

CASE SERIES

Biochemical and molecular characteristics of Malaysian patients with lysinuric protein intolerance

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Abstract

Lysinuric protein intolerance (LPI) is an inborn error of dibasic amino acid transport due to a defect in the dibasic amino acid transporter in the renal and intestine and has a heterogenous presentation. Three Malaysian patients with LPI were studied and their biochemical and molecular findings compared. There were differences and similarities in the biochemical and molecular findings. Molecular analysis of *SLC7A7* gene revealed a novel mutation c.235G>A; p.(Gly79Arg) in exon three in Patient 1 and a mutation c.1417C>T; p.(Arg473*) in exon 10 in patient 2 and 3. The degree of concentration of dibasic amino acids may determine the type of disease of the cell membrane transport, however, a positive molecular confirmation will secure the diagnosis.

Keywords: inborn errors of metabolism (IEM), Lysinuric Protein Intolerance (LPI), *SLC7A7*, orotic acid, dibasic amino acids, amino aciduria

INTRODUCTION

Inborn errors of metabolism (IEM) are genetic diseases causing congenital disorders of metabolism. The majority of cases are due to the defect of single genes that code for enzymes that facilitate conversion of substrates into products. However, lysinuric protein intolerance (LPI) is an inborn error of dibasic amino acid transport due to a defect in the dibasic amino acid transporter in the renal and intestine.¹ Mutations in the *SLC7A7* gene located in chromosome 14q11.2 encoding the light subunit of the heteromeric amino acid transporter system γ -LAT-1 protein have been found to be responsible for this inborn error of metabolism.^{2,3} *SLC7A7* gene contains 11 exons and spans 47 kb of genomic DNA. The codon for translation initiation starts at exon three.^{3,4} There are 50 different mutations of *SLC7A7*³ that have been reported. Lysinuric protein intolerance has heterogenous clinical presentation. Amongst the clinical presentations are failure to thrive with protein intolerance of varying degrees, muscular hypotonia, hepatomegaly and mental retardation. The biochemical abnormalities are generalized amino aciduria with an exaggerated excretion of the dibasic amino acids; lysine,

arginine and ornithine. However, the biochemical abnormalities may not be appreciated if the test request was not accompanied by the patient's clinical features. Plasma amino acid analysis in these patients may show elevated neutral amino acids; citrulline, alanine, glycine and glutamine.⁵ Nevertheless, absence of the abnormalities of these neutral amino acids in the plasma should not exclude the presence of the disease. The concentrations of the dibasic amino acids; lysine, arginine and ornithine in the plasma are usually low but it is not uncommon to be within the reference interval.^{2,6} The objective of the study was to examine and compare the biochemical and molecular characteristics in 3 Malaysian patients with lysinuric protein intolerance (LPI). Consent were obtained from parents of all patients in the study.

MATERIALS AND METHODS

Patient 1

Patient 1 was a four-year-old boy, the offspring of a non-consanguineous marriage. He presented with delayed milestones, recurrent diarrhea and failure to thrive. He started to walk at 19 months old but had frequent falls. At the time

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of diagnosis, he presented with another episode of diarrhea and vomiting. Physical examination revealed that he was severely malnourished with thin hair, pallor, kyphosis, hepatomegaly, muscular hypotonia with unsteady gait. His weight, height and occipito-frontal circumference were less than three standard deviation (-3SD) from the population mean. He became comatose after introduction of high protein diet. The detail clinical and laboratory characteristics of this patient have already been published.⁷

Patient 2

Patient 2 is a male who presented at the age of 4 years with global developmental delay and dysmorphism with flat nasal bridge and full malar region. He had failure to thrive and pallor associated with hepatosplenomegaly not requiring blood transfusion. He passed loose stools intermittently 4 to 5 times a day. At the age of 5 and 6 years old, he sustained left femoral fracture and right supracondylar fracture after falls. His school performance was poor needing special class attendance. He developed aversion to high protein food since young. His height, weight and head circumference were below the third centile. Laboratory investigations showed anemia (Hb 8.6 g/dL) with hypochromia, microcytosis and anisopoikilocytosis without H inclusion. Alpha thalassemia genetic testing detected heterozygous alpha plus 3.7 deletion. His ferritin was elevated but iron concentration was normal. Liver function test showed low albumin but normal enzymes. Abdominal ultrasound showed hepatomegaly with bilateral multiple small renal cysts.

