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# Argininosuccinate Lyase Deficiency – Argininosuccinic Aciduria and Beyond

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## Abstract

The urea cycle consists of six consecutive enzymatic reactions that convert waste nitrogen into urea. Deficiencies of any of these enzymes of the cycle result in urea cycle disorders (UCD), a group of inborn errors of hepatic metabolism that often result in life threatening hyperammonemia. Argininosuccinate Lyase (ASL) is a cytosolic enzyme which catalyzes the fourth reaction in the cycle and the first degradative step, i.e. the breakdown of argininosuccinic acid to arginine and fumarate. Deficiency of ASL results in an accumulation of argininosuccinic acid in tissues, and excretion of argininosuccinic acid in urine leading to the condition argininosuccinic aciduria, ASA.

ASA is an autosomal recessive disorder and is the second most common urea cycle disorder. In addition to the accumulation of argininosuccinic acid, ASL deficiency results in decreased synthesis of arginine which is in common with all UCDs except argininemia. Arginine is not only the precursor for the synthesis of urea and ornithine as part of the urea cycle but it is also the substrate for the synthesis of nitric oxide, polyamines, proline, glutamate, creatine and agmatine. Hence, while ASL is the only enzyme in the body able to generate arginine, at least four enzymes use arginine as substrate: arginine decarboxylase, arginase, nitric oxide synthetase (NOS) and arginine/glycine aminotransferase. In the liver, the main function of ASL is ureagenesis, and hence, there is no net synthesis of arginine. In contrast, in most other tissues, its role is to generate arginine that is designated for the specific cell's needs. While patients with ASA share the acute clinical phenotype of hyperammonemia, encephalopathy and respiratory alkalosis common to other UCD, they also present with unique chronic complications most probably caused by a combination of tissue specific deficiency of arginine and/or elevation of argininosuccinic acid.

This review article summarizes the clinical characterization, biochemical, enzymatic, and molecular features of this disorder. Current treatment, prenatal diagnosis, diagnosis through the newborn screening as well as hypothesis driven future treatment modalities are discussed.

### Keywords

Argininosuccinic aciduria; Argininosuccinate Lyase; urea cycle; arginine; nitric oxide

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## INTRODUCTION

The urea cycle (Fig. 1) has two main functions: the detoxification of waste nitrogen into excretable urea and the *de novo* biosynthesis of arginine [Brusilow and Horwich, 2001]. Deficiencies of all of the enzymes of the urea cycle have been identified, and although each specific disorder results in accumulation of different metabolites, they (except for hyperargininemia) usually present in the newborn period or in early infancy with hyperammonemic encephalopathy and hyperglutaminemia (Brusilow et al., 1980;Msall et al., 1984;Maestri et al., 1999). The deficiencies of all enzymes involved in the urea cycle are inherited in autosomal recessive manner except for ornithine transcarbamylase that is X-linked (Maestri et al., 1999). The overall prevalence of these conditions is estimated to be of 1 in 8,200 in the United States (Brusilow and Maestri, 1996). Ornithine transcarbamylase deficiency has the highest prevalence of the six urea cycle defects while hyperargininemia and N-acetyl glutamate synthetase deficiency are the least frequent.

Argininosuccinic Aciduria (OMIM 207900) is caused by a defect in the enzyme Argininosuccinate Lyase, ASL (OMIM 608310) that cleaves argininosuccinate to fumarate and arginine. It has an estimated incidence of 1 in 70,000 live births [Brusilow and Horwich, OMMBID] and is the second most common urea cycle disorder (UCD) (Tuchman et al., 2008). The first documented cases of this condition were published in 1958 in the *Lancet* as a disease, probably hereditary, characterized by severe mental deficiency and a constant gross abnormality of amino acid metabolism (Allan et al., 1958). The clinical features originally described included mental and physical retardation, convulsions, episodic unconsciousness, liver enlargement, skin lesions, and dry and brittle hair demonstrating trichorrhexis nodosa (Allan et al., 1958). In 1964, it was noted that in the U.S., where arginine is probably supplied adequately by the usual diet, brittle hair may not occur as often as in the United Kingdom, where the average protein intake is less ample (Coryell et al., 1964). These observations together laid the bases for ASA diagnosis and treatment.

