**HYPEREXCRETION OF HOMOCITRULLINE IN A MALAYSIAN PATIENT WITH LYSINURIC
PROTEIN INTOLERANCE**

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ABSTRACT

Lysinuric Protein Intolerance (LPI, MIM 222700) is an inherited aminoaciduria with an autosomal recessive mode of inheritance. Biochemically, affected patients present with increased excretion of the cationic amino acids; lysine, arginine and ornithine. We report the first case of LPI diagnosed in Malaysia presented with excessive excretion of homocitrulline. The patient was a 4 years old male who presented with delayed milestones, recurrent diarrhea and severe failure to thrive. He developed hyperammonemic coma following a forced protein-rich diet. Plasma amino acids analysis showed increased glutamine, alanine and citrulline; but decreased lysine, arginine and ornithine. Urine amino acids showed a marked excretion of lysine and ornithine together with a large peak of unknown metabolite which was subsequently identified as homocitrulline by tandem mass spectrometry. Molecular analysis confirmed a previously unreported homozygous mutation at exon 1 (235G>A, p.Gly79Arg) in the *SLC7A7* gene. This report demonstrates a novel mutation in the *SLC7A7* gene in this rare inborn error of diamino acid metabolism. It also highlights importance of early and efficient treatment of infections and dehydration in these patients. *Conclusion:* The diagnosis of LPI is usually not suspected by clinical findings alone and specific laboratory investigations and molecular analysis is important to get a definitive diagnosis.

Keywords: Lysinuric Protein Intolerance (LPI); dibasic amino acids; homocitrulline

INTRODUCTION

Lysinuric Protein Intolerance (LPI, MIM 222700) is an inherited aminoaciduria caused by a defective cationic amino acid transport at the basolateral membrane of epithelial cells in the intestine and kidney [8]. Mutations in the *SLC7A7* gene which encodes the γ^+ LAT-1 protein have been found to be responsible for this transport defect [8]. Finland, southern Italy and northern Japan have a high prevalence of these cases [7]. Clinically, patients may present with a variety of phenotypic changes ranging from near normal growth with minimal protein intolerance to severe cases with growth delay, hepatosplenomegaly, alveolar proteinosis, osteoporosis [3] and muscular hypotonia [7]. Biochemically, urinary excretion of lysine is hugely increased with moderate excretion of arginine and ornithine. Plasma concentrations of these dibasic amino acids are usually low but sometimes can be within the normal range [3]. Review of literature revealed only two descriptions of homocitrullinuria in LPI patients [1,2]. Here, we report

the clinical, biochemical and molecular findings in a child with LPI who had significant homocitrulline excretion in the urine.

CASE HISTORY

The patient was a 4 years old boy who was born at term after an uneventful pregnancy and delivery with birth weight of 2.87 kg. Antenatal history and the neonatal period were unremarkable. He was the eldest of three siblings. Parents were non-consanguineous but originated from the same village. He had delayed developmental milestones and had recurrent diarrhea, chest infection and severe failure to thrive since infancy. He could sit without support and started to walk at 19 months. He had frequent falls upon walking unaided at 2 years of age. At the age of 3 ½ years old, he presented with lower limb weakness and, loss of bladder and bowel control. He was diagnosed as having transverse myelitis and was treated with corticosteroid which resulted in clinical recovery.

He presented again at the age of 4 years old with another episode of diarrhea and vomiting and was noted to be severely malnourished. On clinical assessment, his weight was 8.5kg (< - 4 SD), height 71cm (< - 4 SD), and occipitofrontal circumference 46 cm (-3 SD). He was found to have thin hair, pallor, kyphosis, mild hepatomegaly, muscular hypotonia and unsteady gait. A high protein diet was prescribed for him and this had resulted in altered sensorium. Subsequently he became comatose and required mechanical ventilation. MRI brain was done and showed severe brain atrophy (Figure 1a). At this point a metabolic screen was done (Table 1). X-ray of spine and hand showed severe osteopenia and delayed bone age (Figure 1b,1c). Chest X-ray was reported normal. His younger sister also had history of recurrent diarrhea and was admitted to hospital at 8 months of age. She became drowsy and comatose after feeding with a high protein diet and unfortunately passed away a few days later.