Patient 3

Patient 3 was a female sibling of patient 2 and was asymptomatic. Biochemical screening was performed at the age of 8 months old with subsequent molecular studies. The parents of patients 2 and 3 had a consanguineous marriage.

Analytical methods

Biochemical

Urine and plasma for amino acids were analyzed using cation-exchange chromatography by dedicated amino acid analyzer. Urine orotic acid was analyzed using the reverse phase HPLC system.

Molecular analysis

DNA was isolated from peripheral blood lymphocytes by standard methods. Polymerase

chain reaction amplification and direct sequencing of all coding exons including the flanking intronic regions of the *SLC7A7* genes were performed.

RESULTS

Biochemical studies

Urine amino acids for all three patients showed generalized aminoaciduria. All three patients had marked excretion of lysine and ornithine. Patient 2 and 3 had marked excretion of arginine unlike Patient 1. Plasma amino acids for Patient 1, 2 and 3 showed elevation of glutamine, however only patient 1 and 2 had elevation of alanine. Patient 2 had normal plasma levels of citrulline whereas there is mild elevation of citrulline (77 $\mu\text{mol/L}$) in patient 1 and marked elevation of citrulline (219 $\mu\text{mol/L}$) in patient 3 which was most likely due to treatment. Only patient 2 had concentration of ornithine, arginine and lysine outside the reference interval in the plasma. Plasma cystine were normal in all three patients. Urine cystine were slightly elevated for all three patients but did not reach significant levels. Urine orotic acids were marked elevated in all patients. Plasma ammonia was elevated in patient 1 but normal in patient 2. Plasma ammonia result was not available for patient 3. Table 1 and Figure 1 show details of blood and urinary profiles.

Molecular study

Mutation analysis of the *SLC7A7* gene revealed that Patient 1 had a homozygous mutation c.235G>A; p.(Gly79Arg) in exon 3. Both parents were heterozygous carriers for this mutation. Further bioinformatic analysis by MutationTaster software predicted the p.(Gly79Arg) mutation to be disease causing. Patient 2 and 3 had homozygous mutation c.1417C>T; p.(Arg473*) in exon 10. This mutation has been previously reported in the Human Gene Mutation Database (HGMD).⁸ Figure 2 show details of mutational findings.

DISCUSSION

In a typical patient the diagnosis may be evident on the first clinical presentation. However, in malnourished patients the usual abnormalities of hyperammonemia, orotic aciduria and plasma amino acid abnormalities might be masked.¹ Glomerular and tubular involvement is common in this condition but renal cortical cysts on renal imaging as seen in patient 2 has never been reported. The challenge in diagnosis of LPI is due to its phenotypic variations. In Finnish

TABLE 1: Laboratory findings in three patients with lysinuric protein intolerance

	Patient 1	Patient 2	Patient 3	Reference range
Age at diagnosis	4 years	4 years	10 months	
Gender	M	M	F	
Glutamine ^p	1017	862	951	86-567 µmol/L
Alanine ^p	1086	942	219	91-560 µmol/L
Citrulline ^p	71	19	219	3-42 µmol/L
Lysine ^p	73	24	65	56-256 µmol/L
Arginine ^p	<1	14	17	14-140 µmol/L
Ornithine ^p	22	4	8	8-156 µmol/L
Cystine ^p	12	16	10	5-46 µmol/L
Lysine ^u	967	548	1317.2	10-68 mmol/mol creatinine
Arginine ^u	2	153	105.9	0-7 mmol/mol creatinine
Cystine ^u	27	22	25	4-15 mmol/mol creatinine
Ornithine ^u	51	27	16.11	0-7 mmol/mol creatinine
Urine orotate	2147	602.4	62.15	0.5-3.3 mmol/mol creatinine
Homocitrulline ^u	206	ND	ND	<10 mmol/mol creatinine
Mutation	c.235G>A p.(Gly79Arg)	c.1417 C>T p.(Arg473*)	c.1417 C>T p.(Arg473*)	

