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Review

Argininemia: Pathophysiology and Novel Methods for Evaluation of the Disease

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Abstract: Argininemia or arginase-1 deficiency constitutes a rare, genetic, metabolic disorder caused by mutations in arginase 1—the last enzyme of the urea cycle—that hydrolyses L-arginine to ornithine and urea. The disease is associated with progressive development of spasticity and other symptoms, including seizures, developmental delay, cognitive impairment, and hepatic pathology. The present review attempts to summarize the current knowledge on the pathophysiology of the disease and highlight novel methods for its evaluation. Different factors, such as the accumulation of arginine, ammonia, and guanidino compounds, act as neurotoxins and may account for the neurological sequelae observed in the disease. New markers, such as arginine/ornithine ratio along with metabolomics, machine learning algorithms, and genetic methods, can be useful in the early diagnosis of argininemia, while mobile phone apps can assist argininemic patients in adhering to the strict diet required. Neurophysiology, multi-modal imaging, and new modelling methods, such as induced pluripotent stem cells, hold promise for providing new insights into the pathophysiology of the disease. There are still many uncertainties regarding the underlying mechanisms of argininemia, but the use of novel modelling methods and new technology can lead to the decipherment of its pathophysiology, improvement of diagnostic accuracy, and better disease management.

Keywords: argininemia; arginase-1 deficiency; urea cycle disorders; inborn errors of metabolism; guanidino compounds; neurotoxicity; arginine/ornithine ratio; metabolomics; machine learning/apps; neurophysiological examinations

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1. Introduction

Argininemia, also known as arginase-1 deficiency (ARG1-D) (MIM number: 207800), constitutes a rare, autosomal recessive, metabolic disorder of the urea cycle with a progressive and debilitating nature [1]. The condition results from inborn mutations in the ARG1 gene that encodes L-arginine-urea-hydrolase (EC 3.5.3.1), leading to either partial or complete absence of activity of the enzyme [2]. Argininemia is characterized by persistent pathological elevation of arginine levels in both plasma and cerebrospinal fluid (CSF). Clinically, individuals with ARG1-D commonly exhibit manifestations such as spasticity (primarily affecting the lower limbs), seizures, developmental delay, and cognitive impairment [3].

The disease exhibits a median prevalence of 1:1,000,000 births, which varies according to different researchers from 1 in 2,200,000 to 2 out of 100,077 children screened [3–5]. Classified among the rarest urea cycle disorders (UCDs), it comprises an estimated 0–27.1% of these disorders, with a median occurrence of 3.8% [3]. However, the frequency of argininemia within UCDs ranges widely, from 27.1% in a US study to ≤2% [6,7]. In the latest systematic review, an almost 1:1 male-to-female prevalence ratio was reported [3].

Argininemia is regarded as one of the few treatable causes of progressive spasticity. Nonetheless, due to the scarcity of this ailment, its diagnosis may be delayed [8]. Simultaneously, the landscape as far as its pathophysiology is concerned remains largely vague [9]. The present review aims to summarize the known literature regarding the etiopathogenetic mechanisms underlying ARG1-D. It also tries to accentuate the existing novel methods for the evaluation of the disease.

2. Clinical Manifestations and Physical History

ARG1-D manifests with a combination of both neurological and extra-neurological symptoms along with an elevated risk of premature mortality [10]. Despite considerable variability in disease severity and progression rates among affected individuals, there is a strikingly consistent pattern of clinical manifestations over time [3]. Various cases have been described, for instance, the case of a 46-year-old female patient who had a history of frequent falls, and delayed development of speech ability at the age of 1 with subsequent progressive lower limbs spasticity, and dependency on a wheelchair by the age of 14. In contrast, there are cases such as that of a 16-year-old girl who also had a history of frequent falls, spastic gait, repetitive vomiting, and global developmental delay at the age of 1, followed by epileptic seizures at the age of 5, but after treatment with sodium benzoate, and a low-protein diet demonstrated no further clinical deterioration [11].

Neurological symptoms associated with this syndrome encompass motor signs such as progressive spastic diplegia/paraparesis—which constitutes the hallmark of the disease and typically begins in the first decade of life [11,12]. Motor symptoms can also present between 1 to 5 years as hyperreflexia, clonus, toe walking, and other gait abnormalities, with 80% of the patients showing upper motor neuron involvement. Less frequently, ataxia, dystonia, and athetoid movements may be observed [8,13]. Progressive lower limb spasticity can lead to loss of ambulation and functional mobility -including fine motor skills decline, particularly in adult patients [10,13]. The rate of neurologic deterioration is not associated with the age of the symptoms' onset [14].

