

## Molecular study is an important tool in the confirmation of Inborn Errors of Metabolism

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### INTRODUCTION

Inborn errors of metabolism are genetic disorders due to impaired activity of enzymes, transporters, or cofactors resulting in accumulation of abnormal metabolites proximal to the metabolic block, lack of essential products or accumulation of by-products.

Kidney glutaminase is responsible for converting glutamine from the liver to urine ammonium. However, about 80% of the excreted nitrogen is in the form of urea which is produced exclusively in the liver, in a series of reactions that are distributed between the mitochondrial matrix and the cytosol. The series of reactions that form urea is known as the Urea Cycle and operates only to eliminate excess nitrogen.

The urea cycle disorders (UCD) are caused by defects in the metabolism of waste nitrogen from the breakdown of protein and other nitrogen-containing molecules. Severe deficiency or total absence of activity of any of the first four enzymes (CPS1, OTC, ASS, ASL) in the urea cycle, or the cofactor producer (NAGS), results in the accumulation of ammonia and other precursor metabolites during the first few days of life (figure 1). Infants with a severe UCD are normal at birth but rapidly develop cerebral edema and the related signs of lethargy, anorexia, hyper- or hypoventilation, hypothermia, seizures, neurologic posturing, and coma. Deficiency in one of the enzymes results in a specific UCD.