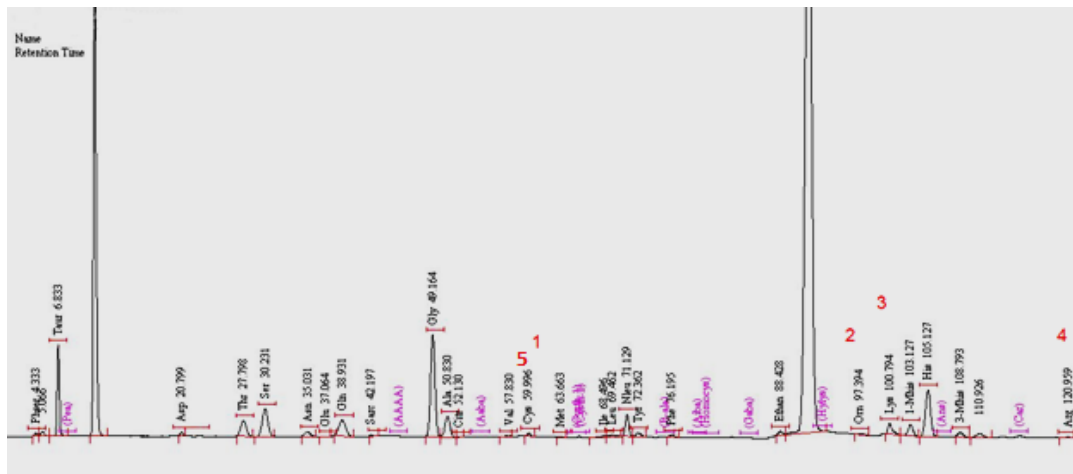
p = plasma; u= urine; ND= not detected

patients with LPI, who had the Finnish mutation in homozygosity, the genotype/phenotype correlations were not achieved due to a wide range of phenotypic severity.⁹

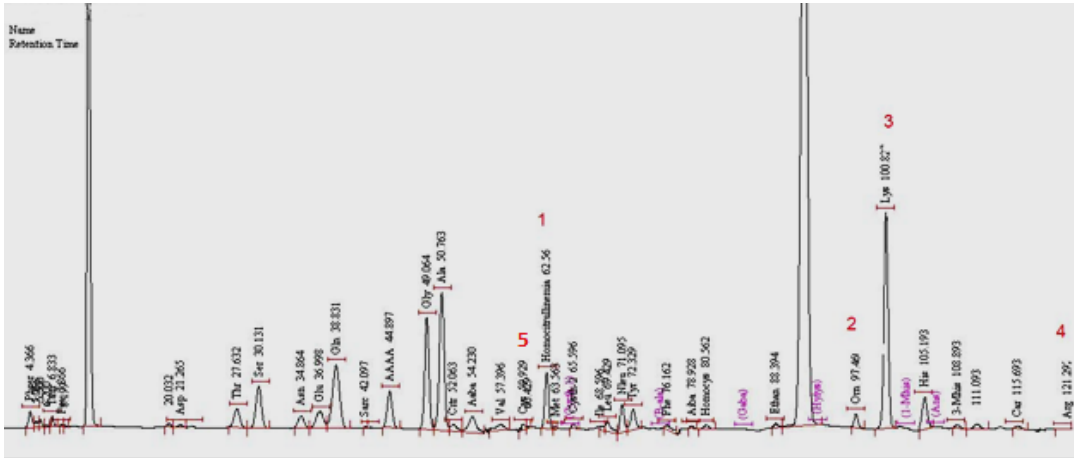
In LPI, the main biochemical abnormalities are generalized amino aciduria. There is hyperexcretion of lysine with moderate excretion

of ornithine and arginine in the urine on close inspection. However, arginine levels can be within the reference interval in some cases. This is due to massively increased urinary excretion and renal clearance of lysine.¹⁰ Defective intestinal absorption in addition to increased renal loss led to low plasma levels of lysine, arginine