MATERIALS AND METHOD

Urine and plasma for amino acid were analyzed using cation-exchange chromatography by dedicated amino acid analyzer. Urine orotic acid was analyzed using a reverse phase HPLC system. Two ml of urine blotted on Whatmann 903 filter paper was sent to laboratory for confirmation of the homocitrulline by tandem mass spectrometry using the method described by Pitt *et al* 2002. DNA was isolated from peripheral blood lymphocytes by standard methods. Polymerase-chain-reaction (PCR) amplification and direct sequencing of all coding exons including the flanking intronic regions of the *SLC25A15* (ORNT1, chrom. 13q14) and *SLC7A7* genes were performed. We proceeded with

mutation analysis of the *SLC7A7* genes of the parents sample in our molecular laboratory.

RESULTS

Biochemical studies

Plasma amino acids showed marked elevation of glutamine, 1017 $\mu\text{mol/L}$ (reference range 86-567) and alanine at 1086 $\mu\text{mol/L}$ (91-560) with mild elevation of citrulline at 77 $\mu\text{mol/L}$ (3-42). Plasma ornithine, arginine and lysine were low at 22 $\mu\text{mol/L}$ (27-96), <1 $\mu\text{mol/L}$ (14-140) and, 73 $\mu\text{mol/L}$ (85-256) respectively. Urine amino acids showed generalized aminoaciduria with marked elevation of lysine, 967 mmol/mol creatinine (reference range 10-68) and moderate elevation of ornithine, 51 mmol/mol creatinine (reference range <7) but normal arginine, 2 mmol/mol creatinine (reference range <7). There was no argininosuccinic aciduria detected. A large unknown peak was found which was later identified as homocitrulline, 206 mmol/mol creatinine (reference range <10). Urine orotic acid quantified by reverse-phase HPLC was 2147 mmol/mol creatinine (reference range <3.3).

Molecular study

Mutation analysis of *SLC25A15* gene did not identify a pathogenic mutation. Mutation analysis of the *SLC7A7* gene revealed the patient was homozygous for a mutation at exon 1: c.235G>A (p.Gly79Arg) whereas heterozygous mutation at the same site was found in both parents. In a comprehensive review on the mutations in *SLC7A7*, Sperandeo *et al* 2008 has described 43 different mutations of *SLC7A7* which did not include this patient's mutation.

DISCUSSION

LPI is an autosomal recessive disorder with great phenotypic variations and poor genotype-phenotype correlation. Biochemically, LPI is characterized by excessive renal excretion of lysine and moderate excretion of ornithine and arginine in urine together with poor intestinal absorption [6] which leads to low plasma levels of lysine, arginine and ornithine. Deficiencies of these urea cycle intermediates cause a malfunction of the urea cycle in LPI [7]. It has also been observed that plasma concentration of neutral amino acids such as serine, glycine, citrulline, proline, alanine and glutamine may also be increased in these patients [3].

The ingestion of moderate to high protein meals in patients with LPI has been observed to cause episodic hyperammonemia and orotic aciduria [6]. Patients with LPI usually present during the weaning period after

introduction of formula or cow's milk. Patients may present with protein intolerance and growth delay, respiratory symptoms, osteoporosis and hepatosplenomegaly [7]. The majority of patients have normal intelligence, although few patients were found to have mental retardation [6]. In our patient, the initial clinical suspicion of urea cycle disorder was based on the history of protein intolerance, failure to thrive and growth delay. The initial biochemical findings; elevated plasma ammonia with marked elevation of urine orotic acid and glutamine were suggestive of an underlying enzyme deficiency of the urea cycle. The mildly raised plasma citrulline with elevated alanine and low arginine was suspicious of argininosuccinic acid lyase deficiency, but was later excluded due to the absence of argininosuccinic aciduria. Oyanagi et al has showed that patients with LPI has significant marked increased in urinary excretion rates and impairment of intestinal absorption of lysine compared to control subjects following oral loading of l-lysine HCL [2]. They also found that the urinary excretion rates of homocitrulline in LPI were ten to fifteen times higher than the control [2]. The urea cycle malfunction in LPI has been compared with HHH and in both condition the blockage of the urea cycle produces accumulation of carbamyl phosphate that results in the formation of orotic acid in the cytosol by carbamylation of aspartate and of homocitrulline by carbamylation of lysine [7].

Samples taken from our patient during periods of hyperammonemia at 4 and 5 years showed increased in both urine orotic acid and homocitrulline (Table 1), indicating significant accumulation of carbamyl phosphate. Homocitrullinuria has been considered a characteristic of HHH syndrome which originally led us to consider this diagnosis. However, the biochemical abnormalities in this patient illustrate that it may be preferable to think of homocitrullinuria as a relatively non-specific finding in disorders affecting the urea cycle.

