Inherited hyperammonemias: a Contemporary view on pathogenesis and diagnosis

Evelina Maines, Giovanni Piccoli, Antonia Pascarella, Francesca Colucci & Alberto B. Burlina

Published online: 27 Dec 2017.

Submit your article to this journal

Article views: 259

View Crossmark data

Taylor & Francis
Taylor & Francis Group

REVIEW

OPEN ACCESS · Check for updates

Inherited hyperammonemias: a Contemporary view on pathogenesis and diagnosis

Evelina Maines[a], Giovanni Piccoli[b], Antonia Pascarella[c], Francesca Colucci[c] and Alberto B. Burlina[c]

[a]Pediatric Unit, Provincial Centre for Rare Diseases, Department of Women's and Children's Health, Azienda Provinciale per i Servizi Sanitari, Trento, Italy; [b]CIBIO - Centre for integrative biology, Università degli Studi di Trento, Italy & Dulbecco Telethon Institute, Trento, Italy; [c]Division of Inherited Metabolic Diseases, Reference Centre Expanded Newborn Screening, Department of Women's and Children's Health, University Hospital, Padova, Italy

ABSTRACT

Introduction: Hyperammonemia is a marker for a disturbance either in the ammonia detoxification or less likely in the ammonia production. The symptoms are due mainly to ammonia's effect on the brain. The prognosis depends on several factors, including the developmental stage, the magnitude and duration of exposure.

A prompt ascertainment is crucial at any age for a good prognosis.

Areas covered: This review article will briefly summarize the most recent advances in the understanding of hyperammonemia due to disorders of the urea cycle and related enzymes. Major focus will be on the neuropathological mechanisms and new diagnostic approaches.

Evidence shows that ammonium central nervous system exposure alters several amino acid pathways and neurotransmitter production, nitric oxide synthesis, signal transduction, cerebral energy metabolism, oxidative/nitrosative stress, and channels and transporters activity.

Newborn screening programs for inherited hyperammonemias and next-generation sequencing panels of genes associated with hyperammonemia are already established diagnostic tools.

Expert opinion: In the light of the recent discovery of the role of the amino acid arginine in ammonia metabolism, urea cycle disorders may reveal new insights.

In the absence of a method to achieve a genetic correction of the defective metabolic pathways, new therapeutic strategies for the neurological complications of hyperammonemia are still an urgent need and deserve further investigation.

1. Introduction

Ammonia is a molecule with many properties. Depending upon pH, it is an ion (NH_4^+) with an ionic radius and properties similar to that of K^+ or a gas (NH_3) with free access across cellular and subcellular membranes. The ratio of NH_3/NH_4^+ is a function of pH as defined by the Henderson-Hasselbach equation: $\log_{10}(NH_3/NH_4^+) = pH–pKa$. At 37°C, the pKa of ammonia is 9.15. Consequently, under normal physiological conditions (pH 7.4), more than 98% of ammonia is present as NH_4^+.

Ammonia is an important substrate for several enzymatic reactions and is produced in all tissues via different biochemical pathways [1]. Large quantities of ammonia normally enter the portal vein circulation from protein digestion in the gastrointestinal system. However, plasma ammonia levels are maintained at low concentrations (<50 μM in healthy children and adults, and <110 μM in healthy neonates), a finding which illustrates the efficiency of hepatic clearance of gut-derived ammonia [2].

Hyperammonemia (>110 μM in healthy neonates, >50–80 μM after the neonatal period) is usually due to a disturbance in the ammonia detoxification; nevertheless, in rarer circumstances, it originates from ammonia overproduction [2].

Increased ammonia concentrations have deleterious effects on central nervous system (CNS) function. Depending on the age at the disease onset and the magnitude and duration of exposure, ammonia toxicity may result in severe neurological symptoms including seizures, lethargy, coma and in neuronal cell damage and loss. The most important mechanism for ammonia-induced neurologic dysfunction is brain edema, causing increased intracranial pressure. As a consequence, death by brain herniation is a frequent complication of severe acute hyperammonemia [3].

Hyperammonemia due to defects in ammonia detoxification is usually differentiated in two types. Primary hyperammonemia is due to loss-of-function defects of any of the urea cycle enzymes. These comprise three mitochondrial enzymatic defects (*carbamoylphosphate synthetase 1 deficiency* [CPS1D, OMIM #237300], *ornithine transcarbamylase deficiency* [OTCD, OMIM #311250], *N-acetylglutamate synthase deficiency* [NAGSD, OMIM #237310]), three cytosolic enzymatic defects (*citrullinemia type 1 or argininosuccinate synthetase deficiency* [ASSD, OMIM #215700], *argininosuccinic aciduria or argininosuccinate lyase deficiency* [ASLD, OMIM #207900], *arginase 1 deficiency or argininemia* [ARG1D, OMIM #207800]), and two mitochondrial transport defects (*hyperornithinemia-hyperammonemia-homocitrullinuria syndrome* [HHH, OMIM #238970])

