Short Report

5-Oxoprolinase deficiency: report of the first human \textit{OPLAH} mutation


Gamma-glutamyl cycle is a six-enzyme cycle that represents the primary pathway for glutathione synthesis and degradation. 5-Oxoprolinase deficiency is an extremely rare disorder of the gamma-glutamyl cycle with only eight patients reported to date. Debate continues as to whether this is a benign biochemical defect because of the heterogeneity of the clinical presentation which ranges from normal to significant neurological involvement. Here, we report the first molecularly characterized patients with 5-oxoprolinase deficiency due to a mutation in \textit{OPLAH} (which encodes 5-oxoprolinase). The largely benign clinical course of the patients described herein despite persistent 5-oxoprolinuria highlights the importance of establishing a molecular diagnosis in the few cases with abnormal neurological outcome to exclude potentially overlapping biochemical defects and to explore potential genotype/phenotype correlation.

Conflict of interest
Authors declare no conflict of interest.

The importance of the gamma-glutamyl cycle, a six-enzyme cycle that represents the primary pathway for glutathione synthesis and degradation, comes from the critical role of glutathione as an antioxidant (Fig. 1). Glutathione synthase deficiency is known to cause oxidative damage of red blood cell membrane in an autosomal recessive fashion and is commonly accompanied by accumulation of 5-oxoproline and its increased excretion (5-oxoprolinuria). 5-Oxoprolinuria is also an important feature of 5-oxoprolinase deficiency, another known metabolic defect of the...
gamma-glutamyl cycle affecting an ATP hydrolyzing enzyme (5-oxo-L-prolinase) encoded by \textit{OPLAH} gene. In addition, acquired causes of 5-oxoprolinuria have been reported such as malnutrition, pregnancy, type 2 diabetes, patients on artificial diets and drugs such as vigabatrin (1).

5-Oxoprolinase deficiency is an extremely rare autosomal recessive disease characterized by 5-oxoprolinuria and a variable clinical presentation (2). Eight patients from six families have been reported in the literature, all were diagnosed based on high 5-oxoproline in the urine, normal glutathione synthase cellular enzyme levels and absence of metabolic acidemia (2). Here, we report the first molecularly confirmed 5-oxoprolinase deficiency patients and compare their clinical and biochemical presentation to those previously reported.

\textbf{Clinical report}

Index is a 10-month-old infant, the second child to healthy first cousin Indian parents. His 8-year-old sister is not known to have any medical problems. He was the product of a full-term pregnancy that was complicated by H1N1 infection 20 days prior to delivery for which his mother received full course of Tamiflue and Augmentine. Delivery was a vacuum-assisted vaginal delivery at 42 weeks of gestation. APGAR scores were 8 and 9 at 1 and 5 min, respectively. His length (56 cm), weight (3.77 kg), and head circumference (34 cm) were within normal range. Patient was transferred to the NICU shortly after birth because of transient hypoglycemia. The additional findings of indirect hyperbilirubinemia and mild metabolic acidemia raised concerns for neonatal sepsis although all cultures tested negative later. A full course of antibiotic was administered and the mild indirect hyperbilirubinemia responded adequately to phototherapy. Metabolic acidosis was mild with pH ranging from 7.31 to 7.35 and lowest HCO$_3$ recorded was 15.9. Extended newborn metabolic screening revealed moderately elevated 5-oxoproline in the urine (see below). However, complete blood count was normal as was the reticulocyte count.

He was discharged in a very good condition with follow-up in the clinic for genetics and metabolism. Severe eczema developed at the age of 2 months with only partial response to steroid therapy. Allergy testing (RAST) revealed strong allergy to multiple food items but maternal abstinence did not appear to influence the severity of the symptoms. In addition, his head circumference (normal at birth at 34 cm) showed some deceleration, but then paralleled the third centile curve throughout his follow-up to the age of 10 months. His motor, social and cognitive development continued to be normal for age. Repeated blood gas measurements were normal but he continued to have elevated 5-oxoproline levels in the urine.

