Lysinuric Protein Intolerance Presenting with Hepatosplenomegaly and Pancytopenia

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Abstract

Lysinuric protein intolerance (LPI) commonly presents with hyperammonemic encephalopathy and failure to thrive. We report a case of a three-and-a-half-year old boy who presented with failure to thrive, recurrent respiratory tract infections, anemia, thrombocytopenia, hepatosplenomegaly and fracture on trivial trauma, indicating towards differential diagnosis of lysosomal storage diseases like Gaucher disease, Niemann-Pick disease, saposin deficiency etc. A homozygous pathogenic variant identified in the *SLC7A7* gene, through next-generation sequencing-based exome sequencing of the child, suggested the diagnosis of lysinuric protein intolerance (LPI). We conclude that LPI should be considered as a differential diagnosis in a patient with presentation of hepatosplenomegaly with pancytopenia.

Introduction

Lysinuric protein intolerance (OMIM #222700) is a primary inherited aminoaciduria with an autosomal recessive mode of inheritance predominantly reported in Finland (Simell et al., 2002). The incidence of the disorder is around 1 in 60,000 new-borns in Finland. There are many cases reported from Japan also. where the prevalence of the disorder is around 1:60,000. There are two cases reported from India, where the patients presented with recurrent hyperammonemic encephalopathy, diagnosed through molecular genetic testing and/or metabolic profile (Bijarnia-Mahay et al., 2016; Moosa et al., 2005). Absence or reduced levels of ornithine and arginine lead to functional deficiency in the urea cycle, causing increased levels of ammonia and may precipitate encephalopathy. The presentation in our patient was totally different with the patient following up with the hematologist for hepatosplenomegaly, pancytopenia and recurrent infections. It is only after detailed analysis and molecular genetic testing that we could confirm the diagnosis.

Case report

A three-and-a-half-year-old boy, born to a consanguineously married couple, presented with distension of abdomen since the age of 6 months and history of recurrent infections and hospitalization since 6 months. Distension of abdomen was generalized and gradually progressive. Patient required hospitalizations at 12 months, 15 and 18 months of age for lower respiratory tract infections. Child required packed cell transfusion once for severe anemia. Child had a fracture of the left arm 10 days prior to visit to the Genetics OPD. Child was born at term gestation, with birth weight of 3.25 kg. Developmental milestones were normal for age. Dietary history revealed strong aversion to proteins as the child was not eating any non-vegetarian foods and pulses and only preferred rice and vegetables.

On examination, anthropometric parameters revealed: weight 10.7 kg (between -2 to -3 SD), height 83 cm (-2 to -3 SD) and head circumference 47 cm (-2 to -3 SD), according to WHO growth charts. Child was pale and had hepatosplenomegaly. Investigation of complete blood counts (CBC) revealed pancytopenia with hemoglobin of 6.2 gm/dl, total leucocyte count of 3,400/cu mm and platelet count of 40,000/cu

mm. Hemoglobin electrophoresis was normal. Bone marrow biopsy was unremarkable with few hemophagocytes being seen. Serum ferritin was normal (97 ng/mL; reference 17.9-464 ng/mL) and other clinical features did not support the diagnosis of hemophagocytic lymphohistiocytosis. Liver function tests including ALT of 55 IU/L (0-60 IU/L) and total bilirubin of 1.3 mg/dl, were normal. In the serum lipid profile, cholesterol was 170 mmol/dl and triglycerides were 230 mmol/dl; which were both elevated above the normal range.

Considering pancytopenia with hep-atosplenomegaly, failure to thrive, recurrent lower respiratory tract infections and fracture humerus, differential diagnosis of lysosomal storage diseases like Gaucher disease or Niemann-Pick disease type B was considered. Though bone marrow biopsy did not reveal any storage cells, absence of storage cells cannot rule out these diseases. We wanted to rule out small molecule diseases based on history of aversion to protein rich foods. Initial tandem mass spectroscopy (TMS) did not show any abnormality. Because of an unclear phenotype, NGS-based exome sequencing was done for the child which showed a homozygous, 'likely pathogenic' one-base pair duplication c.110dupT in exon 3 of the *SLC7A7* gene (transcript id ENST00000397532) that results in frameshift and premature truncation of the protein at codon 38 (p.Ser38LeufsTer4). This frameshift variant is not reported in the 1000 Genomes (`https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes`), gno-mAD (`https://gnomad.broadinstitute.org`) and ClinVar (`https://www.ncbi.nlm.nih.gov/clinvar/`) databases. This variant is predicted to be 'disease-causing' by the MutationTaster software (`www.mutationtaster.org`). After receiving the genetic report, we performed plasma amino acids assay and urine amino acid analysis. Plasma high performance liquid chromatography (HPLC) of amino acids revealed low levels of arginine (2.67 nmol/ml; normal range 10-140 nmol/ml) and glutamine (226 nmol/ml; normal range 254-823 nmol/ml). Urine amino acids assay by HPLC revealed increased excretion or levels of ornithine (124 nmol/ml; normal range 2-91 nmol/ml), lysine (4565.89 nmol/ml; normal range 34-894 nmol/ml), arginine (1044 nmol/ml; normal range 7-133 nmol/ml), and citrulline (283 nmol/ml; normal range 0-90 nmol/ml).