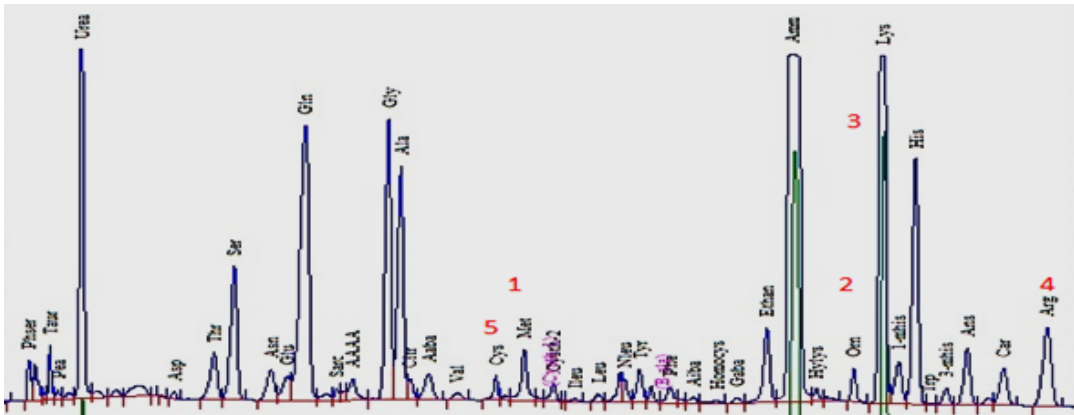
1A: Normal control



1B: Patient 1



1C: Patient 2



1D: Patient 3

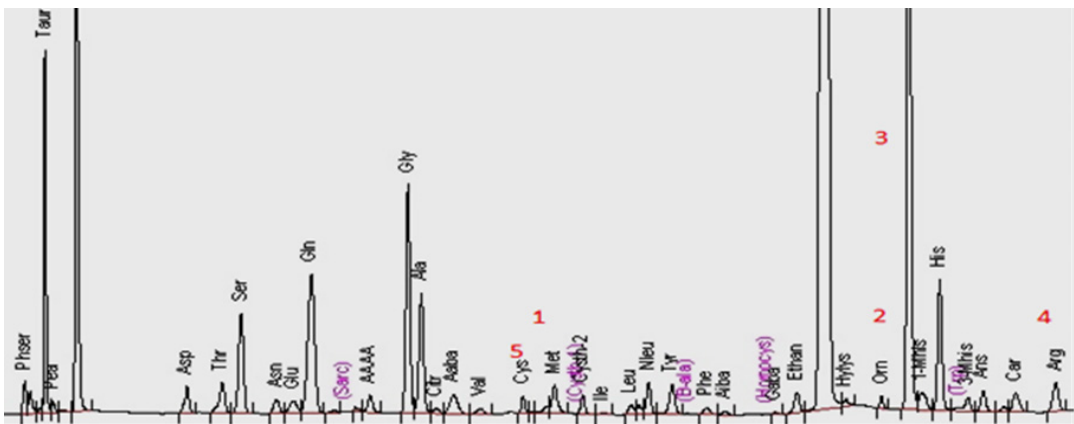


FIG. 1: Urine amino acid profile by cation-exchange chromatography. Peak 1: Homocitrulline (absent in the control, patient 2 & 3), Peak 2: Ornithine, Peak 3: Lysine, Peak 4: Arginine, Peak 5: Cystine. (1A) normal person, (1B) patient 1, (1C) patient 2, (1D) patient 3