Cognitive deficits present as stagnation or regression of cognitive development over time. Additional cognitive and psychiatric symptoms that can be detected in argininemic individuals involve intellectual disability, attention-deficit/hyperactivity disorder (ADHD), aggressive behavior, pervasive developmental disorder, and impaired memory recollection, especially in adult patients [10,15]. Seizures, experienced by approximately 60–75% of the patients, most commonly present as tonic-clonic episodes, and their onset ranges from 4 months to 30 years [16–18]. Catabolic states, including infections, increased protein intake, and certain medications like valproate, can induce intermittent episodic hyperammonemia, which can be of variable severity and even result in hyperammonemic encephalopathy or death [10,19,20].

Extra-neurological symptoms, though relatively rare, primarily affect the liver and skeletal system. Hepatic dysfunction can range from mild to severe, encompassing neonatal jaundice, hepatomegaly, acute liver failure, liver fibrosis/cirrhosis, and hepatocellular carcinoma [2,21,22]. Due to increased chronic spasticity, scoliosis, kyphosis and lordosis may also be present [23].

In early infancy, ARG1-D is typically asymptomatic. Infants may display intermittent episodes of irritability, feeding difficulties, protein aversion, anorexia, vomiting, and decreased alertness when introduced to cow's milk [24]. The disease's first manifestations become evident in late infancy or early childhood (between 1–3 years) and progress in severity over time [1]. Adult-onset cases have also been reported [25,26]. During early childhood

(3 months–4 years), patients may be of short stature and exhibit clumsiness, psychomotor impairment, loss of developmental milestones, global developmental delay, and regression after initial normal neurodevelopment [27,28]. Loss of bowel and bladder control can also occur [29]. Hearing and visual impairments have not been documented [13].

Imaging studies in individuals with ARG1-D have revealed the following: cerebral and cerebellar atrophy, cerebral edema, a thin corpus callosum, dysmyelination, and corticospinal tract degeneration [8,30–32]. When compared to other UCDs, this syndrome is associated with a slower progression, and its symptoms are not evident at birth. Episodes of symptomatic or severe hyperammonemia, especially catastrophic neonatal cases, are less common [13]. When compared to cerebral palsy, argininemia is linked to a more gradual progression of spasticity and additionally leads to decline of the cognitive and language functions and aversion to high-protein foods. Furthermore, in the case of ARG1-D a clear history of risk factors for hypoxia at birth or during the neonatal period is absent [14].

Apart from cerebral palsy, differential diagnosis should also include hereditary spastic paraplegia. Especially the complex forms of this disease resemble argininemia due to being associated with slowly deteriorating lower limb spasticity and weakness along with other neurological symptoms/signs, such as developmental delay and intellectually disability. Similar clinical manifestations may be recognized in other treatable inborn errors of metabolism (IEMs) as well, including hyperornithinemia-hyperammonemia-homocitrullinuria syndrome (HHH syndrome), adrenoleukodystrophy, biotinidase deficiency, and cerebrotendinous xanthomatosis [8,12,33].

3. Pathophysiology

3.1. Urea Cycle Disorders

The urea cycle, initially described by Krebs in 1932, stands as the first metabolic cycle elucidated. Primarily taking place in the liver, the urea cycle comprises five consecutive reactions catalyzed by enzymes situated either in the mitochondria (carbamoyl phosphate synthase 1 (CPS1) and ornithine transcarbamylase (OTC)) or the cytosol (argininosuccinate synthase (ASS), argininosuccinate lyase (ASL), and arginase 1 (ARG1)) (Figure 1). The urea cycle plays a crucial role in both the detoxification of waste nitrogen and the synthesis of L-arginine [9]. UCDs represent a collection of rare congenital deficiencies affecting any of the five principal urea cycle enzymes. These deficiencies disrupt normal ureagenesis from ammonia, which in turn leads to inadequate removal of ammonia. As a result, hyperammonemia of varying severity occurs [34,35].

3.2. Arginase 1/Arginase 2

Arginase, initially identified in mammalian liver tissue in 1904, serves as the fifth and final enzyme of the urea cycle [36]. It catalyzes the hydrolysis of L-arginine to ornithine and urea. Ornithine is subsequently recycled for future urea production and serves as a precursor to polyamines, proline, and other products. Meanwhile, urea is transported through blood to the kidneys to be excreted in urine [23].

Two major isoforms of arginase exist, namely ARG1 and arginase 2 (ARG2), which are encoded by different genes in mammals. While the two isoforms share enzymatic properties, they differ in approximately 40% of their amino acid sequence, as well as in their cellular location, tissue distribution, and metabolic functions [19,36,37].