Article highlights

Hyperammonemia is a marker for a disturbance either in the ammonia detoxification or less likely in the ammonia production. The extent of brain damage depends on several factors, including the developmental stage, the magnitude and duration of exposure. The underlying mechanisms of hyperammonemic encephalopathy are not completely understood. Evidence shows that ammonium central nervous system exposure alters several amino acid pathways and neurotransmitter production, nitric oxide synthesis, signal transduction, cerebral energy metabolism, oxidative/nitrosative stress, and channels and transporters activity. Understanding the mechanisms of ammonia neurotoxicity is important for optimizing the treatment of hyperammonemic crisis and for unrevealing new therapeutic strategies. Because of the unspecific clinical presentation of hyperammonemia, alternative measures to clinical diagnosis are needed. Newborn screening programs for inherited hyperammonemias and next-generation sequencing panels of genes associated with hyperammonemia are already established diagnostic tools.

This box summarizes key points contained in the article.

and *citrullinemia type 2 or citrin deficiency* (Citrin-D, OMIM #605814 and #603471) [4,5].

L*ysinuric protein intolerance* (LPI, OMIM #222700) and *ornithine aminotransferase deficiency* (OAT, OMIM #258,870) have a tangential relationship with urea cycle disorders (UCDs), leading to a secondary low activity of the urea cycle pathway due to deficiencies of crucial intermediates [4,5].

Recently other two disorders due to defects of ancillary enzymes of urea cycle have been described: the deficiencies of pyrroline-5-carboxylate synthase (P5CS) and carbonic anhydrase VA (CA-VA).

Pyrroline-5-carboxylate synthase deficiency (P5CSD, OMIM #610652) is a disorder caused by a defect in the first two steps of ornithine/proline biosynthesis, leading to a lack of urea cycle substrates and thus functional urea cycle insufficiency. In P5CSD patients, hyperammonemia is more prominent during fasting, consequently to a more pronounced deficiency of urea cycle intermediates [6].

CA-VA deficiency (OMIM #615751) results in dysfunction of all four mitochondrial enzymes to which CA-VA provides bicarbonate as substrate (carbamoylphosphate synthetase 1 [CPS1] and three biotin-dependent carboxylases). It leads to hyperammonemia combined with hyperlactatemia and metabolites suggestive of multiple carboxylase deficiency [7].

On the other hand, secondary impairment of NH_3 detoxification results from conditions where glutamate (Glu) or acetyl-CoA are decreased such as in fatty acid oxidation defects (FAODs), mitochondrial diseases, and valproate therapy, or where toxic acyl-CoAs are increased such as in organic acidurias (OAs).

Glutamine deficiency due to glutamine synthetase (GS) deficiency (OMIM #610015) also highlights the role of glutamine (Gln) synthesis in regulating the ammonia concentration [8].

Decreased ammonia detoxification may also result from acquired deficiency of key enzymes and transporters of urea cycle, such as in liver injury, or from bypasses of the liver.

This review article will briefly summarize the most recent advances in the understanding of hyperammonemia due to disorders of the urea cycle and related enzymes. Major focus will be on the neuropathological mechanisms and new diagnostic approaches.

2. Mechanisms of hyperammonemia

2.1. Main sources of ammonia production

2.1.1. The role of the skeletal muscle

Ammonia production by the skeletal muscle depends on physiological conditions. During the fed state, corresponding to an anabolic condition, amino acids are mainly used for protein synthesis. On the contrary, during catabolic conditions such as prolonged starvation, stress, accident or surgery, muscular proteins undergo breakdown and release high levels of amino acids and, eventually, ammonia [2,9].

Amino acids catabolism requires the removal of the amino group through two processes: transamination or deamination [10]. Amino acids are transaminated by specific aminotransferases or transaminases into corresponding 2-ketoacids and Glu using α-ketoglutarate (αKG) as a nitrogen acceptor (Figure 1). In this pathway, the amino groups are funneled to Glu and there is no formation or consumption of ammonia, thusly no net change in the nitrogen amount of the body [2].

Glu produced by amino acid transamination enters mitochondria and gives up its amino group for Gln production by the enzyme GS. Glu may also be a substrate for the transamination of pyruvate, derived from muscle glycolysis, giving alanine (Ala) and αKG to be further recycled in transamination reactions (Figure 1). Ala leaves the skeletal muscle and reaches the liver, where it mainly serves as substrate for gluconeogenesis. Gln is transported in the blood to the intestine, liver, and kidneys and represents the most important source of nitrogen to be disposed via the urea cycle [9,10].

Some amino acids and other nitrogen-containing compounds do not undergo transamination reactions, being directly deaminated to generate free ammonium ions. In particular, Glu is deaminated at a relatively high rate by the enzyme glutamate dehydrogenase (GDH). The coupling of Glu/αKG-utilizing aminotransferases with the GDH reaction channels the excess of amino acid derived nitrogen toward ammonia and, eventually glutamine synthesis [11].

Aspartate (Asp) deamination through the purine nucleotide cycle may also play a role during starvation and exercise [12].

