脂肪食物有特殊愛好，同時對於高碳水化合物的食物感到厭惡，並會出現神經精神症狀。血漿氨基酸分析對於檢測尿素循環障礙病很有幫助，也可進行*SLC25A13*基因突變檢查來確認診斷。

kg/d) and escalated to 18 g/d. Nor did they change in response to dietary protein intakes ranging from 20 to 80 g daily or to N-acetylcysteine 600 mg twice daily. Peanuts 160 g twice weekly was given in accordance with his usual habit. A relatively low carbohydrate diet containing 218 g carbohydrate (43% daily caloric intake), 75 g protein (15%), 96 g fat (42%) was allowed. He was referred for a drug abstinence programme and consideration of liver transplantation. However, because of his other problems, he was not considered a suitable candidate. Till August 2010, he continued to be repeatedly admitted for recurrent episodes of mental confusion due to metabolic encephalopathy.

Discussion

We report here a case of citrin deficiency (type II citrullinaemia) in a Hong Kong Chinese adult with a newly identified mutation. Citrin deficiency is related to the urea cycle disorders. It was primarily reported in the Japanese population. In adults it is characterised by transient intrahepatic cholestasis in the neonate and a myriad of neuropsychiatric features, ranging from aggression, irritability, delusions, disorientation, drowsiness, loss of

TABLE. Plasma amino acid analysis of the patient

Plasma amino acid	Concentration (μmol/L)	Reference range (μmol/L)
Citrulline	311	19-47
Arginine	225	28-96
Threonine	226	92-180
Serine	78	89-165
Threonine-to-serine ratio	2.9	0.9-1.2
Valine	158	179-335
Leucine	87	113-205
Isoleucine	53	46-90
Tyrosine	71	37-77
Phenylalanine	82	46-74
Aromatic amino acids to branched-chain amino acids ratio	1.95	2.76-4.08

memory, flapping tremor, seizures, and coma. Often the disorder is unmasked by sepsis, alcohol intake, surgery, drugs such as sodium valproate, or urinary tract infection with urease-producing organisms.

The urea cycle consists of a series of biochemical reactions that converts nitrogen, the waste product of protein metabolism, into urea. In mammals, the urea cycle takes place primarily in the liver, and to a lesser extent in the kidneys. In urea cycle disorders, the body's nitrogenous waste products accumulate in the form of ammonia, which is neuro-toxic. Urea cycle dysfunction may occur as a result of inborn errors of metabolism, or liver failure from any cause.

Type I citrullinaemia, one of the classical urea cycle disorders, is caused by mutations in the argininosuccinate synthetase (ASS) gene in liver and other organs. Argininosuccinate synthetase is one of the cytosolic enzymes involved in the urea cycle, catalysing the conversion of citrulline and aspartate to argininosuccinate. The latter is further converted to arginine and fumarate by argininosuccinate lyase. In type I citrullinaemia, plasma amino acid measurement reveals a pattern of elevated citrulline and decreased arginine concentrations.

In contrast, type II citrullinaemia is caused by mutations in the *SLC25A13* gene on chromosome 7q21.3, which codes for citrin. Citrin is a mitochondrial solute carrier that is expressed in the liver, heart and kidneys, and plays a role in: (i) transporting aspartate from the mitochondria to the cytosol, where it reacts with citrulline in the process of urea, protein and nucleotide synthesis; (ii) the malate-aspartate shuttle, transporting nicotinamide adenine dinucleotide plus hydrogen (NADH) reducing equivalent from the cytosol into the mitochondria; and (iii) gluconeogenesis from lactate. Apart from hyperammonaemic encephalopathy, other clinical features seen in our patient and most other cases can be explained by the metabolic disturbances secondary to citrin deficiency. For example, cytosolic aspartate deficiency and defective protein synthesis may account for the craving for high protein, especially beans and peanuts that are rich sources of aspartate and asparagine. The accumulation of NADH in the cytosol leads to inhibition of glycolysis, fatty acid oxidation, galactose and alcohol metabolism. This possibly explains why some of these patients have an aversion to carbohydrates, experience neuropsychiatric symptoms after alcohol, have fatty livers and hyperlipidaemia, and why the galactosaemia is encountered in some neonates. Hypoglycaemia in neonates with this consideration may be caused by the inhibition of gluconeogenesis from lactate.[1,2]

Unlike in type I citrullinaemia, in citrin deficiency both the precursor levels of citrulline and the product arginine for ASS are elevated. Arginine in this condition is thought to be produced by renal ASS, since in citrin deficiency the ASS deficiency is confined to the liver. The reason for the slow decline and the organ specificity of ASS deficiency in citrin deficiency is unknown, although these are postulated to account for its late presentation in adulthood, usually between 20 and 50 years of age.