ARG1 is mainly expressed in the liver cytosol, but can also be detected in erythrocytes, vasculature, and immune cells, such as M2-like macrophages [19,38,39]. It is a 195 kDa metalloprotein composed of three similar subunits, which requires manganese (Mn^{2+}) metal ions for maximal catalytic activity and structural stabilization. Each subunit contains a highly conserved binuclear Mn^{2+} cluster with metal-coordinating histidine and aspartic acid residues at the active site [23,40].

Mutations in the ARG1 gene lead to deficient or absent ARG1 activity, resulting in the accumulation of arginine and other nitrogenous metabolites. Argininemia is characterized by an almost 50-fold elevation of intracellular hepatic arginine [41].

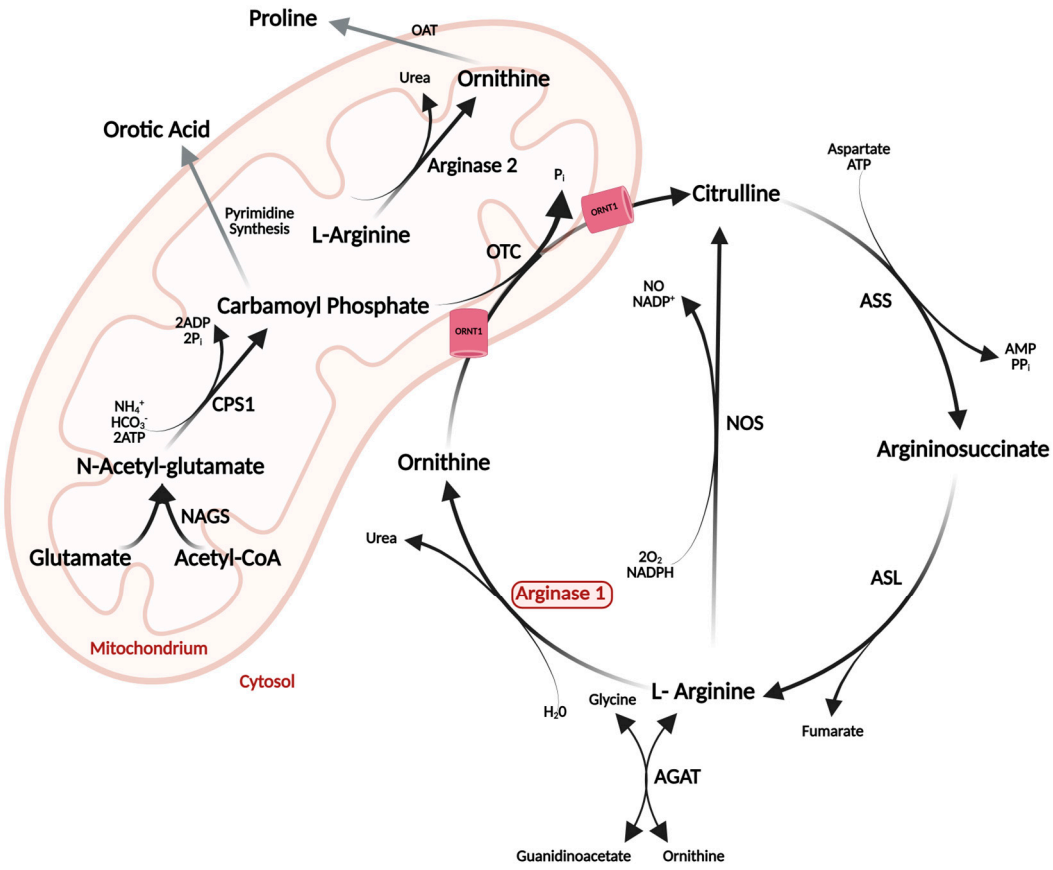

Figure 1. Overview of the urea cycle, L-arginine metabolism, and associated reactions. (NAGS: N–acetylglutamate synthase, CPS1: carbamoyl phosphate synthetase 1, OTC: ornithine transcarbamylase, ORNT1: ornithine transporter 1, OAT: ornithine aminotransferase, ASS: argininosuccinate synthase, ASL: argininosuccinate lyase, NOS: nitric oxide synthase, AGAT: arginine:glycine amidinotransferase).

ARG2 is found in the mitochondria of the kidneys and the prostate, as well as in lower amounts in other extrahepatic tissues, including the brain, gastrointestinal tract, lactating mammary glands, and macrophages [42–44]. Its role encompasses arginine homeostasis and the biosynthesis of polyamines, proline, creatine, citrulline, γ-aminobutyric acid (GABA), glutamate, and nitric oxide (NO) [43,45].

