Case Report

Lysinuric protein intolerance in a family of Mexican ancestry with a novel SLC7A7 gene deletion. Case report and review of the literature

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ABSTRACT

Lysinuric protein intolerance (LPI) is a rare autosomal recessive disorder caused by mutations in the SLC7A7 located on the chromosome 14q11.2. LPI is most prevalent in Finland (1:50,000), Northern Japan (1:60,000) and Italy. Cases have also been reported in Spain and the United States. Here we report two siblings of Mexican descent. The older child was diagnosed at the age of three with severe chronic respiratory insufficiency leading to her demise. In contrast, the younger child was diagnosed soon after birth and dietary therapy has led to a stable life. Genetic analysis revealed a previously unreported deletion in the SLC7A7 gene. Additional research is needed to clarify the role of lysine in the pathophysiology of pulmonary proteinosis and herpes infections.

1. Introduction

Lysinuric protein intolerance (LPI) is a rare autosomal recessive disorder characterized by dysfunctional cationic amino acid (CAA) transport by the heteromeric amino acid transporter y + LAT-1/4F2hc at the basolateral membrane of epithelial cells of the kidney and small intestine [1]. Mutations in the y + LAT-1 gene SLC7A7 located on the chromosome 14q11.2, codes for the light chain portion of the transporter, and are responsible for the LPI disease phenotypes [2,3]. LPI is most prevalent in Finland (1:50,000), Northern Japan (1:60,000) and Italy, and cases have also been reported in Spain and the United States [4]. Although rare, LPI is of particular interest because of the varied types of mutations affecting the SLC7A7 gene and the wide phenotypic range seen in the disease. LPI is a multisystem disorder with a variety of symptoms, none of which manifest before weaning. The most common symptoms are acute nausea and vomiting following a protein-rich meal and aversion to protein-rich foods. Other symptoms may include hepatomegaly, growth retardation, mental retardation, osteoporosis, and muscle weakness [5]. Some patients present with symptoms suggestive of immune system dysfunction including glomerulonephritis, pulmonary alveolar proteinosis (PAP), increased susceptibility to hemophagocytic lymphohistiocytosis and severe varicella infections [6–8]. Impaired intestinal and renal absorption of the cationic amino acids lysine, arginine and ornithine result in low plasma levels and increased urinary excretion of CAAs. The urea cycle is disrupted, leading to hyperammonemia and increased orotic acid excretion [1]. Here we report two sibs of Mexican descent with different presentations. The older of the two presented at the age of three, with severe chronic respiratory insufficiency of unknown etiology. The younger of the two did not express this phenotype and has remained stable throughout his life. Genetic testing was performed on the younger sibling revealing a previously unreported deletion in the SLC7A7 gene.

2. Case reports

The sister and brother were the only two children born to non-consanguineous Mexican parents who immigrated to the United States. There was no family history of pulmonary or liver disease prior to the birth of the older sibling.

3. Case 1

This was a three year eight month old female who presented to Phoenix Children's Hospital (PCH) with chronic respiratory insufficiency of unknown etiology since the age of one year old and a resolving varicella rash. Genetic analysis had shown no mutations in SP-B, SP-C, ABCA3 or GM-CSF. The patient was previously treated with GM-CSF and pulse steroids with no respiratory improvement. Recently the patient also had a mild increase in hepatic transaminases at an outside