## CLINICAL CHARACTERISTICS

The clinical presentation of patients with ASA is marked by clinical heterogeneity. In general, there are two forms, a severe neonatal form and a late onset form. The clinical presentation of the severe neonatal onset form is indistinguishable from that of other urea cycle disorders and is characterized by hyperammonemia within the first few days after birth. Tachypnea leading to a central respiratory alkalosis, hypothermia, vomiting, seizures and lethargy are commonly observed clinical features. In contrast, the manifestations of the late onset form presentation range from episodic hyperammonemia triggered by acute infection, to cognitive impairment, behavioral abnormalities, learning disabilities in patients without any documented episodes of hyperammonemia (Brusilow, 2009). With the advent of comprehensive newborn screening, there are increasing numbers of patients who are being diagnosed presymptomatically.

While manifestations secondary to hyperammonemia are common to all urea cycle disorders, many patients with ASL deficiency present with a more complex clinical phenotype. The increased incidence of neuro-cognitive deficiencies, hepatitis, cirrhosis, systemic hypertension and trichorrhexis nodosa (coarse and brittle hair) are unique to ASA and appear to be unrelated to the severity or duration of hyperammonemic episodes (Ficicioglu et al, 2009; Mori et al., 2002; Saudubray et al., 1999)

In a cross sectional study of patients with UCD, it was observed that patients with deficiency of ASL had a significant increase in disabilities and neurological abnormalities as compared to patients with OTC deficiency (Tuchman et al., 2008). Patients with ASA also had an

increased incidence of attention deficit hyperactivity disorder, developmental disability, seizure disorder and learning disability as compared to all other UCD (Tuchman et al., 2008). Though neuro-cognitive deficits are more common in ASA, they are not universally present as many patients treated with protein restriction and arginine have normal cognition and development (Ficicioglu et al.; Widhalm et al.). The increasing wide availability of newborn screening programs allows the evaluation of benefits of early treatment on disease progression, especially in the late onset form. In a recent study, the long term outcome of 13 patients who were diagnosed between 4–6 weeks of age by newborn screening was evaluated. All patients had low activity of ASL and in spite of optimal therapy with protein restriction and arginine supplementation, four patients out of the 13 had learning disability, three had mild developmental delay, three had seizures and six patients had abnormal EEG that included abnormal sharp irregular background activity, frequent bilateral paroxysms and increased slow wave activity (Ficicioglu et al.,). In an Austrian cohort of 17 ASA patients diagnosed by newborn screening, IQ was average/above average in 11 (65%), low average in 5 (29%), and in the mild intellectual disability range in 1 (6%) patients. Four patients had an abnormal EEG without evidence of clinical seizures (Mercimek-Mahmutoglu et al., 2010).

The second unique feature of ASA that again appears to be independent of the defect in ureagenesis is the increased incidence of liver disease. The spectrum of hepatic involvement ranges from hepatomegaly, elevations of liver enzymes to severe liver fibrosis (Billmeier et al., 1974; Mori et al., 2002; Tuchman et al., 2008; Zimmermann et al., 1986). The histological features of liver involvement include swollen pale hepatocytes, abundance of glycogen, periportal and bridging fibrosis (LaBrecque et al.,; Mori et al.,). The liver involvement has been noted even in patients with no significant hyperammonemia who were treated with protein restriction and arginine supplementation (Personal observation, B.Lee; Mori et al., 2002; Mercimek-Mahmutoglu, 2010)

Recently, it has been noted that hypertension is more commonly observed in patients with ASA (Brunetti-Pierri et al., 2009), though this has not been noted in the few long term follow-up studies (Ficicioglu et al., 2009; Parsons et al., 1987; Widhalm et al., 1992).

While many of the unique distinct manifestations in ASA could be the result of toxicity of argininosuccinate, they raise the possibility that these may also be the result of a cell autonomous deficiency of ASL that perhaps contributes to the deficiency of a compartmentalized urea cycle intermediate such as arginine in various tissues (Scaglia et al., 2004).

## BIOCHEMICAL FEATURES

Hyperammonemia with respiratory alkalosis is the classical finding seen during periods of metabolic decompensation in all UCD including ASA.