3.3. Mechanisms of the Disease

In contrast to other UCDs, the precise pathophysiology underlying ARG1-D remains unclear with only a few things being known as to how the signs and symptoms detected in affected individuals arise.

The disease is characterized by the build-up of arginine within liver cells, which is then released into plasma and accumulates in other organs. Elevated arginine levels are known to be neurotoxic [24,41]. Arginine is a common substrate for arginase and nitric oxide synthase (NOS), thus arginase activity limits the available substrate for NO production. In arginase deficiency, excess L-arginine may trigger oxidative stress, resulting in excessive nitric oxide production. Oxidative stress in turn can reduce the activity of Na^+/K^+-ATPase, which is of pivotal importance for neural excitability [46]. Especially, in argininemia endothelial cells overproduce NO contributing in excessive vasodilation and enthothelium-related abnormalities, since NO plays a significant role in neurotransmission, vascular function and immune regulation [39,47].

Concerning ammonia, it is evident that when accumulated contributes to neurological pathology by causing cerebral edema, and consequently brain damage, which mainly affects the parietal, occipital, and frontal lobes [10]. However, there is consensus on the

fact that ammonia accumulation is not the only one to be blamed for the neurological disorders observed in argininemic patients, due to episodes of hyperammonemia occurring infrequently in ARG1-D [23].

Guanidino compounds, including polyamines, nitrous oxide, and agmatine, are increased in both plasma and CSF/brain tissues of patients with argininemia [48,49]. These compounds, such as guanidinoacetic acid (GAA), β-guanidinopropionic acid (β-GPA), and γ-guanidinobutyric acid (γ-GBA), are produced through a transamidination reaction catalyzed by arginine:glycine amidinotransferase (AGAT) or other types of reactions (e.g., acetylation, transamination, hydrogenation) [50]. Alongside ammonia, guanidino compounds, acting as neurotoxins, potentially contribute to the development of the neurological sequelae (e.g., spasticity, intellectual disability) observed in hyperargininemic patients [50,51]. Moreover, guanidino compounds exhibit epileptogenic properties, which could be attributed to inhibition of glycine, GABA, acetylcholinesterase, and butyrylcholinesterase in central nervous system, decrease in synaptosomal membrane fluidity in cerebral cortex, and reduction in the Na^+/K^+-ATPase activity, which causes excitotoxicity [52–55]. Additionally, as observed in uremia, some guanidino compounds block transketolase activity, which may elicit demyelination and subsequently upper motor neuron signs [56]. Lastly, guanidino compounds (N-acetylarginine, argininic acid, homoarginine) along with arginine induce oxidative stress and interfere with the activity of enzymes (catalase, superoxide dismutase, glutathione peroxidase) that normally protect the brain against free radicals' damage [57].

The decrease in ornithine may impair the enzymatic activity of OTC, leading to the accumulation of orotic acid. Excess carbamoyl phosphate is then utilized in the pyrimidine synthetic pathway [58]. In addition, the reduction in mitochondrial ornithine levels may account for the oligodendrocyte degeneration, dysmyelination, and corticospinal tract immaturity found in patients with ARG1-D [59].

Various mechanisms have been proposed to explain weight differences between hyperargininemic and healthy individuals. The aminostatic hypothesis suggests that pathological arginine levels in these patients may trigger an abnormal satiety mechanism in the brain, resulting in reduced appetite [60,61]. Another plausible explanation for loss of appetite is that increased ammonia levels could suppress food intake via insulin activity, which can act as an anorexigenic [62]. Prolonged reduced food intake can disturb amino acid homeostasis, leading to undernutrition, which may become life-threatening [23].

The role of ARG2 gene in the context of argininemia as well as its interplay with ARG1 is not clearly understood. It has been reported that the milder clinical manifestations, the typical absence of fatal neonatal hyperammonemia, and the prolonged life span observed in ARG-1 deficient patients in contrast to other UCDS can be attributed to the persistence of some ureagenesis due to ARG2 activity. Additionally, the increase of ARG2 levels has been proposed as a potential gene therapy strategy for the disease [63]. In other words, in an attempt to compensate for hyperargininemia, ARG2 activity may be elevated, although some researchers have failed to detect such compensatory increase [64]. Furthermore, based on the results from homologous ARG2 deficient mice, which were viable, and practically indistinguishable from wild-type mice, apart from the fact that they were hyperargininemic, it was suggested that ARG2 deficiency in otherwise healthy humans is possibly benign [36,65]. However, it can be of clinical importance by contributing to neuroinflammation, and pain in the case of a previous injury [66].