\textbf{Biochemical studies}

Determination of the 5-oxoproline in the urine is detailed in the supporting information (Fig. S1 and Appendix S1). Briefly, we compared the relative abundance of 5-oxoproline with an internal standard on Gas Chromatography-Mass Spectrometry (GC-MS) in the patients with an average ratio obtained for 21 controls. The ratio in the index (7.60) was markedly higher than the average ratio in the controls (0.06).

\textbf{Molecular studies}

To investigate the cause of this persistent 5-oxoprolinuria, we performed sequence analysis of the glutathione synthase gene as well as \textit{OPLAH} gene which encodes the enzyme 5-oxoprolinase. Genomic DNA was purified from the patient’s whole blood using Gentra Puregen Blood Kit. Primer sequences and PCR conditions are available upon request. Glutathione synthase gene sequence was normal. However, sequence analysis of \textit{OPLAH} revealed the presence of a single base pair insertion (NM_017570.3:c.2601_2602insC) with resulting frameshift and premature truncation of the enzyme [p.(His870Profs*92)] (Fig. 2). Both parents were found to be heterozygous for this mutation which is not listed as a SNP in any of the public databases including the 1000 genomes. Surprisingly, we found that the sister is also homozygous for this mutation. As a result, she was brought
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Discussion

5-Oxoprolinase enzyme is an ATP-dependent enzyme which catalyzes transformation of 5-oxoproline to glutamate in the gamma-glutamyl cycle (3). This enzyme is found in microorganisms, plants, as well as most mammalian tissues. Its substrate, 5-oxoproline, can be normally detected at low levels in the urine (4). However, high values are considered the whole mark of the enzyme deficiency states. The extreme rarity of the 5-oxoprolinase deficiency is reflected in the presence of only eight cases in the literature, the last being reported more than a decade ago. Extended newborn screening was not performed on her as a neonate, but our GC/MS analysis of her urine revealed increased 5-oxoproline excretion (3.76 vs 0.06 in control; see above). Her blood gas and the rest of her extended metabolic screen were normal.

Our patient, her young age and borderline head circumference notwithstanding, displayed normal development. Along with her normally developing sister, the number of patients with apparently normal psychomotor development increased to seven. Although we did not assay 5-oxoprolinase in our patients, it is expected to be severely impaired because the frameshift mutation almost completely removes the oxoprolinase domain (amino acids 733–1260) which has been found to mediate the oxoprolinase activity (10). The fact that our patients are the only patients to date in whom 5-oxoprolinase deficiency was confirmed at a molecular level is important because the biochemical diagnosis made in the previous reports may have been confounded by the presence of other biochemical defects. For example, the biochemical diagnosis of 5-oxoprolinase deficiency may have been the result of unsuspected cofactor deficiency that was detrimental to the enzyme activity but also resulted in neurological symptoms. Phenylketonuria (PKU) is a good example where neurological deterioration can be a result of cofactor tetrahydrobiopterin deficiency as well as the decreased activity of the phenylalanine hydroxylase.

Thus, it will be of interest to molecularly test previously reported patients to establish whether or not those with neurological involvement do indeed have 5-oxoprolinase deficiency and not other biochemical defects. Not only will this facilitate the delineation of the phenotype of this controversial metabolic condition but may also establish a genotype/phenotype correlation.

Supporting Information

The following Supporting information is available for this article: Fig. S1. The characteristic ion spectrum for 5-oxoproline showing the predominant ions m/z 156 and 258.

Fig. S2. 5-Oxoprolinuria OA profile vs a normal OA profile. In panel A (normal), 5-oxoprolines levels are detected at very low levels and the abundance is much lower than the IS. In panel B (patient), 5-oxoproline is detected as a higher abundance peak than that of the IS.

Appendix S1. Detailed materials and methods for the determination of the 5-oxoproline level in urine.

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References