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institution (values not available), which led to her transfer to PCH for a lung and possible liver biopsy. Upon admission, the patient weight was 13.7 kg/30 lb 3 oz (5–10th percentile) and the height 85 cm/2′9 ft (<5th percentile). She was on 5 L of oxygen with a pO_2 of 69.2 mm Hg and a pCO_2 of 40.3 mm Hg. Imaging showed diffuse ground glass posterior opacities with thickening of interlobular septae (Fig. 1). The patient's hospital admission was complicated by an episode of streptococcal pneumonia and respiratory failure on BiPAP. She also experienced a tonic–clonic seizure prior to discharge which was attributed to withdrawal from sedatives. The right upper lobe lung biopsy histological examination (Fig. 2) was characterized by marked architectural distortion with alveolar proteinosis, septal thickening, type II pneumocyte hyperplasia and chronic inflammation. Cholesterol granulomas were noted in many alveolar spaces. The pulmonary histological findings were consistent with alveolar proteinosis. The liver biopsy contained a few clusters of hepatocytes with clear waxy cytoplasm. The cytoplasmic material was Periodic Acid-Schiff (PAS) positive and diastase sensitive. The remainder of the hepatic histoarchitecture was unremarkable. The histopathological findings were consistent with focal glycogenosis. Follow up urine amino acid analysis revealed elevated levels of ornithine (58.5, expected: 3.5–10.3), lysine (1419.3, expected: 17.3–71.6) and arginine (100.8, expected: 3.5–12.3) and the clinical diagnosis of lysinuric protein intolerance was confirmed. LDH, triglycerides and ferritin analysis were not performed. She immediately began a low protein diet (1.45 g/kg daily) with citrulline (300 mg/kg daily) supplementation. Unfortunately, she expired a few years later.

4. Case 2

The younger sibling of the index case was born following a full term pregnancy. The patient was born after the diagnosis of his sister, and when he exhibited poor growth at one month of age he was found to have LPI based on aminoaciduria, with decreased plasma ferritin, lysine, arginine, and ornithine. LDH and triglyceride analysis was not performed. Based on this history and laboratory findings, the patient started on the same low protein diet and citrulline supplementation. He never had a hyperammonemic decompensation. His follow up physical examinations between the ages of four and seven years of age found him to be intellectually intact, moderately hypotonic and below the 5th percentile for height and weight. He had no history of pulmonary disease. His peripheral blood was submitted for Sanger sequence analysis (see Acknowledgement) of all exons, adjacent splice junctions and promoter region. Results were interpreted according to sequence data published in GenBank accession number NT_026437.11 transcript
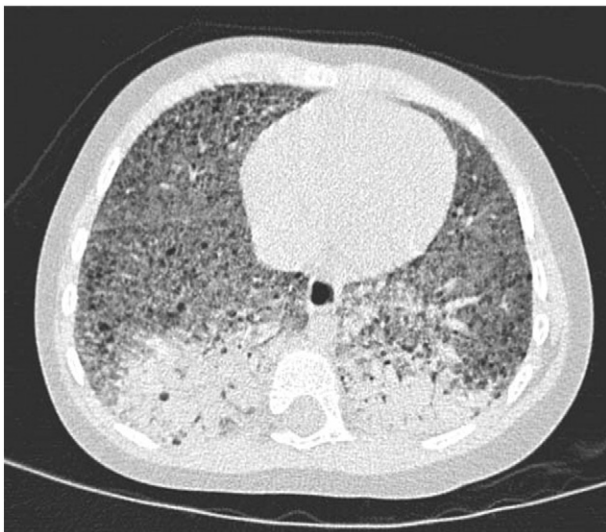

Fig. 1. HRCT of lungs, patient 1.

NM_003982. The patient was reported as homozygous for the deletion. The parents were not tested.

5. Discussion

LPI is grouped with a few additional disorders that control the transport of urea cycle intermediates. LPI patients will usually present with recurrent episodes of vomiting and diarrhea. Most of the times, first symptoms manifest after weaning from breast milk. With time, many complications involving multiple systems become apparent. Plasma ammonia levels may be normal in the fasting state and only rise after a protein-rich meal. Urinary excretion of cationic amino acids as seen in the reported older sibling supports the diagnosis.

