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Arginine: The Key Driver of Pathophysiology and Progression in Arginase 1 Deficiency

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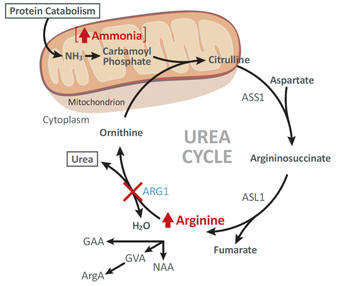
Introduction

- Arginase 1 Deficiency (ARG1-D) is a rare, progressive inborn error of metabolism and distinct urea cycle disorder (UCD) that results in persistent hyperargininemia and debilitating cognitive and mobility impairments[1,2]
  - Mutations in *ARG1* lead to impaired or absent arginase 1 activity and accumulation of arginine and arginine metabolites[1,3] **(Figure 1)**
  - The classic phenotype of ARG1-D involves insidious onset with symptoms becoming evident in the first years of life and progressing over time[2,4-7] **(Figure 2)**
  - Hyperammonemia is a potentially life-threatening complication of most UCDs; however, symptomatic hyperammonemia is relatively uncommon in ARG1-D[8,9]
  - The distinct clinical profile of ARG1-D suggests an alternative biochemical driver of disease pathology compared with other UCDs

Figure 2. **Urea Cycle Dysfunction in Arginase 1 Deficiency**



ARG1, arginase 1; ArgA, argininic acid; ASL1, argininosuccinate lyase 1; ASS1, argininosuccinate synthase 1; GAA, guanidinoacetate; GVA, alpha-keto-delta-guanidinovaleric acid; NAA, N-alpha-acetylarginine.

Figure 2. **Typical ARG1-D Phenotype**

Infancy (birth to 1 year)	Toddler age (2–5 years)	Childhood (5–10 years)
• Initial 6–12 months often uneventful[2,10] • May present with[10]: ○ Seizures ○ Episodes of hyperammonemia with irritability, feeding difficulties, poor appetite, nausea/vomiting, decreased alertness	• Spasticity in lower limbs (mainly tiptoe walking)[2] • Seizures (usually generalized tonic clonic)[10] • Developmental delay and/or intellectual disability (eg, delay or interruption of developmental milestones)[2,9,10] • Spontaneous avoidance of high protein foods (common)[11,12]	• Progressive spasticity[2] • Variable decline in growth[11] • Variable neuromotor decline and worsening of intellectual disability[5,9,12] ○ Gait abnormalities ○ Decrease in vocabulary or loss of spoken language

Management of ARG1-D

- Persistently elevated arginine, >300 µmol/L (normal, <115 µmol/L), is typical in ARG1-D
- Guideline-recommended plasma arginine (≤200 µmol/L)[8] is rarely achieved with available management strategies
  - Urea Cycle Disorder Consortium (UCDC) data revealed that nearly all on-treatment arginine levels were >115 µmol/L; very few were ≤200 µmol/L[14]
  - Continued progression of ARG1-D is observed despite standard-of-care treatment (eg, worsening of spasticity, gait abnormalities, and ultimately, loss of ambulation)[4,7,9]
- Management of other UCDs is focused on reducing risk of hyperammonemia,[8] but this is not sufficient to improve long-term outcomes in patients with ARG1-D

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Evidence Implicating Arginine in ARG1-D Pathophysiology

- Neurotoxic effects of persistent high arginine in ARG1-D and a mechanistic role in the development and progression of neurologic manifestations have long been speculated, and are supported by both anecdotal and empirical evidence[3,11,15,16]

Clinical Observations

- Results of a UCDC natural history study of multiple UCDs also suggests a role of arginine in development and progression of ARG1-D manifestations[18]:
  - ARG1-D patients were at greater risk for low IQ and poor performance in all domains assessed
  - Global and memory domain deficits were more tightly associated with arginine than with other biochemical markers
  - Higher arginine level was significantly correlated with worse motor composite score
  - Increasing cumulative exposure (ie, duration of disease) was an indicator of worse neuropsychiatric outcome

Clinical Observations

- Multiple mouse models of arginase deficiency demonstrate markedly elevated plasma arginine and phenotypic abnormalities similar to disease manifestations observed in ARG1-D patients
- Gene delivery systems that restore arginase 1 activity in *ARG1* knockout mice reverse pathology
  - *ARG1* gene therapy with an adeno-associated viral vector (AAV) normalized arginine and body weight, and increased long-term survival rates[19]
  - In *ARG1* homozygous knockout mice, postnatal white matter and synaptic abnormalities that are hypothesized to contribute to phenotypic neuromotor abnormalities are normalized by AAV vector–mediated replacement of *ARG1*[20]
- Comparison of wild-type, untreated *ARG1* knockout, and AAV-treated *ARG1* knockout mice provided a compelling demonstration of the role of arginine in ARG1-D manifestations **(Table 1)**
  - Serum arginine in untreated knockout mice was nearly 10-fold that of wild-type littermates and knockouts treated with *ARG1*-expressing AAV vector[21]
  - The restoration of arginase 1 activity in ARG1 knockout mice resulted in near-complete resolution of metabolic abnormalities and absence of phenotypic abnormalities suggestive of brain dysfunction (weight loss, tail tremor, seizure-like activity, and gait instability progressing to inability to stand[21]