The accumulation of argininosuccinic acid (and its anhydrides), the substrate proximal to the metabolic block is the biochemical hallmark of ASL deficiency. Argininosuccinic acid is not detectable in body fluids of normal individuals. The typical levels observed in ASA patients range between 50–110 μmol/L in the plasma, and > 10,000 μmol/gm of creatinine in the urine (Ficicioglu et al., 2009). The argininosuccinate chromatographic peak may co-elute with leucine or isoleucine, resulting in an apparent increase in one of these amino acids, but its anhydrides eluting later in the run should facilitate the correct identification of argininosuccinate. Plasma citrulline levels are elevated typically in the range of 100–300 μmol/L as compared to levels greater than 1000 μmol/L in citrullinemia (Brusilow and Horwich, 2009). In addition to increased argininosuccinic acid and citrulline, elevations of alanine, glutamine and glycine that are reminiscent of defects in urea formation are

commonly observed in the plasma amino acid profile. The elevations in glutamine in ASA tend to be lower than those seen with proximal metabolic blocks such as OTC and CPS deficiencies (Tuchman et al., 2008).

Arginine is converted to guanidinoacetate (GAA) and creatine by sequential action of two enzymes, glycine amidotransferase and guanidinoacetate methyl tranferase, respectively. Decreased amounts of GAA prior to initiation of arginine therapy and subsequent normalization of levels with arginine supplementation have been described in patients with UCD including ASA (Arias et al., 2004).

Orotic aciduria is a feature that may be seen in this condition (Brosnan and Brosnan 2007; Gerrits et al., 1993). The metabolic block in ASA is far removed to cause an accumulation of carbamoyl phosphate due to simple feedback inhibition but the impaired recycling of ornithine seems to contribute to the increase in carbamoyl phosphate leading to overproduction of orotic acid (Brosnan and Brosnan, 2007).

Increase in serum levels of aspartate transaminase and alanine transaminase is more common in ASA as compared to other UCD (Tuchman et al., 2008); hence, this needs to be monitored with serial measurements. However, the levels of bilirubin, alkaline phosphatase, total proteins, and prealbumin are comparable to other UCD.

Enzyme activity in ASA can be measured from flash frozen liver biopsy sample or more conveniently from skin fibroblasts or the red blood cells (Shih et al., 1969). There seems to be no correlation between enzyme activity and the neuro-clinical outcome as patients with undetectable residual enzyme activity have normal intellect while those with higher activities present with cognitive impairment (Ficicioglu et al., 2009; Mercimek-Mahmutoglu et al., 2010). This clearly supports the contribution of dominant genetic and/or environmental modifiers. ASL enzyme activity in fibroblasts as measured by incorporation of $^{14}$C-citrulline into proteins has been suggested as a better prognostic indicator but the test is not available on a clinical basis (Ficicioglu et al., 2009; Kleijer et al., 2002). In general, clinical history, biochemical testing and molecular testing are sufficient and enzyme assay is not generally required for the diagnosis of ASA.

## PATHOLOGY

Hepatomegaly can be seen even in patients with ASA who are on optimal therapy and have not had any episodes of hyperammonemia (Zimmermann et al., 1986). Elevations of aspartate and alanine transaminases, AST and ALT respectively is a biochemical marker for progressive liver injury and fibrosis, a complication of ASL deficiency (Mori et al., 2002). Biopsy performed in patients with persistent liver dysfunction has revealed liver fibrosis in the periportal and central area which extend into the liver lobule (Mori et al., 2002). In a recent study, 13 percent of ASA patients with late disease onset, had abnormal liver function tests and/ or evidence of hepatic steatosis (Mercimek-Mahmutoglu et al., 2010). Neuropathology changes described in ASA include astrocyte transformation to Alzheimer type II glia, a finding that may be a consistent feature of any form of hyperammonemia (Lewis and Miller, 1970). Trichorrhexis nodosa, one of the unique clinical features of ASA, is the formation of nodes along the hair shaft at which breakage readily occurs. The normal hair contains 10.5% arginine by weight. The deficiency in arginine resulting from ASL deficiency produces weak hair with a tendency to break. Microscopic evaluation of hair from patients with ASA, reveal nodular swellings on the hair shafts and frayed cortical fibers (Fichtel et al., 2007).

## PATHOGENESIS OF DISEASE

As stated before, it is unlikely that elevated plasma ammonia is the only toxic compound in ASA because neuro-cognitive delays, liver fibrosis, hypertension and renal dysfunction have been described even in patients with no documented hyperammonemic episodes. In addition, these clinical features are unique to ASA patients and are not seen at all or to the same magnitude in other urea cycle disorders, supporting the hypothesis that the phenotype in ASA is likely attributable to a combination of the increase in argininosuccinic acid together with the additional roles of ASL in generating endogenous arginine in other tissues outside the liver.