4. Novel Methods of Evaluation

4.1. Current Methods of Diagnosis and Treatment

Diagnosing ARG1-D involves a combination of high clinical suspicion and specific biochemical findings. Confirmatory methods include genetic testing or the inability to detect red blood cell arginase enzyme activity, procedures also followed in the context of newborn screening (NBS) [22,67,68].

Laboratory methods used for diagnosis encompass measuring arginine and other amino acid levels, as well as metabolites and even ammonia levels [3]. Most frequently arginine levels are measured in plasma using mass spectrometry, but also other types of samples, such as CSF, urine, and leukocytes can be used. Red blood cell arginase activity is evaluated in fresh or dried blood samples [67]. In the case of argininemia, arginine levels can be as high as four times the normal (>300 μmol/L) and red blood cell arginase activity less than 1% of normal levels [3,22]. Assessing the levels of other amino acids, such as ornithine, glutamine, phenylalanine, aspartic acid, lysine, and threonine—that are typically abnormal in hyperargininemic patients—in plasma or erythrocytes can help validate the diagnosis. Metabolites like orotic acid, guanidino compounds, glutathione, prealbumin, and pyrimidine can also be measured in adequate samples, such as plasma, serum, erythrocytes, or urine, as reported in some studies [3].

Evaluating ammonia levels can also prove helpful during the process of diagnosing argininemia. Most studies support the measurement of ammonia in the context of NBS or in combination with other laboratory tests that detect the absence of red blood cell arginase activity and/or increased plasma arginine. Increased ammonia levels have also been used as triage, followed by genetic or laboratory testing to confirm the diagnosis [3]. Genetic testing—conducted by sequence analysis, deletion/duplication analysis or with a multi-gene panel containing ARG1 gene in tandem with other genes—in order to discover pathogenic mutations in the ARG1 gene is widespread [67,69]. Physical examination, electroencephalogram (EEG), brain imaging, and family history of ARG1-D provide additional diagnostic clues [3].

NBS for argininemia—in countries where it is performed—is crucial for early disease detection and prompt initiation of treatment [5]. For instance, on the US Recommended Uniform Screening Panel ARG1-D is incorporated as a secondary target and its diagnosis is established by assessing arginine levels in dried blood spots via tandem mass spectrometry [68]. In cases of known ARG1 gene mutations, prenatal diagnosis is feasible through mutation analysis in chorionic villous tissue, amniocytes, or percutaneous umbilical blood sampling, given that ARG1 is expressed in fetal red blood cells as early as 16–20 weeks of pregnancy at comparable levels to postnatal levels [67,68].

As far as the treatment is concerned, the primary goal in argininemia is to reduce arginine concentration in plasma below 200 μmol/L. Achieving this objective necessitates severe dietary protein restriction, coupled with essential amino acid supplementation, which covers up to 50% of the protein requirement [67].

While hyperammonemia occurs less frequently in hyperargininemic patients compared to other UCDs, nitrogen scavengers, such as benzoate, phenylbutyrate, and phenylacetate, can be administered to reduce the risk of such crises or treat them when they occur [3,67]. Nitrogen scavengers provide an alternative pathway for waste nitrogen excretion by facilitating the formation and excretion of hippuric acid and phenylacetylglutamine [70]. Ornithine supplementation proves useful by replenishing hepatocellular ornithine—therefore preventing hyperammonemia—and simultaneously by inhibiting the formation of neurotoxic guanidino compounds through blockage of AGAT [71]. Lysine supplementation can possibly increase argininuria and can also compete with arginine for uptake in the brain, potentially lowering brain arginine levels [72,73].

Liver transplantation effectively treats ARG1-D in the liver, normalizes arginine and ammonia levels, and halts neurological deterioration, rendering strict protein restriction and nitrogen scavengers unnecessary [74]. It constitutes the ultimate treatment option for patients with recurrent hyperammonemia episodes [75]. However, it is a high-risk operation, particularly for those with acute liver failure or encephalopathy, and—as expected—is resource-intensive [65].In certain cases, dialysis and blood transfusion have been employed to acutely decrease arginine and ammonia levels in plasma, but the clinical benefits of such interventions last for only a few months [76,77]. Enzyme replacement therapy is another treatment option that is currently under investigation, involving intravenous injections of pegylated human recombinant arginase 1 (pegzilarginase) [78]. Lastly, symp-

tomatic treatment should also be considered. The management of spasticity may include botulinum toxin injections and orthopedic surgery [12,79]. For seizures, phenobarbital or carbamazepine can be administered [10].