2.1.2. The role of the intestine

Ammonia is produced in the gut by three sources: urease-positive bacteria in the large bowel that hydrolyze urea into CO_2 and NH_3, bacterial oxidation of amino acids in the fed state, and intestinal epithelial glutaminase (GA), that converts Gln to Glu and ammonia. Approximately, 75% of this ammonia returns to the urea pool through the hepatic urea synthesis [9].

The direct elimination of ammonia from the gut via purgatory treatments and the reduction of bacterial flora represent the mainstay of hepatic encephalopathy (HE) therapy [13] and have also an important role in the treatment of some inherited metabolic disorders (IMDs).
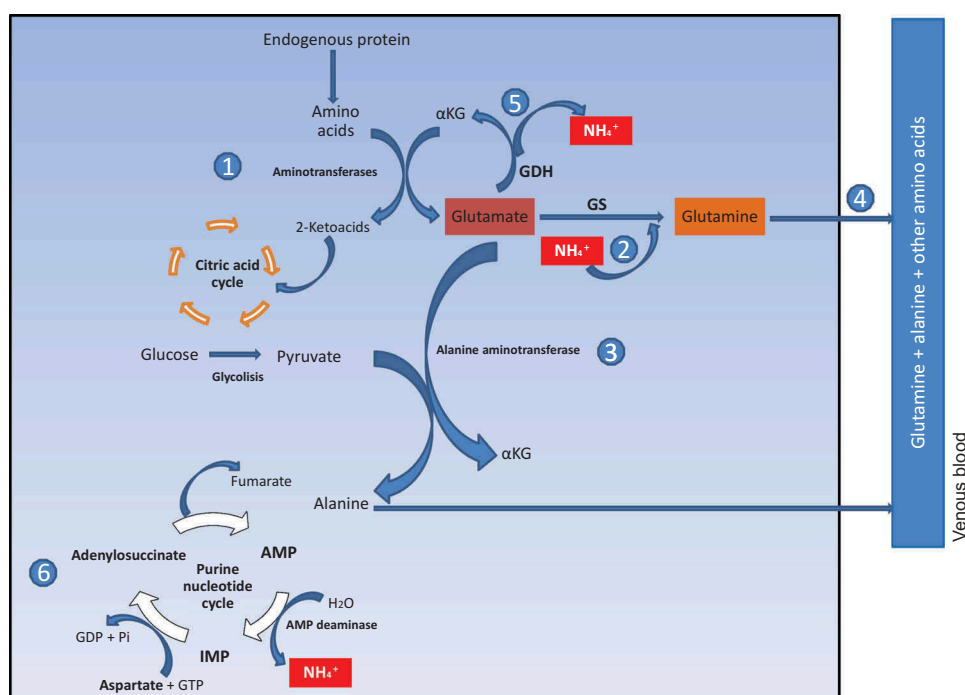
Figure 1. Schematic representation of the role of the skeletal muscle in ammonia homeostasis. During catabolic conditions, muscular proteins undergo breakdown releasing high levels of amino acids and, eventually, ammonia. Amino acids are transaminated by specific aminotransferases or transaminases into corresponding 2-ketoacids, the 'carbon skeletons' of amino acids, and glutamate using α-ketoglutarate (αKG) as a nitrogen acceptor **(1)**. Glutamate produced by amino acid transamination enters mitochondria and gives up its amino group for glutamine production by the enzyme glutamine synthetase (GS) **(2)**. Glutamate may also be a substrate for the transamination of pyruvate. This reaction produces alanine and αKG to be further recycled for transamination reactions **(3)**. Glutamine is transported in the blood to the intestine, liver, and kidneys **(4)**. Glutamate may be also directly deaminated to generate free ammonium ions by the enzyme glutamate dehydrogenase (GDH) **(5)**. Aspartate deamination through the purine nucleotide cycle is a source of ammonia during starvation and exercise **(6)**.

2.1.3. The role of other organs

GDH is expressed in other tissues (pancreas, heart, intestine, spleen, brain, skin, leukocytes, fibroblasts, lymph nodes, testicular tissue). Since Glu deamination via GDH results in a free ammonium ion production, these organs may contribute to ammonia production [2].

2.2. Main sources of ammonia detoxification

2.2.1. The role of the liver

In humans, there are two main ways to neutralize ammonia: urea synthesis and Gln synthesis. Their efficacy is different: renal excretion of urea allows the clearance of waste nitrogen whereas synthesis of Gln only sequesters it.

The urea cycle represents the major pathway of nitrogen disposal from the body and is the unique pathway able to transform ammonia into a nontoxic product, the urea. Hosting both the mechanisms, the liver is the main player in ammonia detoxification. The urea cycle takes place exclusively in the liver, in particular in the periportal hepatocytes, and therefore is tightly related to the integrity and functionality of this organ [9]. It converts ammonia and carbon dioxide (under the form of bicarbonate) into urea and water through a five-enzymatic-step process (Figure 2). One activating enzyme (N-acetylglutamate synthase, NAGS) and two transport proteins on the mitochondrial membrane are also required: ornithine carrier (ORNT) allows ornithine (Orn) import and citrulline (Cit) export, and citrin allows export of Asp and Glu import. The urea cycle

is mostly regulated by N-acetylglutamate (NAG) controlling the CPS1 activity and by the availability of Orn [5].