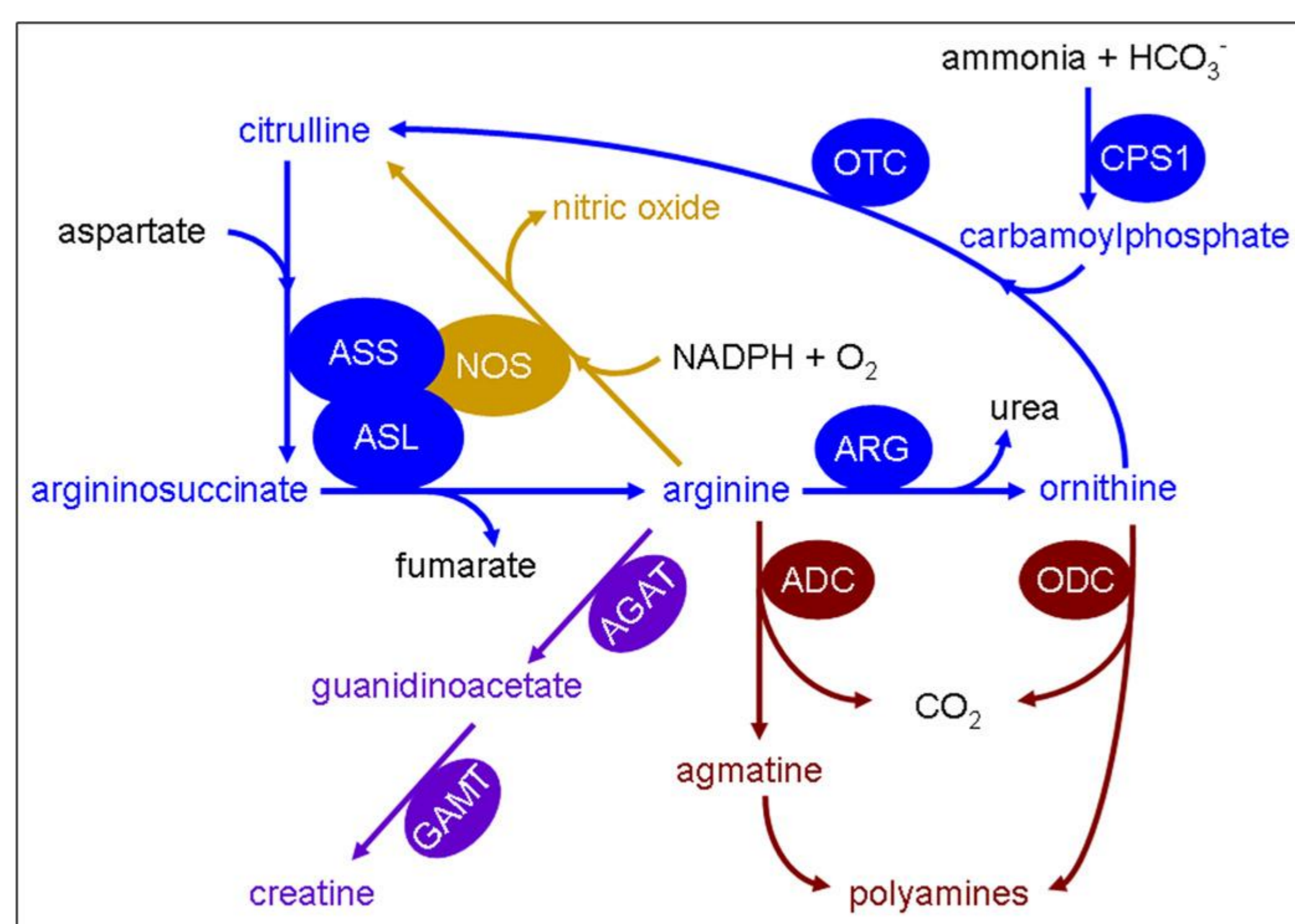


FIGURE 1. Schematic illustration of the involvement of ASL in various metabolic and biochemical reactions. (1)