4.2. Novel Evaluation Methods

4.2.1. Use of Arginine/Ornithine Ratio

In newborns with ARG1-D, plasma arginine levels may appear normal or near-normal due to the lingering effects of maternal arginase or due to arginase 2, which can increase greatly when arginase 1 is deficient [3,80]. Therefore, relying solely on elevated plasma arginine for the diagnosis of argininemia may potentially lead to the omission of some positive cases, something that would understandably be associated with detrimental consequences for the neurophysiological development of the falsely diagnosed individuals [80].

To address the aforementioned issue, the arginine to ornithine ratio (Arg/Orn ratio) has been investigated as a secondary diagnostic marker in newborns with a positive NBS result for hyperargininemia and arginase deficiency [81]. It was revealed that increased plasma arginine when combined with an Arg/Orn ratio of $\geq$1.4 correctly identified all the arginase cases in the study. Furthermore, it was observed that during the first 31 days of life, plasma arginine increased at a rate of 0.94 µmol/L/day, while ornithine remained essentially unchanged. Consequently, the Arg/Orn ratio exhibited a similar increasing trend (0.01/day) [81].

The researchers concluded that the combination of both plasma arginine and plasma Arg/Orn ratio serves as a more effective diagnostic marker compared to plasma arginine alone. This improved diagnostic approach enhances sensitivity for identifying ARG1-D in newborns with hyperargininemia, allowing for a timely diagnosis and early initiation of adequate treatment before the onset of symptoms [80,81].

4.2.2. Use of Metabolomics and Machine Learning

Emerging diagnostic methods for metabolic disorders leverage untargeted metabolomics data and rely on disease-specific networks derived from profiling data [6,82,83]. A group of researchers, with the aid of untargeted metabolomic analysis, identified multiple novel potential biomarkers for ARG1-D, that could prove useful when it comes to the monitoring of the treatment's efficacy [6]. Another notable achievement in this field involved the development of an automated computational method, which enables accurate diagnosis of 16 different IEMs, including argininemia [83].

This novel model appears promising particularly for cases where patients remain undiagnosed using current available methods. It quantifies the similarity of individuals' metabolite perturbations with patterns observed in various diseases, allowing for the recommendation and ranking of candidate diagnoses. Furthermore, the model proves valuable in interpreting variants of uncertain significance identified through exome sequencing. This computational method offers competitive diagnostic accuracy when compared to rule-based biomarker modeling approaches, has the potential to replace pathway-based modeling approaches, and significantly improves the speed and confidence with which clinical laboratory directors make diagnostic and treatment decisions [83].

4.2.3. Use of Genetic Databases

Genetic population databases have been employed to determine the prevalence of ARG1-D. Through this approach, researchers identified a global birth prevalence of 2.8 cases per million live births and a population prevalence of 1.4 cases per million people. Birth prevalence estimates varied based on population demographics and consanguinity rates [84].

These findings led the authors to conclude that ARG1-D might be more common than previously believed, especially when compared to prevalence calculations made in NBS studies. This observation raises the possibility of underdiagnosis or misdiagnosis of ARG1-D in the absence of comprehensive genetic population data [12,85].

4.2.4. Use of Apps for Diet Monitoring

Patients with IEMs, including argininemia, should adhere to a strict diet to maintain acceptable metabolic control and hinder organ damage [67]. However, following such a diet can be challenging for various reasons, such as limited information on the disorder-specific nutrient content of foods, the availability and cost of special products, and difficulties in reliably calculating and tracking dietary intake [86]. To address these challenges, mobile phone apps tailored to the specific needs of these patients can be immensely helpful.

One example of such an app is the Metabolic Diet App, which was developed based on the MetabolicPro food database by the Genetic Metabolic Dietitians International (GMDI) Technology committee. This app provides features like creating a personalized management plan with specific nutrient goals, counting nutrient intake, adding custom foods and homemade recipes, maintaining a daily food diary, and offering feedback. Such apps play a crucial role in supporting patients to manage their dietary requirements effectively [87].

4.2.5. Use of Neurophysiological Methods and Multi-Modal Imaging

Neurophysiological assessment in patients with ARG1-D has been infrequently reported to date, but it holds the potential to offer new insights into the pathophysiological mechanisms underlying the disease. Additionally, it can aid physicians in monitoring and managing patients more efficiently. Examples of neurophysiological assessment tools include motor evoked potentials (MEPs), somatosensory evoked potentials (SEPs), F-wave, electromyography, and nerve conduction velocity (NCV) [30].