Gln synthesis by the cytosolic enzyme GS plays a secondary role in ammonia detoxification in healthy humans. Its relevance changes under specific metabolic conditions (e.g. UCDs) and depends on the acid-base status [14].

Häussinger et al. in 1983 provided the first evidence supporting the perivenous localization of GS and the predominantly periportal localization of GA and urea synthesis. Their study on substrate and enzyme activity gradients along the liver lobule, provided the basis for an intercellular Gln cycling [15].

GS detoxifies approximately one-third of the total portal blood ammonia, although this percentage can vary depending on the acid-base status.

Due to this hepatic Gln synthesis, plasma Gln is usually high in primary UCDs, though there is a large variability. On the acid-base status contrary, low or normal Gln levels are typically found in hyperammonemia due to OAs. This is related to the inability to maintain adequate levels of Gln precursors, in particular αKG, through a dysfunctional Krebs cycle [16].

2.2.2. The role of the kidney

Hepatic urea travels to the kidneys and it is removed in the urine.

Locally, ammonia is produced upon Gln hydrolysis by GA. This ammonium may be released into the systemic circulation (renal vein) or excreted into the urine depending predominantly on acid-base balance and potassium (K^+) concentration. In healthy
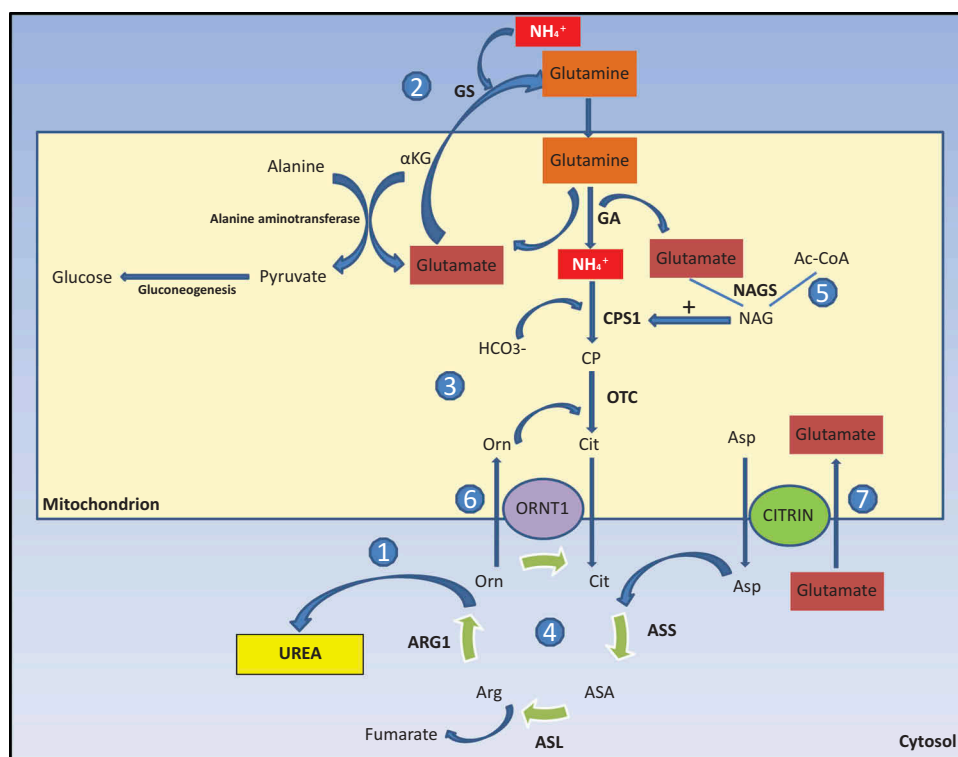
Figure 2. Schematic representation of the role of the liver in ammonia homeostasis. The urea cycle represents the major pathway of nitrogen disposal from the body and is the unique pathway able to transform ammonia into a nontoxic product, the urea **(1)**. Glutamine synthesis **(2)** plays in the liver a secondary role. Urea cycle consists of a five-enzymatic-step sequence, the first two taking place in the mitochondrion (carbamoylphosphate synthetase 1, [CPS1]; ornithine trancarbamylase, [OTC]) **(3)** and the last three in the cytosol (argininosuccinate synthase, [ASS]; argininosuccinate lyase, [ASL]; arginase 1, [ARG1]) **(4)**. One activating enzyme (N-acetylglutamate synthase, [NAGS]) **(5)** and two transport proteins on the mitochondrial membrane are also required: the ornithine/citrulline antiporter (ORNT1) allows ornithine (Orn) import and citrulline (Cit) export **(6)**, and citrin allows export of aspartate (Asp) and Glu import **(7).**

individuals under normal acid-base balance conditions, total kidney ammonium production is approximately half-divided between urine and venous blood [2]. On the contrary, metabolic acidosis increases urinary NH_4^+ excretion while metabolic alkalosis induces the opposite effect. K^+ depletion enhances urinary NH_4^+ excretion by the kidney. Conversely, the administration of K^+ supplements decreases urinary NH_4^+ excretion [2,17].