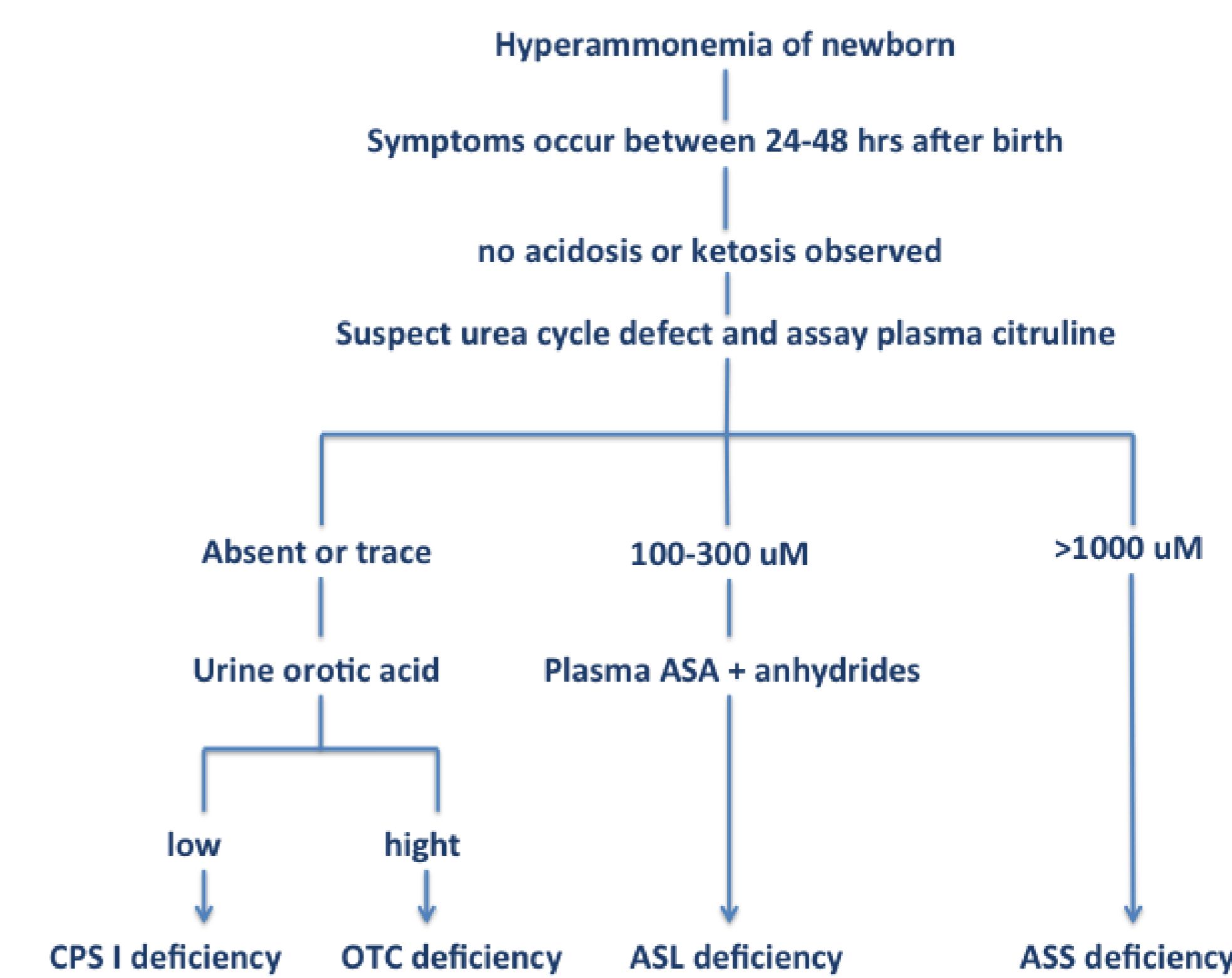


FIGURE 2. Schema for the differential diagnosis of neonatal UCDs

Citrullinemia and argininosuccinic aciduria are autosomal recessive disorders that lead to the accumulation of nitrogen as ammonia, alanine, glutamate, and other intermediate metabolites. The diagnoses of these disorders are based on clinical suspicion and biochemical and molecular genetic testing. Plasma and urine quantitative amino acid analysis, determination of plasma concentrations of ammonia and measurement of urinary orotic acid can distinguish between the specific urea cycle defects (figure 2).

A definitive diagnosis of a urea cycle defect depends on either molecular genetic testing or measurement of enzyme activity.

### PATIENT AND METHODS

The authors present a symptomatic Brazilian case that came to our lab with a diagnosis the citrullinemia. Patient in the first days of life presented poor feeding with subsequent progression to coma and death. The amino acid profile obtained by MS/MS shows an increase of citrulline in plasma and the levels of plasma ammonia are also increased. To clarify this case the genes ASS1 and ASL, that encode the enzyme argininosuccinate synthetase and argininosuccinate lyase, were studied by reported methods.

### RESULTS

The molecular study of ASL has allowed the identification of the **homozygous mutation** in this patient: the splicing mutation c.524+2T>G. This mutation is already described in the literature (2). No mutations were found in the molecular study of the ASS1 gene.

### DISCUSSION

The finding of elevated Citrulline in amino acid analysis suggests one of two metabolic defects: Argininosuccinic Acid Synthetase Deficiency or Argininosuccinate Lyase Deficiency. Both are associated with severe metabolic decompensation with episodic hyperammonemia.

Quantitative amino acid chromatography is the key to diagnose this disorders. In general, an increase of citrulline level is a useful way to the diagnosis of ASS and ASL deficiency. In ASL plasma citrulline is usually between 100 and 300 uM and higher in ASS deficiency. The increased argininosuccinic acid and two anhydrides in the plasma and urine of a patient with hyperammonemia establishes the diagnosis of ASL (3). However, unless the retention time of argininosuccinic acid is known, this compound may not be recognized since it can have the same retention time as other amino acids.

The onset and severity of UCDs is highly variable. This depends on the specific mutation involved and correlates with the amount of urea cycle enzyme function. Severe mutations result in zero to very little enzyme function and ability to detoxify ammonia, and cause severe UCD. Mild to moderate mutations represent a broad spectrum of enzyme function, providing some ability to detoxify ammonia, and result in mild to moderate UCD. This mutation causes the complete exon 6 skipping with a deletion of 26 amino acids in critical regions of the protein which is in agreement with severe presentation of the patient.

Genetic analysis should play an important role for prenatal diagnosis of argininosuccinic aciduria. We strongly recommend molecular genetic testing in these patients in order to provide adequate prenatal counseling to the future pregnancies in their families. In this case the molecular study was essential to the correct diagnosis and to provide future prenatal counseling. On the other hand, on mild phenotypes or on late-onset form, the molecular study is important as well as reported in the poster Marcão et al.