Furthermore, multi-modal imaging can possibly greatly contribute to the research of argininemia [31,32,88]. Combining different neuroimaging modalities, such as EEG, functional magnetic resonance imaging (fMRI), and functional near-infrared spectroscopy (fNIRS), enables an in-depth study of both hemodynamic and electrical neural activity alterations in affected individuals. This approach opens new horizons in researching the underlying neurocognitive impairments associated with the disease. Moreover, the combination of such novel neuroimaging modalities with machine learning methods holds promise in supporting physicians' decision-making process and identifying the neural signature of UCDs, including ARG1-D [88].

4.2.6. Discovery of Novel Mutations

ARG1, the gene encoding the enzyme ARG1, is located on chromosome 6q23 and consists of 8 exons [89]. It was first cloned in 1986 and to date more than 43 pathogenic mutations have been identified [69,90]. The majority of these mutations is missense/nonsense or small deletions, but also whole-gene deletion and complex rearrangement have been described [69,91,92]. The identified mutations are fairly uniformly distributed across the eight exons as well as at several exon-intron boundary splice sites [69]. The mutations disrupt the binuclear Mn^{2+} cluster and interfere with the metal-activated hydroxide mechanism, affecting the active site, bridging residues, creating steric clashes, and influencing regions essential for oligomerization -with the latter hindering the assembly of the protein trimer [93,94].

The disease is autosomal recessive, with almost half of the patients being compound heterozygotes and the other half homozygous [23]. Most affected individuals have private mutations, although prevalent mutations have been identified in specific populations (e.g., Portuguese, French-Canadian) [95,96].

While the genotype-phenotype correlation remains vague, a surprising correlation exists between responsiveness to dietary protein restriction and different genetic mutations of the ARG1 gene [97,98]. Patients with at least one moderately mutated allele respond well to dietary treatment, as reflected by a great reduction in plasma arginine levels (<300 µmol/L), while patients with two severely mutated alleles respond poorly with their plasma arginine levels remaining high (>400 µmol/L). The characterization of a mutation as moderate or severe is based on the degree of metabolic defect that it causes [98].

Recently, case reports of argininemic patients with novel mutations of the ARG1 gene have been published. The researchers mainly used technics such as whole exome sequencing to identify these mutations [99,100].

4.2.7. Use of New Modeling Methods

Modelling methods, including knockout mice and induced pluripotent stem cells (iPSCs), have been employed in an attempt to decipher the pathophysiology of the disease.

Both ARG1-knockout mice, and double ARG1/ARG2-knockout mice have been created by deletion of exon 4 of ARG1 gene, deletion of exon 4 of ARG1 gene and part of exon 4 and 5 of ARG2 gene, and conditional deletion of exons 7 and 8 of ARG1 gene. These mice models replicated multiple features of human argininemia, thereby providing new insights in the ARG1-D pathogenesis, revealing for example the implication of guanidino compounds in the emergence of the neurological aberrations of the disorder [50,64,101–103]. However, there are still many open questions regarding the exact sequence that leads to the development of argininemia, and therefore new types of knockout mice (such as tissue-selective knockout mice) should be created [23].

The newest method of modelling diseases could be applied in the context of ARG1-D, namely using appropriately differentiated iPSCs reprogrammed from patients (usually from fibroblasts), that carry the identical genetic information as patients, combined with genome editing technologies to create 'repaired' cells that are genetically identical except for the gene mutation site [104,105]. Given the rarity of the disease and the difficulty to obtain an adequate supply of tissue (e.g., liver, blood, brain) from the patients, it is evident that the utilization of such modern modelling methods is of utmost importance [23]. This approach would allow for comparisons at various levels (e.g., cellular, transcriptomic, proteomic, metabolomic) between gene-edited and parental-mutated iPSCs that have been differentiated to hepatocyte-like cells and/or other cell types expressing ARG1 (e.g., neuronal cells) and facilitate the study and discovery of the underlying mechanisms of the disease as well as novel methods for its treatment [23,104,106].

Table 1 summarises findings from selected studies that propose novel methods of diagnostic evaluations of people with ARG1-D.

Table 1. Selected studies presenting novel methods of diagnostic and therapeutic evaluation of patients with ARG1-D.