Including the above family, 62 mutations in the SLC7A7 gene resulting in LPI have been reported in the 2014 edition of the Human Genome Database (HGMD) including missense, nonsense, splice site, small duplications/insertions, small deletions, and large genomic rearrangements [4,9,10]. All of the mutations are found in the SLC7A7 gene and none has been reported in the SLC3A2 gene, which codes for the heavy chain of the cationic amino acid (CAA) transporter, 4F2hc. The specificity of the CAA transporter arises from the light chain y + LAT1 encoded by SLC7A7, while the 4F2hc chain participates in five other amino acid transporters and has a role in β-integrin function [11]. Given the amount of essential functions allowed by 4F2hc expression, it is not surprising that the SLC3A2 knockout confers a lethal phenotype in the mouse model [12]. In our patient, a novel E5-E11 deletion was detected. This deletion is most similar to large deletions found in the Spanish population [9] and appears to be associated with a more severe phenotype. While we observed a severe phenotype including pulmonary alveolar proteinosis and respiratory failure in one sibling, the younger sibling has not exhibited the same presentation. Furthermore, the two novel large deletions reported in the Spanish population [9] were mediated by an *Alu*-mediated recombination between intronic *Alu* repeats at introns 3 and 5 with an identical *Alu* repeat at the 3′ end of SLC7A7. *Alu* repeats are the most common repetitive DNA elements in the genome, and are prone to recurrent genomic rearrangement through *Alu–Alu* recombination. Several types of cancer like neurofibromatosis as well as non-ketotic hyperglycinemia are linked with disruptive *Alu* recombination events [13,14]. Our patient would require further analysis to determine the nature of the novel deletion detected. The discovery of an *Alu* recombination event could point to an inherited recombination hot spot passed from Spanish ancestors to this Mexican family.

In this case, the older sibling had a delayed diagnosis and showed a severe phenotype leading to her demise. In contrast, the younger child did not show the same phenotype under an adequate therapeutic support and despite a large gene deletion. Neither patient exhibited symptoms until weaned from breast milk, an observation that is consistent with all LPI case reports. Patients with LPI typically begin to show progressive deviation from growth curves and delayed skeletal maturation, in addition to protein intolerance and evidence of urea cycle disruption upon introduction to cow's milk. Breast milk has lower protein content than cow's milk. However, infants with LPI also react to dilute cow's milk and formula [15]. Boyd et al. suggest the interesting possibility that mRNA in microvesicles existing in maternal milk may provide temporary protection against the LPI phenotype [16]. Studies based on cow's milk have show that mRNA in similar microvesicles is resistant to acidic environments mimicking the gastrointestinal tract and is capable of being transferred into cultured cells [17].

As mentioned, the older sibling in this family was not diagnosed until the age of 3.5 yo, and once diagnosed immediately began her dietary treatment. This treatment did not correct all symptoms and she continued to deteriorate over time with failure to thrive and respiratory insufficiency. Failure to thrive has been proposed to be a result of persistent lysine deficiency [18]. In rats, a lysine deficient diet has been shown to result in growth retardation and decreased levels of
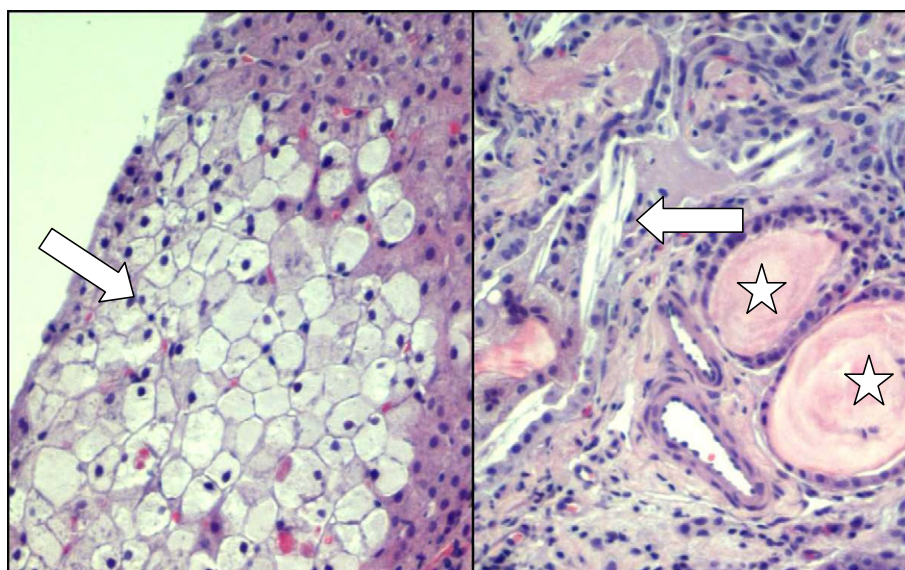
Fig. 2. 40× H&E of liver with focal glycogenosis (left) and lung with alveolar proteinosis (right). The liver biopsy contains a few clusters of hepatocytes with clear waxy cytoplasm (left arrow). The lung biopsy showed alveolar proteinaceous material (stars), septal thickening, type II pneumocyte hyperplasia and chronic inflammation. Cholesterol granulomas (right arrow) were also noted in many alveolar spaces.

serum growth hormone [19]. However, SLC7A7 −/− mice fetuses that died with IUGR exhibited down-regulated hepatic Igf1 [20]. Since the SCL7A7 −/− mice received maternal lysine levels via umbilical blood, the mechanism of growth retardation may be more directly linked to another failure of the SLC7A7 −/− phenotype.