Figure 2. **Reversal of the ARG1-D Phenotype in *ARG1* Knockout Mice**

Manifestation*	ARG1-D Patients	ARG1-D Mouse Model		
		Wild-type	*ARG1* knockout: untreated	*ARG1* knockout: *ARG1* restored (AAV)
Impaired arginase 1 activity	+	–	+	–
Markedly elevated pArg	+	–	+	–
Failure to thrive (weight loss)	+	–	+	–
Spasticity (tremor)	+	–	+	–
Seizure (seizure like activity)	+	–	+	–
Gait abnormalities/loss of mobility (gait instability/ inability to stand)	+	–	+	–

*Or analog in mouse model; pArg, plasma arginine.

Clinical Impact of Reducing Plasma Arginine

Dietary Restriction

- Dietary protein restriction to minimize exogenous arginine is usually not sufficient to achieve guideline-recommended plasma arginine levels and most patients deteriorate over time. However, even suboptimal lowering of arginine improves patient outcomes
  - In late-treated ARG1-D patients, reducing natural protein intake successfully lowered plasma arginine and led to improvement in manifestations. Specifically, neurologic and cognitive function improvement, decreased spasticity, and accelerated growth was noted despite advanced disease[22,23]
- Treatment from birth has been shown to reduce or delay progression, with some patients not developing any overt signs or manifestations of ARG1-D through 5 years of age[6,24]
- Lapses in treatment or arginine control in older patients with established disease result in worsening of impairments that subsequently improve upon reinstatement of therapy and return to patients' typical plasma arginine range[16]

Experimental Enzyme Therapy

- Evidence from a prospective Phase 1/2 trial of a human enzyme therapy for ARG1-D demonstrated a clear link between plasma arginine and clinical manifestations[25,26]
  - At **baseline** while receiving standard-of-care treatment[25]:
    - Median plasma arginine (389 µmol/L) was >3-times the upper limit of normal and nearly twice the level recommended by current ARG1-D guidelines
    - Lower-limb spasticity was present in 75% of patients
    - **88% of patients demonstrated mobility impairment** on ≥1 of 3 assessments of functional tasks performed while sitting, standing, walking, running, and jumping
  - After **20 weeks** of treatment with enzyme therapy[25]:
    - Plasma arginine was significantly reduced, with median levels in the normal range and nearly all patients below the guideline-recommended level
    - **Lowering of plasma arginine was accompanied by functional improvements**. Most patients achieved clinically meaningful improvement on ≥1 mobility assessment, and one-third achieved meaningful improvement on ≥2 assessments
  - After 1 year on treatment with enzyme therapy[26]:
    - Plasma arginine reductions were sustained, with most patients within the normal range and all patients below the guideline-recommended level
    - **Clinically meaningful improvement in functional ability was sustained**

Clinical Perspective

- ARG1-D is often detected late, after manifestations are established, which may account for poor neurodevelopmental outcomes
  - If diagnosed at and effectively treated from birth, patients may have a much better prognosis since owing to reduced exposure to excess circulating arginine
  - Early treatment in ARG1-D may be more effective than in other UCDs since ammonia levels tend to be more manageable and the degree of hyperammonemia less severe in ARG1-D; however, despite the focus on arginine levels as a driver of disease pathogenesis, the risk of mortality from hyperammonemic events remains an important concern
- The aggregate data from the UCDC highlight the difficulty in maintaining adequate arginine levels with the current standard of care, even at highly specialized centers
- Changes in the severity of disease manifestation during acute decompensation events support the reversibility of some of the neurological impacts of hyperargininemia and the need to keep arginine levels low using all available tools

Summary and Conclusions

- Together, the available evidence implicates arginine as the driver of pathology and progression in ARG1-D:
  - The ARG1-D biochemical profile and clinical manifestations are distinct from other UCDs in which hyperammonemia is the primary concern
  - Persistent high plasma arginine is accompanied by progressive and debilitating neurologic manifestations and functional impairment, whereas reducing plasma arginine improves manifestations even in patient with established disease
- There is an urgent need for therapies that maintain long-term reduction of arginine levels in patients with ARG1-D