2.2.3. The role of other organs

GS is also expressed in brain (astrocytes), keratinocytes, gastrointestinal cells, lymphocytes [2] and skeletal muscles [18]. In patients with severe liver disease and portalsystemic shunting, ammonia-laden portal blood bypasses the liver and is diverted into the systemic circulation. Under these circumstances, skeletal muscles may become the most important organ in ammonia homeostasis [18].

Ammonia in gas phase has also been detected in human skin [19] and exhaled air [20,21], denoting that both the skin and the lungs may participate in nitrogen elimination.

3. Pathogenesis of brain ammonia toxicity

Notwithstanding increased systemic concentration, the symptoms of hyperammonemia are due mainly to ammonia's effect on the brain. Anyway, deleterious effects of ammonia have been observed in several cell types [22].

The extent of brain damage due to hyperammonemia depends on several factors, including the developmental stage, the concentration of ammonia that is reached, the rate of ammonia increase, the duration of hyperammonemia, and the co-presence of other pathological conditions (e.g. inflammation) [3,23,24].

In children with symptomatic hyperammonemia, the pattern and extent of brain magnetic resonance imaging (MRI) abnormalities correlate with clinical neurological outcome. Bireley et al. observed a trend in distribution of brain MRI abnormalities related to the severity of neurological sequelae. The peri-insular region is the first involved, extending into the frontal, parietal, temporal and, finally, the occipital lobes [25].

Biochemical changes in brain chemistry may be observed even before the development of clinical symptoms [26,27]. Proton magnetic resonance spectroscopy (^1H MRS) studies in symptomatic and severely affected OTCD patients have demonstrated that some metabolites are altered specifically: Gln is elevated, while myoinositol and choline are decreased. Interestingly, the myoinositol concentration has been linked to the development of ammonia-induced injury [26].

Neuropathological evaluation reveals an alteration of astrocyte morphology including cell swelling in acute hyperammonemia and Alzheimer type II astrocytosis in chronic hyperammonemia [23,28]. The rise of Gln in astrocytes that overcomes osmoregulation capacity plays an important role in both mechanisms [29].

Ammonia toxicity is also associated in childhood with neuronal loss, defects in neuritic growth, nerve cell migration abnormalities, and hypomyelination. These alterations are usually not observed in adult patients exposed to ammonia. For this reason, neurological symptoms are largely reversible in adults when NH_4^+ returns to normal levels [3,23].

The pathogenetic mechanisms underlying the brain ammonia toxicity are not still fully understood. Recent evidence shows that CNS exposure to ammonium alters several amino acid pathways and neurotransmitter production, nitric oxide (NO) synthesis, signal transduction, cerebral energy metabolism, oxidative/nitrosative stress (ONS), and channels and transporters activity [3].

3.1. Glutamine-induced toxicity

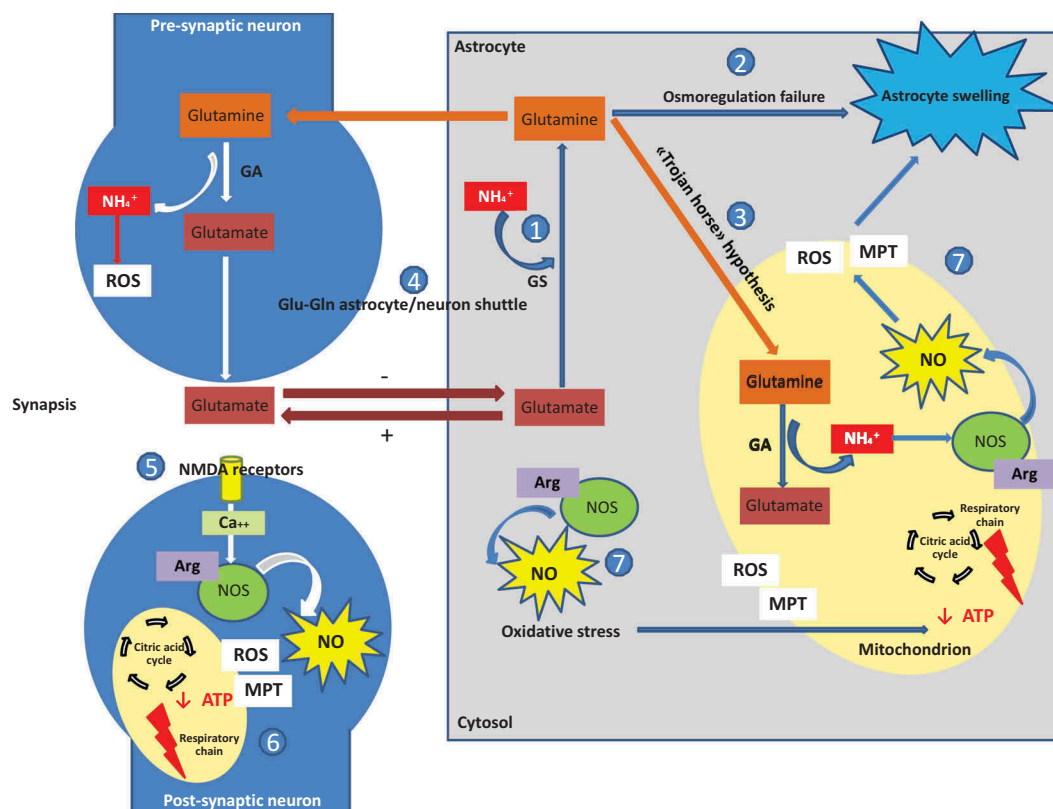Ammonia toxicity in the brain is tightly connected to Gln metabolism in astrocytes [28].