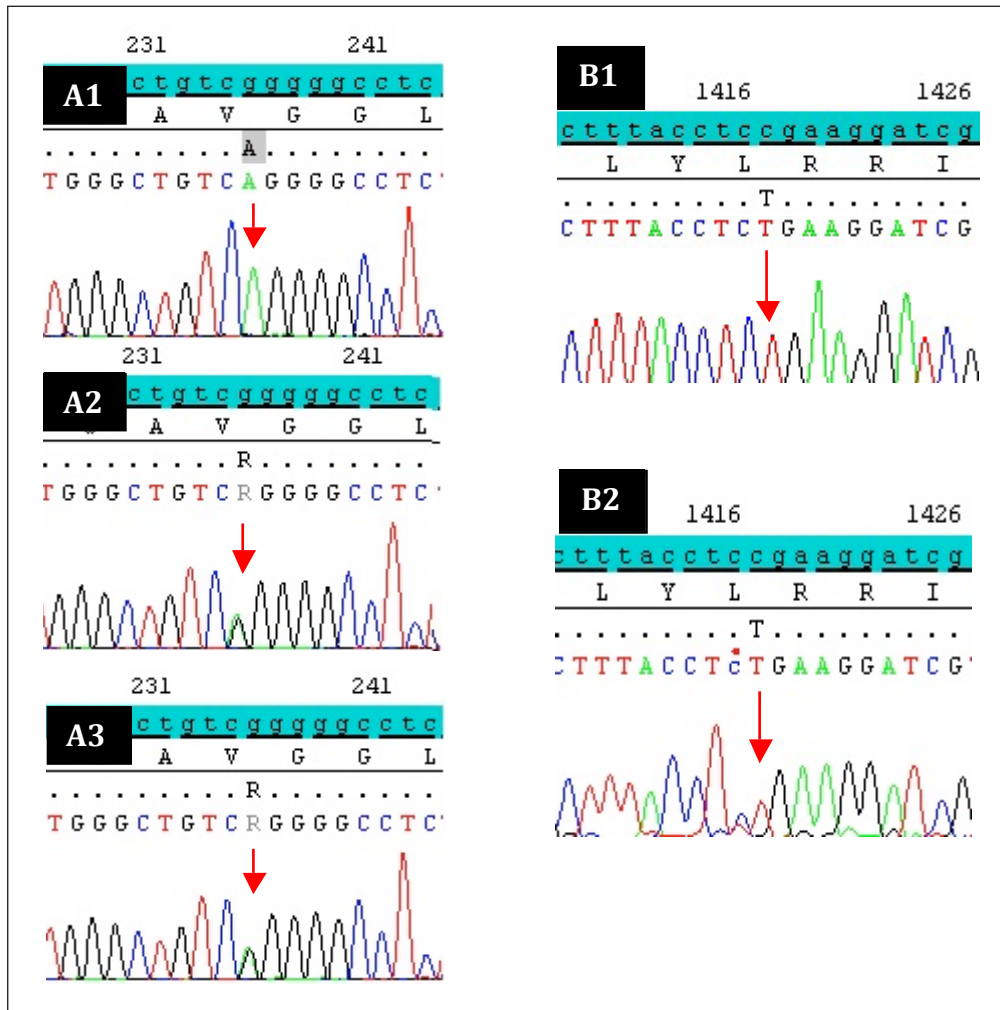


FIG. 2: Mutation analysis of Patient 1 showed a homozygous mutation at c.235 G>A in Exon 3 (A1), resulting in amino acid substitution from glycine to arginine at codon 79. Similar mutation was found in the parents in heterozygous state (A2 and A3). Mutation analysis of Patient 2 (B1) and Patient 3 (sibling) (B2) showed a homozygous mutation at c.1417 C>T in Exon 10, introducing amino acid substitution from arginine to a stop codon at codon 473

and ornithine.⁵ In contrast to dibasic amino acids, the plasma concentrations of the neutral amino acids; serine, citrulline, proline, alanine and glutamine are increased in patients with LPI.² In some patients there is also hyperexcretion of homocitrulline^{7,11} which was present in patient 1. Due to the presence of homocitrulline in patient 1, the initial diagnosis was hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome. However, the diagnosis was revised after a negative mutation analysis of the *SLC25A15*. LPI has also overlapping biochemical features with cystinuria in which dibasic amino acids are hyperexcreted together with cystine. Renal reabsorption of lysine is comparable in LPI and cystinuria, whereas the hyperexcretion

of arginine and ornithine are lower in LPI than in cystinuria.¹² The dibasic amino acids and cystine in the plasma are also lower in patients with cystinuria.² Defective transport of cystine and dibasic amino acids (lysine, arginine and ornithine) across the apical membranes of proximal renal tubules and jejunal epithelial cells due to mutated *SLC3A1* or *SLC7A9* caused cystinuria.^{13,14} Patients with LPI do not excrete significant amount of cystine.¹² In all three patients in this study, symptoms manifested after the weaning period, which is consistent with reported literature.^{1,2} Both Patient 1 and 2 had mental retardation although some patients were reported to have normal intelligence.²

Conclusion

The degree of concentration of dibasic amino acids may determine the type of disease of the cell membrane transport. These cases illustrate how a positive molecular confirmation secured the diagnosis.

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