Long term treatment of LPI consists of dietary protein restriction, nitrogen scavenger drugs and supplementation with citrulline and lysine [5]. In our patient, he was given feeding via gastrostomy and the dietary protein intake was restricted to 1.5g/kg/day. He also received oral sodium benzoate 175mg/kg/day, sodium phenylbutyrate 175mg/kg/day, citrulline 250mg/kg/day, arginine 350mg/kg/day, vitamins and calcium supplements. Biochemical and clinical improvement were minimal and compliance was an issue (Table 1). He died at 6 years of age when there was a delay in seeking medical treatment following an episode of diarrhea. It is likely that this patient's younger sister also had LPI given the similarities in her clinical course. Unfortunately, no material was available to confirm the diagnosis in the sister. The fatal course in these two siblings underlines the importance of aggressive treatment during periods of metabolic decompensation and careful monitoring of protein intake.

CONCLUSION

The diagnosis of LPI is usually not suspected by clinical findings alone and specific laboratory investigations and molecular analysis is important to get a definitive diagnosis. Early and efficient treatment of infections and dehydration in these patients is crucial to prevent fatality.

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Conflict of interest

This case study was funded by a diagnostic fund from the Ministry of Health where most of the authors work (except author from Australia). We declare no conflict of interest.

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TABLE LEGEND

Table 1. Serial growth measurements and laboratory data

FIGURE LEGEND

Fig. 1. Radiological investigation that were obtained at 4 years old. (a). The axial view of T1-weighted image showing severe brain atrophy. (b). Lateral view of the lumbar spine showing deossified vertebrae with dense and thin endplates. (c). Anteroposterior view of hand showing osteopenic bone and delayed skeletal maturation. Variable bone age from 1 year to 2 years 8 months.

TABLE 1

Growth parameters		Result		Reference range
Age (years)	4 ^a	5 ^b	5.5 ^c	
Weight (kg)	8.5 (<-4SD)	8 (<-4SD)	8.6 (<-4SD)	
Height (cm)	71 (<-4SD)	74 (<-4SD)	78 (<-4SD)	
Head circumference (cm)	46 (-3SD)	47 (-3SD)	47.2 (-3SD)	
Analyte				
Plasma ammonia	543.2	81	33	0-50 µmol/L
Lactate	2.4	1.41	-	1.0-2.6 mmol/L
pH	7.47	-	7.45	7.35-7.45
pCO ₂	3.1	-	4.5	3.1-4.5 kPa
Bicarbonate	17	-	24	21-26 µmol/L
Urea	4.3	5.2	7.3	0-8.3 mmol/l
Sodium	144	141	139	132-145 mmol/l
Potassium	3.6	3.5	3.6	3.1-5.1 mmol/l
Creatinine	6	12	14	0-106 µmol/L
Alkaline Phosphatase	357	235	163	0-341 U/L
ALT	58	24	75	0-33 U/L
AST	72	25	42	0-48 U/L
LDH	-	965	-	0-850 U/L
Hb	7.5	9.3	11.1	11.1-14.1 g/dL
Ferritin	701	-	-	16.4-323ug/L
MCV	69	77	77.9	72-84 fL
MCH	23	24	25.1	25-29 pg
Triglyceride		4.1	2.1	<2.3 mmol/L
Orotic acid ^u	2147	683	-	<3.3 mmol/mol creatinine
Glutamine ^p	1017	134	331	86-567 µmol/L
Alanine ^p	1086	195	171	91-560 µmol/L
Citrulline ^p	71	23	46	3-42 µmol/L
Ornithine ^p	22	21	54	27-96 µmol/L
Ornithine ^u	51	259	-	<7 mmol/mol creatinine
Lysine ^p	73	29	32	85-256 µmol/L
Lysine ^u	967	761	-	10-68 mmol/mol creatinine
Arginine ^p	<1	8	12	14-140 µmol/L
Arginine ^u	2	1611	-	<7 mmol/mol creatinine
Homocitrulline ^u	206	101	-	<10 mmol/mol creatinine
Argininosuccinic acid	not detected			not detected

p:plasma; u:urine

^aat diagnosis; ^bon oral with sodium benzoate (500mg TDS), sodium phenylbutyrate (500mg TDS), arginine (750mg TDS); ^con oral with sodium benzoate (500mg TDS), sodium phenylbutyrate (500mg TDS), arginine (1g TDS) and citrulline (1g TDS)

212 **FIG. 1**

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