Variable manifestations in lysinuric protein intolerance: a report of two novel mutations from Bahrain

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ABSTRACT

Background: Lysinuric protein intolerance (LPI) is a metabolic disorder resulting from mutations in the SLC7A7 gene that is inherited in the autosomal recessive pattern. The disease has been described sporadically worldwide, including a few cases from Arab countries. The affected patients typically present with failure to thrive, hepatosplenomegaly, and protein intolerance. Various complications such as autoimmune disorders, infiltrative lung disease, hemophagocytic lymphohistiocytosis (HLH), and neurological manifestations could be noted during the disease course.

Methodology: We described patients diagnosed with LPI in Bahrain by reviewing their presentations, complications encountered, genetic variability, and treatment options.

Results: Four patients, two males and two females from three families with an age range between 2 and 14 years, were followed. Failure to thrive and HLH were the main presenting features in all patients. Two novel mutations were detected in the SLC7A7 gene. One of them was a homozygous splice-site mutation of c.1429+1G>C., whereas the second mutation was a homozygous missense mutation of c.168T>G p. (Phe56Leu). Lung complications were found in two patients, autoimmunity observed in two patients, gastrointestinal complication presenting as hemorrhagic gastritis in one patient, and neurological complications were seen in one patient.

Conclusion: The main presenting feature in all the patients was HLH. Two novel mutations in the SLC7A7 gene were detected. Rheumatological complications were variable within the same family members; moreover, hemorrhagic gastritis was reported in one of the patients as a new possible complication related to the disease.

Keywords: Lysinuric protein intolerance, hemophagocytic lymphohistiocytosis, pulmonary alveolar proteinosis, osteoporosis, hemorrhagic gastritis.

Introduction

Lysinuric protein intolerance (LPI; OMIM entry number: 222700) is an inborn error of metabolism disorder that results from mutations in the solute carrier family 7 (SLC7A7) gene on chromosome 14q11, which encodes for the expression of an amino acid transporter known as y+LAT-1 (1–5). It is inherited as an autosomal recessive disorder reported particularly in Finland, Italy, and Japan (2–4). The effect of this gene mutation results in defective transportation of dibasic amino acids lysine, arginine, and ornithine along the basolateral membrane, chiefly in the intestinal tract and renal tubules. In the latter, there is impaired reabsorption resulting in a leakage of these amino acids in the urine, associated with normal to low-level amino acids in the plasma which is the key to the diagnosis (5). The y+LAT-1 transporter is also expressed in other tissues, including the lung and reticuloendothelial system, reflecting some of the notable clinical features and complications of LPI, including pulmonary alveolar proteinosis (PAP) and hemophagocytic lymphohistiocytosis (HLH) (6). A list of some of the reported possible clinical manifestations and/or complications of LPI is shown in Figure 1 (7–9). The treatment aims to prevent hyperammonemia and
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Subjects and Methods

This study was a retrospective analysis of the medical records of patients diagnosed with LPI at Salmaniya Medical Complex in the Kingdom of Bahrain between the years 2004 and 2017. The patient’s characteristics, clinical presenting features, biochemical results, initial treatment, and molecular genetic testing for the SLC7A7 gene were studied. The gene was tested in three of them by Sanger sequencing at the Human Genetic Center/Bioscientia Laboratory. The sequencing is performed with dye terminators. The fragments were separated by capillary electrophoresis. All nine exons, the adjacent splice junctions, and the promotor have been sequenced (3,716 bp), whereas the fourth one was tested through whole-exome sequencing at the CENTOGENE laboratory. HLH diagnosis was based on the diagnostic criteria reported by Henter et al. (10). A rheumatological involvement was diagnosed according to clinical manifestations and confirmed by autoimmune markers. Pulmonary complications were considered based on chest radiograph, lung computerized tomography (CT), and/or lung biopsy. The presence of osteopenia was checked radiologically by plain X-ray and/or bone density with a Z-score < −1 standard deviation (SD). Renal impairment was defined with the presence of microalbuminuria and abnormal estimated glomerular filtration rate (eGFR).