Since the brain lacks the urea cycle, ammonia detoxification relies only on Gln synthesis by the astrocytic enzyme GS. In response to elevated serum NH_4^+ levels, GS activity increases and, consequently, also the Gln content in astrocytes rises. High intracellular Gln concentrations, may overcome astrocyte osmoregulatory mechanisms, leading to astrocyte swelling and, consequently, to brain edema [3,28] (Figure 3). According

to the 'Trojan horse' hypothesis, astrocyte swelling under NH_4^+ exposure may be caused by Gln transport into mitochondria. In this scenario, Gln (the Trojan horse) is transported from the cytoplasm to mitochondria where is cleaved by GA in Glu and NH_4^+ [30].

The glutamine-derived ammonia could interfere with mitochondrial function, give rise to the excessive production of reactive oxygen species (ROS) and induce the mitochondrial permeability transition (MPT) [3]. MPT increases the permeability across the inner mitochondrial membrane, leading to a collapse of the mitochondrial inner membrane potential, osmotic swelling of the mitochondrial matrix, movement of metabolites across the inner membrane, defective oxidative phosphorylation and ATP synthesis, and finally oxidative stress [31].

The 'Trojan horse' hypothesis is supported by the observation that L-histidine, which inhibits mitochondrial Gln uptake, prevents swelling in ammonia-exposed astrocytes [32]. Nevertheless, this observation must be interpreted with caution because L-histidine also protects from oxidative stress [33]. Moreover, other authors have strongly criticized the 'Trojan horse' theory, because CNS GA activity in acutely hyperammonemic rats is low [29].

Interestingly, Gln deficiency upon astrocytes death has been linked to a relevant endogenous brain production of NH_4^+, as observed in some OAs [34,35].

Figure 3. Schematic representation of the main mechanisms of ammonia neurotoxicity. Since the brain lacks the urea cycle, ammonia detoxification relies only on glutamine synthesis by the astrocytic enzyme GS (1). High intracellular glutamine concentration may overcome astrocyte osmoregulatory mechanisms, leading to astrocyte swelling (2). The glutamine transport into mitochondria and its cleavage by glutaminase (GA) in glutamate and NH_4^+ (the 'Trojan horse' hypothesis) seem to play the most important role (3). Ammonia exposure may also induce alterations in the astrocyte-neuron Glu-Gln shuttle (4) and, consequently, in the glutamate extracellular levels (5). Increased glutamate levels lead to neurons hyper-stimulation essentially through the N-methyl-D-aspartate (NMDA) receptors activation (5). That leads to increase in intracellular calcium, which initiates several calcium-dependent processes including NO formation. The excessive formation of NO is the main cause of mitochondrial dysfunction, free radical accumulation and impaired energy metabolism both in neurons (6) and in astrocytes (7).

3.2. Glutamate-induced toxicity

Glu is the major excitatory neurotransmitter in the CNS. Glu has also a vital role in brain ammonia homeostasis, being strictly involved in Gln metabolism and to the astrocyte-neuron Glu-Gln shuttle (Figure 3).

Upon NH_4^+ exposure, the Glu intracellular pool drops in astrocytes and extracellular Glu level rises. The first phenomenon is mainly due to the conversion of Glu and NH_4^+ to Gln by GS [3].

High extracellular Glu level leads to neurons hyper-stimulation and eventually to neurotoxicity, essentially through the N-methyl-D-aspartate (NMDA) receptors activation [36,37] (Figure 3).

NMDA receptors activation increases intracellular calcium levels, which finally affect the activity of different enzymes both in neuronal mitochondria and cytosol. This leads to a wide array of metabolic alterations affecting NO metabolism and sodium-potassium pump (Na^+-K^+-ATPase). Increased NO formation, ATP shortage, mitochondrial dysfunctions, free radical accumulation and oxidative stress lead definitely to cell death (Figure 3) [38].

Involvement of NMDA receptors in ammonia neurotoxicity is confirmed by the observation that acute ammonia brain damage is reduced by NMDA receptor blockade both *in vitro* and *in vivo* [39,40].