Of interest, the older sibling not only persisted the growth delay, but the chronic respiratory insufficiency due to pulmonary alveolar proteinosis progressed to the point of oxygen dependence despite steroid and GM-CSF treatment. Patients with LPI have been observed to have various pulmonary presentations [21]. In a retrospective review of 31 Finnish patients with LPI, four children died in respiratory insufficiency, one adult had an episode of respiratory insufficiency, and another had chronic symptoms. Twenty-five patients remained symptom free, but 1/3 of these had signs suggestive of pulmonary fibrosis on chest X-ray. Of the four children who died in respiratory failure, three showed pulmonary alveolar proteinosis and one had pulmonary hemorrhage with cholesterol granulomas. In light of this, a recent study [22] proposed that defective alveolar macrophages could contribute to the presentation of PAP in LPI patients. SP-D and GM-CSF were shown to increase the uptake of protein and dying cells by LPI alveolar macrophages, but ex vivo supplementation of alveolar macrophage cultures with GM-CSF showed a marked increase in the number of granulomatous structures, an effect mitigated by the addition of SP-D [22]. The same study concluded that the treatment of LPI patients with GM-CSF may not result in the improvement of PAP. The treatment of our patient agrees with this conclusion, as she was not responsive to either a steroid burst or GM-CSF treatment.

LPI may also present with transient episodes of hyperammonemia or Reye-like clinical syndrome. The prevailing theory for the hepatic dysfunction is based on the functional deficiency of urea cycle intermediates arginine and ornithine as a consequence of the poor dietary protein tolerance and absorption failure in the intestine [1]. Of interest, a study of six patients with LPI revealed reduced portal blood flow volume, which was restored to near controls levels after administration of nitrates or L-arginine [23]. The older sibling liver biopsy had small clusters of hepatocytes with clear cytoplasm (Fig. 2). This histopathological finding supports the diagnosis but is not required for the diagnosis of LPI. Interestingly, this finding has also been noted in liver biopsies from patients with other urea cycle defects such as hyperammonemia-hyperornithinemia-homocitrullinuria (HHH) syndrome, ornithine transcarbamylase (OTC) and carbamoylphosphate

synthetase (CPS) deficiencies [23]. Other microscopic findings described in LPI which were not observed in our patient include microvesicular steatosis, fibrosis, cirrhosis, and increased periportal nuclear glycogen. A liver biopsy was not performed in the younger sibling.

Finally, immunodeficiency has been observed in patients with LPI [6–8]. The older sibling presented with a widespread varicella rash, which was resolving at the time of exam at our hospital. Patients with LPI are at an increased risk for generalized varicella infections, and these infections resemble varicella in children who have been treated for acute leukemia or who are immunocompromised [8]. Additionally, varicella infection in this population may induce or aggravate pulmonary changes leading to pulmonary alveolar proteinosis [8]. Patients with LPI respond poorly to the varicella vaccine and have increased susceptibility to infections, suggesting humoral and T cell impairment. Lysine has been studied as a treatment for another herpesvirus, HHV-1 and the lysine:arginine ratio has been shown to affect HSV replication rates in vitro [24,25]. Patients with LPI typically have decreased plasma lysine levels, and the relationship to the increased susceptibility to varicella infection is unknown.

In summary, to our knowledge, this is the first report of lysinuric protein intolerance occurring in a family of Mexican ancestry associated with a novel gene deletion. Early diagnosis and dietary therapy appear to halt the progression of the disease as exemplified by the younger sibling. On this assumption, a cost effective screening program has been proposed in areas affected by a founder mutation [26]. Additionally, research is needed to clarify the role of lysine in the pathophysiology of pulmonary proteinosis and herpes infections.

Acknowledgment

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