Results

Four patients were diagnosed with LPI with an age range between 2 and 14 years, of which two of them were males, one Bahraini (patient A) and one Egyptian (patient B) products of a full-term pregnancy, whereas other two females were preterm Bahraini sisters and products of triplet pregnancy induced by in vitro fertilization (patients C and D, respectively). All of them were born to consanguineous parents with a mean age of diagnosis at 15 months. The main presenting symptoms were failure to thrive (FTT) and hepatosplenomegaly. Besides, one patient (patient C) had vomiting and feed intolerance. They had significantly elevated lysine along with high ornithine and arginine in the urine amino acids (Table 1). Urinary orotic acid was elevated in patients C and D at presentation. The treatment was initiated with a low protein diet (0.8–1.5 mg/kg/day) and L-citrulline (20–50 mg/kg/day). The doses were adjusted according to the serum amino acid level aiming to maintain normal level, in addition to daily folic acid and Vitamin D. The molecular genetic analysis of the SLC7A7 gene revealed two different novel mutations, and one of them, which was present in three Bahraini patients from two different families, is a homozygous splice-site mutation of c.1429+1G>C. The parents of the patients were
heterozygous for the same mutation. The pathogenic relevance of this mutation relies on its involvement of the invariant splice donor and consequently results in changed splicing. The second mutation, which was detected in the Egyptian patient, was a homozygous missense mutation of c.168T>G p. (Phe56Leu) which is predicted to be pathogenic as it was tested in silico parameter program with three-fourth damage in highly conserved amino acid position.

All patients met the criteria for the diagnosis of HLH at presentation as part of autoimmune manifestations, including the presence of pancytopenia, hypertriglyceridemia, hyperferritinemia, hypofibrinogenemia, and hemophagocytosis in bone marrow aspirate. CD25 was elevated only in two patients (patients B and C). A trial of monthly intravenous immunoglobulin and dexamethasone was given at the time of diagnosis for 6 months for patients A, C, and D together with monthly monitoring of HLH biochemical parameters, which showed partial improvement as these patients continued to have mild anemia, neutropenia, thrombocytopenia, and elevated ferritin. No treatment was started for HLH inpatient B as the family was reluctant and left to their original country at the age of 2½ years. Other autoimmune abnormalities were manifested clinically as a lupus-like picture in patient C, who presented with persistent fever, joint stiffness, and erythematous rash on the perineal area that later disseminated all over her body (Figure 2). Patient D had developed joint involvement in the form of joint swelling and reduced range of motion with a period of remission and flare-up consistent with rheumatoid arthritis in the small and large joints that end up with contractures in both the wrist joints. This was managed with the courses of non-steroidal anti-inflammatory drugs. Patient A had no clearly defined clinical manifestations related to autoimmunity; however, he had a positive anti-nuclear antibody, anti-SS-P, and anti-RNP with negative anti-double-stranded DNA, which was also detected in patients C and D.

Pulmonary involvement was significant in the two sisters of the patients. One of them (patient C) had recurrent attacks of cough and hypoxemia soon after her diagnosis requiring ventilator assistance. Her chest CT scan revealed bilateral interlobar septal thickening with ground-glass opacities. Eventually, PAP was diagnosed at the age of 2 years after bronchoscopy and lung biopsy. She underwent whole lung lavage, which resulted in the improvement of lung functions till the age of 9 years when unfortunately her respiratory symptoms recurred requiring an intensive care unit admission. Repeated chest CT scan showed a patchy consolidation and progression of the ground-glass changes in both the lungs which could represent an activation of PAP, especially that there was no evidence of infections (Figure 3). An inhaled granulocyte-macrophage colony-stimulating factor (GM-CSF) was

<table>
<thead>
<tr>
<th>Patient</th>
<th>Plasma amino acids</th>
<th>Urine amino acids</th>
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<tbody>
<tr>
<td></td>
<td>Lysine</td>
<td>Ornithine</td>
</tr>
<tr>
<td>A</td>
<td>83</td>
<td>7</td>
</tr>
<tr>
<td>B</td>
<td>174</td>
<td>11</td>
</tr>
<tr>
<td>C</td>
<td>115</td>
<td>118</td>
</tr>
<tr>
<td>D</td>
<td>110</td>
<td>80</td>
</tr>
</tbody>
</table>

Normal reference range as per our diagnostic laboratory; plasma amino acids (µmol/l): lysine (52–196), ornithine (22–103), and arginine (12–133). Urine amino acids (µmol/mmol creat): lysine (16–69), ornithine (0–8), and arginine (0–8).
used for 1 month with no improvement, and eventually, she ended into respiratory failure and death. Her sister (patient D) started to develop mild attacks of shortness of breath with hypoxemia at the age of 10 years. Her chest CT scan showed lung fibrosis; however, she remained stable clinically and radiologically until the current age, i.e., 14 years.