Arginine is a semi-essential amino acid. The sources of arginine are both exogenous from the diet, and endogenous from the breakdown of proteins in addition to the synthesis from citrulline (Wu and Morris, 1998)(Fig 2). In healthy adults the level of endogenous synthesis generates sufficient arginine so that it is not essential to obtain it through exogenous sources. However in situations such as catabolic stress or in conditions involving kidney/small intestine dysfunction, the arginine production is not commensurate with the requirements and arginine becomes an essential amino acid. The liver is the major site of arginine metabolism wherein arginine generated in the urea cycle is rapidly converted to urea and ornithine. Thus under normal conditions the liver does not contribute to the circulating pool of arginine. Approximately 60% of net synthesis of arginine in adult mammals occurs in the kidney, where citrulline is extracted from the blood and converted to arginine by the action of argininosuccinate synthetase, ASS and, argininosuccinate lyase ASL that are localized within the proximal tubules (Windmueller and Spaeth, 1981). However, many other tissues and cell types also contain both of these enzymes for generating arginine from citrulline (Gotoh, 2004)(Fig 3). In ASA, because all cells and tissues are deficient in ASL, arginine becomes an essential amino acid.

Arginine serves as the precursor for the synthesis of urea, nitric oxide, polyamines, proline, glutamate, creatine and agmatine (Fig 2). Thus, in contrast to the one enzyme that produces arginine- ASL, 4 enzymes use arginine as substrate: arginine decarboxylase ADC, arginase, nitric oxide synthetase NOS and arginine/glycine aminotransferase. NO is the most studied of arginine metabolites. With deficiency of ASL and the resulting deficiency in arginine, one could hypothesize that there would also be deficiency of NO and other metabolites for which it is a precursor. However, ASA patients are supplemented with arginine and hence theoretically, should not be deficient for its metabolites. The “arginine paradox”, describes the observation that despite apparently saturating intracellular levels of arginine, exogenously administered L-arginine is able to increase NO production. This important paradox suggests that L-arginine availability at the site of NO production may be the limiting factor (Cui et al., 2006); (Vukosavljevic et al., 2006). One explanation for this is intracellular compartmentalization of arginine.

Other than its well characterized role in vasodilatation, NO has important roles in many diverse processes including immune response, neurotransmission and adhesion of platelets (Naseem, 2005; Malyshev and Shnyra, 2003). Hence, it is intriguing to further study the effect that NO donors might have on the cognitive delay, liver fibrosis and renal abnormalities seen in ASA patients.

It is important to note that the depletion of arginine as substrate for NO synthesis has the effect of causing increase free radical production due to uncoupling of NOS (Pignitter et al., 2006). Increase in free radical production results in tissue damage with the brain being sensitive to both direct damage as well as an indirect damage caused by increases in intracellular free $Ca^{2+}$ and, possibly, release of excitatory amino acids (Halliwell, 1992).

Finally, free radicals could also interact with argininosuccinic acid to form guanidinosuccinic acid, GSA, a known cellular and neuronal toxin (Aoyagi, 2003; Aoyagi et al., 2001; D'Hooge et al., 1992).

## MOLECULAR CHARACTERISTICS

The gene encoding ASL was cloned in 1986 (O'Brien et al., 1986) and since then, a number of mutations have been found. The cDNA encodes a deduced protein of 463 amino acids with a predicted molecular mass of 52 kD. The active enzyme is a homotetramer of four identical subunits. The *ASL* gene has now been identified in a variety of species including E. Coli, Saccharomyces, algae, amphibia, rat and human (Yu and Howell, 2000). In humans, the protein is expressed predominantly in the liver but is also expressed in multiple other tissues as fibroblasts, kidney, heart, brain, muscle, pancreas and red blood cells.