The child was started on levocarnitine, low dose citrulline and lysine supplements and a protein-restricted diet. He showed marked symptomatic improvement and gained 400 grams of weight after 2 months of follow up. Urinalysis for proteinuria and a chest radiograph and serum ammonia were normal on follow-up.

Discussion

Lysinuric protein intolerance (OMIM# 222700) is a rare metabolic disorder also known as dibasic aminoaciduria, caused by a defective membrane transport of cationic amino acids (CAA) like lysine, arginine and ornithine. This leads to decreased circulating plasma CAA levels and increased excretion in urine (Simell et al., 1975).

Massive urinary excretion of dibasic amino acids, especially lysine, and poor intestinal absorption of these amino acids leads to deficiency of these amino acids (Simell et al., 2002). Protein malnutrition and deficiency of the essential amino acid lysine contribute to the patient's failure to thrive (Nunes & Niinikoski, 2006). Biallelic pathogenic variants in the *SLC7A7* gene are responsible for this disorder. Although more than 65 mutations have been detected in patients across the world, as per the Human Gene Mutation Database (HGMD), only one mutation has been reported from India. Excess intracellular arginine, because of trapping (due to block in transport) may trigger an overproduction of nitric oxide, leading to dysfunction of monocytes and macrophages dysfunction (Nunes & Niinikoski, 2006). This explains the association of this disorder with immunodeficiency features, hemophagocytic lymphohistiocytosis, pulmonary alveolar proteinosis and renal disease seen in older untreated patients with LPI. Though bone marrow showed few hemophagocytes, ferritin was normal in our patient. Our patient's predominant presentation of hepatosplenomegaly with pancytopenia and fracture of the humerus added differential diagnosis of lysosomal storage disease like Gaucher disease, saposin C deficiency etc. (Nunes & Niinikoski, 2006). The bone marrow examination did not reveal any storage cells. Therefore, with an unclear phenotype, exome sequencing was performed which revealed the diagnosis. Reverse phenotyping revealed excess excretion of lysine in the urine, confirming the diagnosis. Our patient also had strong aversion to proteins, eating only cereal based food and minimal vegetables. This patient could really get misdiagnosed to have a malabsorption syndrome, storage disorder or immunological disease,

considering such a variable phenotype. Without NGS-based diagnostic testing, it would have been difficult to identify this as a case of small molecule-associated inborn error of metabolism. The blood ammonia levels were not elevated in our patient, but it is important to counsel regarding the life-threatening phenomenon of hyperammonemia in these patients, so that it can be recognized and treated early enough to be able to prevent further neurological damage and other complications. The standard treatment for LPI involves low protein diet, ammonia-lowering nitrogen scavengers like sodium benzoate (100-250 mg/kg/day in 3 divided doses), levocarnitine (100 mg/kg/day in three divided doses) and low-dose citrulline and lysine supplementation. The metabolic derangements are readily treatable with dietary modifications, but some complications like interstitial lung disease, proximal renal dysfunction, hypercholes-terolemia, hemophagocytic lympho-histiocytosis, pancreatitis, and growth hormone deficiency add to the morbidity and mortality (Nunes & Niinikoski, 2006). As LPI is an autosomal recessive disease, there is 25% chance of recurrence in future offspring of parents of the index case. Thus, genetic counselling will help these families and prenatal genetic testing can be done if the pathogenic variant(s) is/are identified in the proband.

In conclusion, next generation sequencing is a boon to genetic disease diagnostics, especially for diseases like lysinuric protein intolerance which has multisystemic presentation mimicking various conditions as described above, but deep and reverse phenotyping is an irreplaceable tool to aid the molecular diagnostic results.

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