The diagnosis of citrin deficiency is best confirmed by genetic studies, since more than 95% of patients have mutations in the *SLC25A13* gene. In Chinese patients, the three mutational hotspots are: c.851_854delGTAT (also known as mutation [I]) that was detected in our patient, c.1638_1660dup (mutation [III]), and IVS6+5G>A (mutation [X]).[3,4] All of these have previously been found in other patients in our hospital, who presented with the neonatal form of the disease. The other mutation identified in this patient, c.1231G>A, is a novel mutation. Although we have not performed functional studies, this mutation has not been identified in 150 normal subjects studied by us, and we believe it is not a benign polymorphism. To identify novel mutations in citrin deficiency in a cost-effective manner, we recommend that laboratories provide a molecular diagnostic service for this condition based on a two-tier testing strategy. The first-tier testing should be on the three hotspots, followed by second-tier mutational analysis on the rest of the gene only if no or only one heterozygous mutation is detected in the first-tier testing. Among the Chinese, the carrier rates for *SLC25A13* gene mutations have been reported to vary from 1/940 in the north to 1/48 (south of Yangtze River).[3]

In contradistinction to hyperammonaemic encephalopathy caused by cirrhosis, in citrin deficiency a low carbohydrate and high protein diet is advocated.[5] The use of arginine and sodium pyruvate had also been described in some studies.[6] Nevertheless, we were unable to detect any favourable effect after implementing this regimen in our patient. To ameliorate the oxidative stress observed in citrin deficiency,[7] we also tried giving the patient N-acetylcysteine, again to no avail. Liver transplantation offers the only specific therapy for this rare metabolic disease, unfortunately he was considered ineligible because of his drug abuse and personality problems. Whether these problems were secondary to his metabolic disorder remain enigmatic.

Conclusion

Hyperammonaemic encephalopathy is a common clinical condition in Hong Kong. It is often caused by hepatic decompensation. However, in patients who do not have other evidence of hepatic decompensation, analysis of plasma amino acids may be necessary to exclude citrin deficiency or other urea cycle disorders.

References

1. Kobayashi K, Saheki T. Citrin deficiency. In: Pagon RA, Bird TC, Dolan CR, Stephens K, editors. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-. 2005 Sep 16 [Updated 2008 Jul 2]. NCBI Website: http://www.ncbi.nlm.nih.gov/books/NBK1181. Accessed May 2011.
2. Saheki T, Kobayashi K. Mitochondrial aspartate glutamate carrier (citrin) deficiency as the cause of adult-onset type II citrullinemia (CTLN2) and idiopathic neonatal hepatitis (NICCD). J Hum Genet 2002;47:333-41.
3. Lu YB, Kobayashi K, Ushikai M, et al. Frequency and distribution in East Asia of 12 mutations identified in the *SLC25A13* gene of Japanese patients with citrin deficiency. J Hum Genet 2005;50:338-46.
4. Tabata A, Sheng JS, Ushikai M, et al. Identification of 13 novel mutations including a retrotransposal insertion in *SLC25A13* gene and frequency of 30 mutations found in patients with citrin deficiency. J Hum Genet 2008;53:534-45.
5. Saheki T, Kobayashi K, Terashi M, et al. Reduced carbohydrate intake in citrin-deficient subjects. J Inherit Metab Dis 2008;31:386-94.
6. Mutoh K, Kurokawa K, Kobayashi K, Saheki T. Treatment of a citrin-deficient patient at the early stage of adult-onset type II citrullinemia with arginine and sodium pyruvate. J Inherit Metab Dis 2008 Oct 29. Epub ahead of print. Springerlink website: http://www.springerlink.com/content/c3nx211817661209/fulltext.html. Accessed May 2011.
7. Nagasaka H, Okano Y, Tsukahara H, et al. Sustaining hypercitrullinemia, hypercholesterolemia and augmented oxidative stress in Japanese children with aspartate/glutamate carrier isoform 2-citrin deficiency even during the silent period. Mol Genet Metab 2009;97:21-6.