In chronic hyperammonemia, the impaired function of the glutamate-nitric oxide-cGMP pathway associated to NMDA receptors, could be responsible for cognitive impairment [41]. In fact, both NMDA and gamma-aminobutyric acid (GABA) receptors are involved in the electrophysiological mechanisms for cognition, memory and learning [42].

Finally, NH_4^+ can also impact other neurotransmission systems such cholinergic and serotoninergic neurotransmissions [43].

3.3. Arginine and alteration of nitric oxide synthesis

In the CNS, NO is involved in crucial functions such as neurotransmission [44], differentiation of neuronal cells [45], epigenetic mechanisms in developing neurons and migration [46].

NO is produced from arginine (Arg) by NOS, which constitutes together with argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL) the citrulline-NO cycle. The citrulline-NO cycle together with cationic amino acid transporters (CATs, y^+LAT) are well expressed in CNS, and allow a steady supply of Arg to brain cells [47].

The role of Arg is confirmed by multiple observations.

The activation of NMDA receptors by ammonia activates NOS-mediated NO synthesis only in the presence of normal Arg supply [48]. Moreover, astrocytes exposed to NH_4^+ increase their Arg content by the expression of the glutamine/arginine transporter y+ LAT2, thereby stimulating the citrulline-NO cycle [49].

Excessive NO can impair brain function via multiple mechanisms. The most important are the mitochondrial respiration impairment, leading to ATP depletion and oxidative stress [3], and the alteration of blood-brain-barrier (BBB) permeability, thus contributing to vasogenic edema [50].

Suboptimal concentration of Arg, as observed in UCDs except ARG1D, promotes the uncoupling of NOS and eventually the production of superoxide rather than NO. Superoxide is strictly involved in oxidative stress and secondary mitochondrial dysfunction [42]. On the contrary, decreased NO production might affect the regulation of microcirculation [51], protein S-nitrosylation [52], histone methylation and gene expression [53], neurotransmission [44], differentiation of neuronal cells [45], and neuronal migration during development [46].

ASLD is the only inherited condition proven to cause systemic NO deficiency [53,54]. Nevertheless, plasma and urinary NO metabolites (as markers of NO synthesis) have also been detected below the normal range in OTCD [55]. Patients with ASLD experienced a mild neurological improvement after NO therapy [56]. This evidence supports the role of NO deficiency in the pathophysiology of the brain disease in ASLD patients.

Arg is also the precursor for creatine (Cr) synthesis. Consequently, decompensated UCDs can be associated with disturbances in the Cr metabolism both in brain and periphery [57].

3.4. Calcium-induced toxicity

Many studies have shown that one of the earliest events in astrocytes exposed to ammonia is a rise in Ca^{2+} levels [58,59]. Moreover, it correlates with ammonia concentration [59].

The increase in mitochondrial Ca^{2+} levels has been linked to MPT, defective oxidative phosphorylation, oxidative stress, and eventually to cell swelling. Calcium release may also underlie NH_4^+-induced neuronal apoptosis through activation of caspases and calpain (Ca^{2+}-dependent proteases) [60], and eventually bring to alterations in brain microcirculation [61].

3.5. Alterations in the energy metabolism

Ammonia affects several pathways involving energy metabolism, including the tricarboxylic acid cycle (TCA), lactate-malate shuttle, αKG dehydrogenase (αKGDH), and mitochondrial respiratory chain complexes [62,63]. Brain cells exposed to NH_4^+ also show a reduction in the creatine/phosphocreatine/creatine kinase system, due to altered Cr transport and synthesis [57].

The impairment of oxidative metabolism determines a compensatory increase of glycolysis (mainly in astrocytes), which induces an increase in brain lactate production [22]. Nevertheless, nuclear magnetic resonance studies have provided *in vivo* evidence for the absence of a sustained alteration in the concentration of high-energy phosphate compounds during a period of acute hyperammonemia [64].

Several observations argue against the hypothesis that energy failure results primarily by αKG depletion of TCA cycle (related to the conversion of αKG to Glu by GDH). αKG levels are not decreased and Glu levels are not increased in acutely hyperammonemic rat brain [65]. Moreover, [13N] labeling experiment showed that only a small amount of ammonia is incorporated into Glu in rat brain [11].

Ammonia-induced depletion of ATP seems to be prevented by NMDA receptor antagonists [39]. Moreover, acetyl-L-carnitine has been reported to enhance the recovery of cerebral

energy deficits caused by hyperammonemia, through the restoration of cytochrome c Oxidase (or complex IV) activity and free-radical scavenging [66].

3.6. Oxidative/nitrosative stress

ONS, i.e. the excessive and ubiquitous accumulation of ROS and reactive nitrogen species (RNS), is the major outcome of mitochondrial dysfunction and impaired energy metabolism due to hyperammonemia [67].

In the brain, ONS alters astrocytes function and morphology, perturbs astrocyte-neuron cross talk and affects synaptic plasticity, leading to encephalopathy. Moreover, it is a major determinant in the development of brain edema.