Study (First Author, Year of Publication)	Design/Method of Evaluation	Key Findings
Catsburg et al., 2022 [82]	Genetic database analysis to establish the prevalence of ARG1-D	- Global birth prevalence of ARG1-D: 2.8 cases/1,000,000 live births. - Population prevalence of ARG1-D: 1.4 cases/1,000,000 people. - 58% of the alleles responsible for ARG1-D were annotated with ethnic-specific frequencies. - Birth prevalence rates correlated with demographic characteristics, and consanguinity rate of each studied population.
Thistlethwaite et al., 2022 [81]	Use of untargeted metabolomic profiling, and disease-specific networks to diagnose IEMs	- An automated computational method was developed that could accurately diagnose 16 different IEMs, including argininemia. - The method could serve as a toolset for biological interpretation of untargeted metabolomics data, improve performance with new case data, and increase the speed and confidence of diagnostic and treatment decision-making.
Cui et al., 2021 [29]	Neurophysiological characteristics of a patient with ARG1-D	- Neurophysiological methods, such as MEPs, SEPs, F-wave, electromyography, and NCV, could assist in the assessment of a patient with argininemia. - In the presented case the only abnormal finding among all performed neurophysiological examinations was found in MEPs, where prolonged latency of them was observed in all four limbs.

Table 1. *Cont.*

Study (First Author, Year of Publication)	Design/Method of Evaluation	Key Findings
Huang et al., 2021 [79]	Development of Arg/Orn ratio as a secondary diagnostic marker in patients with positive NBS for ARG1-D	- Arg/Orn ratio $\geq$ 1.4, when combined with elevated plasma arginine, could serve as a secondary diagnostic marker, improving the detection accuracy of arginase deficiency in newborns with hyperargininemia. - During the first month of a newborn's life: plasma arginine increased 0.94 µmol/L/day, ornithine was practically unchanged, and Arg/Orn ratio increased 0.01/day.
Sen et al., 2021 [86]	Review of multi-modal imaging in UCDs	- Neuroimaging clinical studies demonstrated that in arginase deficiency non-specific brain abnormalities were observed. - MRS revealed increased glutamine levels and decreased myoinositol levels both in early-onset, and in later-onset forms of the disease. - Multi-modal imaging could help in the research of the pathophysiology of argininemia.
Burrage et al., 2019 [6]	Untargeted metabolomics analysis for discovery of pathway perturbations, and novel biomarkers in UCDs	- Untargeted mass spectrometry-based metabolomic analysis revealed the unique metabolomic signature of ARG1-D. - New biomarkers, potentially useful for monitoring the patient's response to treatment, were identified.
Ho et al., 2016 [85]	Development of an app for diet monitoring in patients with IEMs	- The Metabolic Diet App Suite aimed to aid individuals with IEMs, such as argininemia, to plan and track their meals, in order to adhere to the targeted metabolic nutrition therapy needed. - The feedback derived from patients, and their families was deemed as positive.
Miller et al., 2015 [80]	Untargeted metabolomics analysis for clinical screening of IEMs	- A screening test based on untargeted metabolomic analysis via mass spectrometry was developed that could accurately diagnose 20 IEMs, including argininemia. - The severity of the biochemical phenotype was associated with the severity of the patient's clinical manifestations.

5. Conclusions and Future Perspectives

Argininemia is a rare metabolic disorder resulting from mutations in the ARG-1 gene, leading to deficient or absent ARG1 activity, and elevated concentration of arginine and other nitrogenous metabolites.

The precise pathophysiology associated with ARG1-D remains yet to be unveiled, but the neurotoxicity of increased arginine levels, episodes of hyperammonemia, and the produced guanidino compounds are considered crucial for the development of the disease. Clinically, it manifests through progressive development of spasticity, seizures, developmental delay, cognitive impairment, and extra-neurological symptoms such as hepatic dysfunction. Current diagnostic means include plasma arginine, other amino acid and ammonia levels measurement, red blood cell arginase activity evaluation, genetic testing (also used in NBS), and a couple of clinical assessment methods (mainly physical examination, EEG, brain imaging, and family history). The methods used for treatment include severe dietary protein restriction, nitrogen scavengers, dialysis and blood transfusion, ornithine and lysine supplementations, liver transplantation, enzyme replacement therapy, and symptomatic treatment.

There is a plethora of novel methods for diagnosis and treatment, which include the use of plasma arginine/ornithine ratio, metabolomics and machine learning, genetic databases, and mobile apps for diet management. In addition, advanced neurophysiological methods, multi-modal imaging, the detection of new genes, and the utilization of new modelling methods, like knockout mice and iPSCs, are bringing optimism for the future pathophysiological study of the disease.

Thus, a better understanding of the exact nature of the disease should allow the invention of new more accurate diagnostic methods, and the discovery of innovative and more effective treatments.

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Abbreviations

ARG1-D = arginase-1 deficiency, IEMs: inborn errors of metabolism, Arg/Orn ratio: arginine/ornithine ratio, NBS: newborn screening, UCDs: urea cycle disorders, MRS: magnetic resonance spectroscopy, MEPs: motor evoked potentials, SEPs: somatosensory evoked potentials, NCV: nerve conduction velocity.

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