ASL belongs to a super family of enzymes that have homologous parts and catalyze similar cleavages with the release of fumarate as one of the products. The conserved sequences among the family members are involved in the catalysis. In addition, the crystal structure reveals a common protein fold generating four active sites in each homotetramer (Turner et al., 1997). Among all of the enzyme in the super family, ASL is most closely related to δ–crystallin, a protein found in abundance in the lens of birds and reptiles, with amino acid identity of 64–71% (Vallee et al., 1999; Wistow and Piatigorsky, 1987; Yeh et al., 1988). The thermodynamic stability and ability of ASL to accumulate to high intracellular concentration without precipitation thus allowing transparency, befits its role as the major structural protein in the lens (Brusilow and Horwich, 2009). In ducks, there are two closely related crystallins with high homology to ASL, apparently resulting from gene duplication. As ASL plays a role as both catalytic and structural protein in the duck lens, only one of the crystalline- δD2 has retained the enzymatic activity while the other, δD1 holds a structural function (Lee et al., 1992; Piatigorsky and Wistow, 1989). It should be noted, that although birds lack a urea cycle since they detoxify ammonia by conversion to uric acid, they still have ASL for the generation of arginine, emphasizing the importance of this enzyme outside the urea cycle.

The human ASL gene has been mapped to chromosome 7cen-q11.2 and consists of 17 exons. The first, exon 0 codes only for 5′UTR (Trevisson et al., 2007). The presence of another partial sequence on chromosome 22 was assumed to be a pseudogene but later found to code for Ig-λ like mRNA (O'Brien et al., 1986; Linnebank et al., 2002). Recently, a pseudo gene was located on chromosome 7, ~3 Mb upstream of the ASL gene that includes intron two, exon three and part of intron three (Trevisson et al., 2007).

ASA is caused by heterogeneous mutations in the ASL gene. The type of pathogenic mutations varies and includes nonsense, missense, insertions, deletions and those affecting mRNA splicing. Mutations are scattered through out the gene; however; exons 4, 5, 7, appear to be a mutational hotspots. Analysis of exon 7 flanking sequences did not reveal any specific motif that could explain its susceptibility (Linnebank et al., 2002; Trevisson et al., 2009; Trevisson et al., 2007). Until now, the number of reported mutations is quite small compared to other urea cycle defects, probably because molecular genetic studies are not essential for the diagnosis (Linnebank et al., 2002; Trevisson et al., 2007). Direct correlation between the clinical phenotype and residual ASL activity has been hard to establish most probably due to limited sensitivity of the assay (Brusilow, 2009). In vivo [$^{14}$C] citrulline uptake show better correlation with clinical phenotype but require skin biopsy (Linnebank et al., 2002). Recently, various mutant alleles were characterized by evaluating growth in arginine free medium of yeast deletion mutants (Trevisson et al., 2009). The advantages of this method include its ability to detect low levels of residual activity and to assess the effect

of different allelic complementation. This model was able to demonstrate that patients with late onset form of ASA harbor either significant residual activity or allow the occurrence of intragenic complementation. In these cases, at least one active site was formed in the hybrid tetramer or the mutations partially stabilized each other (Trevisson et al., 2009; Yu and Howell, 2000). The disadvantage of the assay is its inability to study the effect of the patient's genetic background on enzyme activity.

Continued characterizations of different allelic combinations will undoubtedly allow further correlation between clinical phenotype and the molecular changes.

## ANIMAL MODELS

A mouse knockout model for the ASL gene has been generated (Reid Sutton et al., 2003). Metabolic studies of these mice demonstrated that they have the same biochemical phenotype as humans, including hyperammonemia, elevated plasma argininosuccinic acid and low plasma arginine. Not surprisingly, the phenotype of the animals was of neonatal lethality within the first 48 hours of life resulting most probably from hyperammonemia. Recently, we have generated a novel hypomorphic and conditional model of Asl deficiency and preliminary analysis show multi-systemic disease that may be related for an essential role of Asl in nitric oxide metabolism (unpublished observations, B Lee).

## PRENATAL DIAGNOSIS

If the mutations in the ASL gene are known, prenatal diagnosis can be performed by mutational analysis on either chorionic villous tissue or the amniocytes (Haberle et al., 2004). Elevated levels of argininosuccinic acid in the amniotic fluid can also reliably detect affected fetuses (Kleijer et al., 2006; Kamoun et al., 1995, Mandell et al., 1996). Analysis of enzyme by direct methods from chorionic villus tissue or amniocytes or indirect methods such as $^{14}$C-citrulline incorporation in uncultured chorionic villus samples, have been successfully performed for prenatal diagnosis (Pijpers et al., 1990; Kleijer et al., 2002). The enzyme assays are available only at few specialized laboratories precluding their use in clinical settings.