Short stature (height <3rd centile on the growth chart) was documented in all the patients through their follow-up. Osteopenia was noticed in three patients (A, C, and D), which was complicated by recurrent pathological fracture at various sites in one of them (patient D), despite regular supplement of Vitamin D and calcium. Cerebral involvement was manifested as a seizure disorder in patient C; however, her ammonia level remained normal throughout. The brain magnetic resonance imaging revealed volume loss with delayed myelination; moreover, her electroencephalogram showed epileptiform changes in the posterior temporal region. The sister (patient D) experienced recurrent attacks of hyperammonemia, which was started at the age of 10 years. She required daily hyperammonemia treatment including sodium benzoate and carglumic acid that was shifted recently to glycerol phenylbutyrate. The developmental milestones remained appropriate for the age in all patients in both cognitive and motor aspects. Gastrointestinal manifestations developed in patient D presenting as recurrent episodes of epigastric pain with no biochemical evidence of pancreatitis or helicobacter pylori. Her endoscopy showed evidence of hemorrhagic gastritis (Figure 4) which was treated with esomeprazole that controlled her symptoms. No renal impairment was detected in any of the patients as they maintained normal eGFR with an absence of microalbuminuria. The clinical characteristics and manifestations are shown in Table 2.

**Discussion**

**SLC7A7** gene is the only gene reported to be responsible for LPI, which is located on chromosome 14q11.2. It is composed of 11 exons that encode the y+LAT-1 subunit. This subunit is expressed in the basolateral plasma membrane of epithelial cells of proximal renal tubules, small bowel, lung, and leukocytes (6). The disease has been reported worldwide with the majority of patients reported from Finland constituting one-third of the cases (11). Four patients with LPI from Bahrain are discussed in this report. Three of them are of Bahraini descent, whereas the fourth case is of an Egyptian origin. To the best of authors’ knowledge, 10 cases have been identified from five different Arab countries, including 1 Saudi Arab, 2 Moroccan, 1 United Arab of Emirates, 1 Jordanian (13), and 5 Tunisian (14). More and more cases of LPI are being reported worldwide; it may be due to an increased availability of genetic studies and increased awareness of the presentation of metabolic diseases among physicians prompting investigations. Screening of LPI for families with an index case plays a role in discovering more cases as reported by Mauhin et al. (6). All the patients were born to consanguineous parents, which appeared to be consistent with the previous literature (15,16). The average age of the presentation of our cases was 15 months, which was inconsistent with the report of Mauhin et al., where the average age was 4.1 years. This could be explained by the high index of suspicion in our clinical practice necessitating screening for LPI, after the diagnosis of the twin sisters who had early and severe manifestations of the disease. LPI has a variable clinical presentation that is often related to long-standing gastrointestinal symptoms including failure to thrive, hepatosplenomegaly, and protein aversion, extending to immunological and pulmonary manifestations (9). This was similarly observed in the patients whom FTT was a main presenting feature. The literature secondary to the inadequate use of dietary protein combined with urinary losses as well as an aversion of meals rich in protein (17). It appears that patients who develop modified behavior, in which they avoid protein-rich meals, may go undiagnosed until later in life when complications begin to manifest (17).

To date, there have been more than 60 types of genetic pathological variants reported, including small insertions/deletions, large insertions/deletions, missense, nonsense, and splice-site variants (18). The two novel mutations in SLC7A7, which were detected in the patients, will be added to what has been described in the literature. The homozygous splice-site mutation of c.1429+1G>C in the three Bahraini patients from two different families, who expressed different clinical manifestations (PAP, immunological, and neurological), emphasizes the absence of a genotype–phenotype relationship. Interfamilial clinical heterogeneity was also observed between the two sisters. The variable clinical course in the patients brings difficulty in predicting the ultimate prognosis of this disease despite the similar gene mutation. Tringham et al. (19) described that this variable manifestation of the disease is related to the expression of 926 genes other than SLC7A7 which are involved in the basic cellular functions including cationic and neutral amino acid transporters.

HLH is an immunological syndrome related to the uncontrolled activation of macrophages, and it could...
be primary or secondary. LPI is a well-known cause of the second type that might present during disease course or the newborn period. It is characterized by fever, hepatosplenomegaly, cytopenia, and hemophagocytosis in bone marrow, in addition to hyperglyceridemia, hypofibrinogenemia, hyperferritinemia, and elevated CD25, which might progress to a macrophage-activating syndrome with severe hemolysis (9). In our cases, HLH was detected in all the patients at the time of presentation who continued to have chronic mild manifestations thereafter where the manifestations were limited to splenomegaly, thrombocytopenia, elevated ferritin, and anemia with no evidence of hemolysis. There are a few case reports of LPI patients who developed SLE, where the pathophysiology was hypothesized to be related to a defect in humoral immune responses in some patients (20), and it could be a presenting feature or a complication of the disease. Other variable autoimmune manifestations in LPI that have been reported include rheumatoid arthritis and vasculitis (9). In this report, the two sisters (patients C and D) met the criteria for the diagnosis of SLE and rheumatoid arthritis, respectively. Patient C showed partial improvement of SLE manifestations after immunoglobulin infusion which was used as a part of the HLH management protocol which is consistent with the other reports (9). The symptoms of rheumatoid arthritis in patient D were controlled by intermittent courses of non-steroidal anti-inflammatory medications. Other described autoimmune signs in LPI such as vasculitis, hemolytic anemia, and severe viral and bacterial infections were all not noted in our patients (21).