Another unusual scenario involving prenatal counseling would be that of a pregnant female who has ASA. Women with ASA have had uneventful pregnancies when monitored closely and have delivered healthy babies and authors reporting these cases conclude that argininosuccinic acid may not be embryotoxic (Worthington et al., 1996; Mardach et al., 1999; Reid Sutton et al., 2009).

## NEWBORN SCREENING PROGRAM

The success of neonatal screening for detection of inborn errors of metabolism and the availability of new techniques such as tandem mass spectrometry (TMS) have led to inclusion of urea cycle defects in the in newborn screening (NBS) programs. The progress made in the treatment of urea cycle defects presenting in the neonatal period has clearly improved the survival of patients during the first 2–3 years of life and it is the hope that early treatment after detection by newborn screening could also improve the cognitive outcome in these patients. All of the 50 states in the USA are required by law to screen for ASA and have fully implemented the screening

Citrulline measured by TMS is the metabolite used for screening of ASA. Elevation of citrulline can also be seen with citrullinemia type 1 (ASS deficiency), citrullinemia type 2 (citrin deficiency) and pyruvate carboxylase deficiency. Elevated citrulline on NBS should prompt evaluation and vigilance for signs and symptoms of hyperammonemia such as poor

feeding, vomiting, lethargy, hypotonia, tachypnea, and seizures. Referral to metabolic physicians with further evaluation including plasma amino acids, and urine amino acids are the next appropriate measures to be followed. Elevation of both citrulline and argininosuccinic acid on plasma is diagnostic of ASA.

## MANAGEMENT AND TREATMENT

The treatment of ASA involves two different scenarios – 1) rapid control of hyperammonemia during metabolic decompensations and 2) chronic long term management to help prevent episodes of hyperammonemia and the long term complications

During acute hyperammonemic episodes, the management of ASA is not much different than other UCD excepting that a higher dose of intravenous arginine is used for the priming and maintenance infusion. In short the management of hyperammonemia includes discontinuing the oral protein intake, caloric supplementation with intravenous glucose and/or lipids along with initiation of intravenous drugs to scavenge ammonia (2001; Enns et al., 2007). Chronic management of ASA includes dietary restriction of protein and arginine supplementation. Patients who have had frequent metabolic decompensations or elevated ammonia are candidates for additional oral nitrogen scavenging therapy with either sodium benzoate or sodium phenyl butyrate. Diet constitutes a key component of the treatment. The Recommended Daily Allowance (RDA) for dietary protein is often higher than the minimum needed for normal growth. Most patients with a UCD can receive less than the RDA of protein and still maintain adequate growth patterns. There are some contradicting evidences regarding the correlation between compliance with the prescribed diet and outcome. Dietary therapy along with arginine supplementation has been shown to reverse the abnormalities of hair and to improve cognitive outcome including reversal of abnormalities on EEG (Ficicioglu et al., 2009; Kvedar et al., 1986; Coryell et al., 1964). However, dietary therapy has not been shown to influence the outcome of liver disease (Mori et al., 2002, Mercimek-Mahmutoglu et al, 2010). In addition, the efficacy of arginine supplementation in either preventing the hyperammonemic episodes or the chronic complications is not known. While evidence suggests that arginine supplementation may prevent metabolic decompensations in patients with severe early onset disease, long term follow up of patients detected through NBS did not show any discernible difference in outcomes between those on supplementation as compared to those who were not on arginine (Donn et al., 1985; Batshaw et al., 2001; Ficicioglu et al., 2009; Mercimek-Mahmutoglu et al., 2010). While this observation is counterintuitive to the notion of decrease in arginine as a cause for these complications, it may support a unique role for ASL in the subcellular compartmentalization for arginine utilization and the basis for the “arginine paradox” (B. Lee unpublished data).

A theoretical concern with arginine supplementation is that while it compensates for the decreased synthesis, it also generates increased amounts of argininosuccinic acid that is hypothesized to be toxic together with increase in guanidino acetate (Schulze et al., 2001). Magnetic resonance spectroscopy of brain in ASA patients on arginine supplementation has revealed elevations of guanidino acetate with either normal decreased or elevated levels of creatine (Sijens et al., 2006; van Spronsen et al., 2006). Hence, the ideal dosing of arginine and its utility in treatment of ASA is still unclear.

The alternative pathway therapy includes the use of sodium benzoate and sodium phenyl butyrate to stimulate the excretion of nitrogen in the form of hippuric acid and phenylacetylglutamine, respectively (Batshaw et al., 2001). Though there have been no controlled studies, and it is unlikely that there ever will be due to ethical reasons, treatment with alternative pathway therapy appears to have improved survival, biochemical control

and neurologic outcome in patients with urea cycle disorders (Batshaw et al., 1982; Maestri et al., 1991; Maestri et al., 1995). However for reasons mentioned above that point to metabolites other than ammonia that are also involved in long term complications in ASA, the efficacy of nitrogen scavenging therapy in prevention of the same is not known. As many patients with ASA are metabolically well controlled with diet and arginine supplementation, it would seem reasonable to use the phenyl butyrate and benzoate in patients who have had hyperammonemia and have not been controlled with arginine, and at the same time to minimize the effective chronic dosing of arginine.

Long term correction of the defect in urea cycle can be accomplished by orthotopic liver transplantation (OLT) (Lee and Goss, 2001). OLT has resulted in biochemical cure in patients with ASA (Newnham et al., 2008; Robberecht et al., 2006; Marble et al., 2008). However OLT does not correct the biochemical abnormalities including arginine deficiency at the tissue levels or elevation of argininosuccinic acid, two abnormalities that have been hypothesized to account for the long term complications. This being the case, it is our policy to recommend OLT only in patients with recurrent hyperammonemia, metabolic decompensations that are resistant to conventional therapy or in cases with cirrhosis with decompensation.

## CONCLUSION

Argininosuccinic aciduria occurs due to deficiency of Argininosuccinate Lyase and is a treatable inborn error of the urea cycle. This condition may present in the newborn period or as a late onset chronic disease. The common biochemical hallmarks are depletion of arginine and elevations of citrulline and argininosuccinic acid. The accumulation of argininosuccinic acid and related guanidinosuccinic compounds may contribute to the pathogenesis of disease. In addition, NO deficiency and increased free radical production add a layer of complexity to disease severity. The advent of mass spectrography allows screening for this condition in the newborn period. However, even with good dietary compliance and early arginine supplementation, patients can have cognitive and hepatic involvement. Current studies aimed at evaluation and treating NO deficiency may offer new opportunities for treating long term complications unrelated to hyperammonemia.

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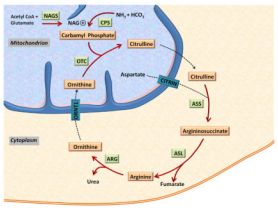


**Figure 1.**

The urea cycle consists of six sequential enzymatic steps in which the nitrogen from ammonia and aspartate is transferred to urea. Deficiencies of all six urea cycle enzymes (depicted by green boxes) have been described. Deficiency of ASL leads to accumulation of argininosuccinate upstream of the block as well as deficiency of arginine downstream of the block. CPS1-CarbamylPhosphate Synthetase I, OTC- Ornithine Transcarbamylase, ASS Argininosuccinate Synthetase, ASL- Argininosuccinate Lyase, ARG- Arginase, ORNT1-Ornithine transporter.

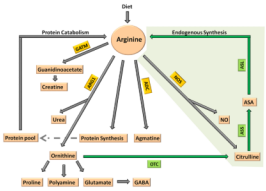


**Figure 2.**

Metabolic fates of arginine: Arginine is derived from dietary sources, protein catabolism, or endogenous synthesis. Arginine serves as the precursor for many biologically important molecules; a decrease in arginine may result in decreased production of compounds for which it serves as a precursor. GATM – Glycine Amidinotransferase, Arg1- Arginase 1, ADC - Arginine Decarboxylase, NOS - Nitric Oxide Synthetase, NO - Nitric Oxide, OTC - Ornithine Transcarbamoylase, ASS – Argininosuccinate Synthetase, ASA - Argininosuccinic acid, ASL - Argininosuccinate Lyase, GABA - γ-Amino Butyric Acid

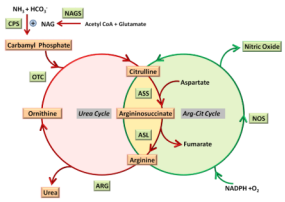


**Figure 3.**

Arginine-Citrulline Cycle. While ASS and ASS are involved in the urea cycle with no net synthesis of arginine in the liver, many tissues depend on these two enzymes for regeneration of arginine in various tissues. In ASL deficiency, arginine becomes an essential amino acid. Note that the production of nitric oxide is closely coupled with this arginine-citrulline cycle.