Pulmonary manifestations in the form of lung fibrosis and PAP are linked to LPI, and often, the radiological findings including interstitial pattern, ground-glass opacities, and interlobular thickening are not correlated with the clinical symptoms. The clinical involvement could vary from being asymptomatic lung fibrosis manifested in CXR and lung CT scan to a severe respiratory failure which was considered to be a bad prognostic sign that could lead to death (6). In our patients, the respiratory complications were observed in the two sisters. Patient C had early severe respiratory complications related to PAP as diagnosed by lung biopsy, while the other sister has mild clinical respiratory manifestations. Both had radiological findings that include lung opacities and ground-glass appearance, which is similar to what has been described in the literature. Lung lavage and GM-CSF therapy were considered to be an effective treatment of the respiratory manifestation, whereas the use of steroids remained controversy (6). In our patient who had PAP, her symptoms improved after lung lavage for many years, whereas GM-CSF therapy had no major effect which is probably due to its administration late in her disease course preventing us from monitoring its effectiveness, or it could be related to the presence of autoimmune manifestations in this patient. The effective use of GM-CSF in PAP was reported in scattered cases in the literature. Furthermore, its use in LPI patients was reported as effective in one Chinese patient, who had primarily pulmonary complications of LPI without other autoimmune complications. It was hypothesized that GM-CSF inhalation could increase the number of macrophages in alveolar fluid (22). Osteopenia is a common complication in LPI, and protein depletion has a suggested role in its development through synthesis defect of osteoblasts.

Table 2. Clinical characteristics and manifestations of patients with LPI.

<table>
<thead>
<tr>
<th>Patient</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Origin</td>
<td>Bahraini</td>
<td>Non-Bahraini</td>
<td>Bahraini</td>
<td>Bahraini</td>
</tr>
<tr>
<td>Consanguinity</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Death</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>FTT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Hepatosplenomegaly</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HLH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Autoimmunity</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PAP</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pathological fractures</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Seizures</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Hemorrhagic gastritis</td>
<td>-</td>
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</table>
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rather than degradation by osteoclasts (23). Three of our patients fulfill the criteria for osteopenia which was defined radiologically by the bone density of a Z-score < -1 SD. Only one of them (patient D) had a recurrent fracture of the long bones which could be related to her rheumatoid arthritis that might be an added factor for such a problem. Cerebral involvement manifested as seizure, behavioral changes, encephalopathy, and loss of consciousness was described concerning attacks of hyperammonemia (20). Patient C experienced attacks of seizure, during which her ammonia levels were normal, unlike her sister who had attacks of encephalopathy started at the age of 9 years which were related to high ammonia. The cause of such late manifestation is not clear, especially that her diet was not changed and the level of plasma amino acids including arginine and ornithine was persistently low from an early age. Gastrointestinal complications were manifested in one patient (D) as recurrent pancreatitis and hemorrhagic gastritis. In the literature, the former is a well-reported problem (6), unlike the latter which is not linked to the disease. Hemorrhagic gastritis in our patient could be related to vasculitis which could be a part of the autoimmunity complications. Renal involvement is a well-known association with LPI, and it could vary from mild proteinuria, proximal tubulopathy, glomerular dysfunction to end-stage renal failure (9). None of our patients exhibit any renal manifestations; however, it is a progressive late complication that mandates continuous screening during the disease course.

Conclusion

This study described LPI cases from Bahrain with various clinical manifestations and two novel mutations. HLH was the main presenting feature in all patients. Besides, PAP was an early complication that carried a poor prognosis. Rheumatological problems were variable within the same family members; moreover, hemorrhagic gastritis is reported in one of the patients as a new possible complication. Persistent hyperammonemia was detected as a late manifestation of the disease.

Acknowledgments

The authors would like to thank the patients and their families.

List of Abbreviations

CT Computerized tomography  
eGFR Estimated glomerular filtration rate  
FTT Failure to thrive  
FM-CSF Granulocyte-macrophage colony-stimulating factor  
HLH Hemophagocytic lymphohistiocytosis  
LPI Lysinuric protein intolerance  
MRI Magnetic resonance imaging  
PAP Pulmonary alveolar proteinosis

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Ethical approval

Ethical approval was obtained from the Health Research Committee for the Ministry of Health, Bahrain.

Consent for publication

Informed consent was taken from the parents of the patients.

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