How ONS may cause astrocyte swelling is not fully understood, but an involvement of MPT has been confirmed. MPT seems not to be a consequence of the opening of a pre-formed pore, but the consequence of oxidative damage to pre-existing membrane proteins [67,68].

Several antioxidants are capable of diminishing the ammonia-induced MPT [3].

3.7. The role of channels and transporters

NH_4^+ is a water-soluble weak acid that crosses cell membranes through various transport systems. Because of the physico-chemical similarity between NH_4^+ and K^+, NH_4^+ can move along K^+ channels and transporters (Na^+-K^+-ATPase, K^+ channels, Na^+-K^+-Cl^- cotransporters), altering the uptake of ions and water [69].

Na^+-K^+-Cl^- cotransporter-1 (NKCC1), shuffles Na^+, K^+, 2 Cl^-, and water. NKCC1 activation upon NH_4^+ exposure increases the entry of water in astrocytes [69] and may contribute to brain edema.

Ammonia can also interfere with aquaporin channels, which might link cerebral metabolism to kidney physiology [70].

3.8. Blood–brain barrier and cerebral blood flow

Apart from a direct cytotoxic effect in the CNS, ammonia may also affect BBB functionality and, consequently, the passage of different molecules across its structures [71].

BBB permeability is strongly influenced by the state of the capillary endothelial cells, which are the first cells in contact with blood ammonia during hyperammonemia. Ammonia affects capillary endothelial cells functionality via a mechanism encompassing ONS and activation of matrix metalloproteinases [50].

Change in the cerebral blood flow, resulting in altered intracranial blood volume, has also been suggested to contribute to the development of brain edema [72].

4. Diagnosis of inherited hyperammonemias today

After a short asymptomatic interval, neonatal inherited hyperammonemias give symptoms which cannot be distinguished clinically from sepsis. Late-onset manifestations may also not be straightforward [4,73], thus making the diagnosis of inherited hyperammonemias a challenge for the clinicians.

Due to the resulting brain edema, a late diagnosis and treatment are important negative prognostic factors. Consequently, a prompt ascertainment of hyperammonemia is crucial at any age for a good prognosis.

Because of the unspecific clinical presentation of hyperammonemia, alternative measures to clinical diagnosis are needed. In recent years, newborn screening programs using tandem mass spectrometry (MS/MS) have extended the number of inherited disorders leading to hyperammonemia identifiable in the first days of life.

Next-generation sequencing (NGS) panels of genes associated with hyperammonemia are also a promising diagnostic tool, because they can improve the timing and the accuracy of the diagnosis.

4.1. Expanded newborn screening (NBS)

Screening programs measuring acylcarnitine and amino acid profiles in newborns through MS/MS can detect more than 40 IMDs. The goal of NBS is the early identification of babies with a high risk for inborn errors of metabolism that may not be clinically evident at birth, but that could have severe consequences if not promptly diagnosed and treated.

Among the IMDs causing hyperammonemia, NBS may potentially recognize OAs, FAODs, and UCDs.

The majority of OAs causing hyperammonemia results from a defect in the branched-chain amino acids (BCAAs) catabolism. The most important are propionic acidemia (PA, OMIM #606054), methylmalonic acidemia (MMA, OMIM #251000), and isovaleric acidemia (IVA, OMIM #243500). Hyperammonemia may be also a feature of severe forms of maple syrup urine disease (MSUD, OMIM #248600) [74]. The diagnosis of these disorders is based on acylcarnitines profiles identified by primary biomarkers [75–78] and in some cases by second-tier testing to improve the positive predictive value of NBS [78,79].

The most common FAODs presenting with hyperammonemia are carnitine uptake deficiency (CUD, OMIM #212140), carnitine acylcarnitine translocase deficiency (CACTD, OMIM #212138), the neonatal and infantile forms of carnitine palmitoyltransferase II deficiency (CPT II deficiency, OMIM #608836, #600649), medium-chain acyl-CoA dehydrogenase deficiency (MCADD, OMIM #201450) and multiple acyl-CoA dehydrogenase deficiency (MADD, OMIM #231680) [80]. While there is the possibility that many patients died without being diagnosed or are symptomatic but undiagnosed, it is also very likely that many undiagnosed individuals with these disorders are asymptomatic. There is considerable evidence that many infants identified by NBS with MCADD remain asymptomatic. Early infancy treatment is not likely the reason. It is therefore probable that many, perhaps most, of the infants identified with MCADD by NBS have a mild and perhaps asymptomatic form of the disorder [81].

Patients with UCDs can be detected by neonatal screening measuring amino acids profile in dried blood spot (DBS).

Increased level of Cit may be suggestive for ASSD, ASLD and citrin-D. In ASLD, argininosuccinic acid (ASA) will also be present. An increased Arg is suggestive for ARG1D [82], while a low Cit concentration can be detected in patients with CPS1D, NAGSD and OTCD.

However, mitochondrial enzymatic defects of the urea cycle have frequently been reported even in cases with normal NBS Cit concentration [83] and the specificity and sensitivity of the test currently remain low for these disorders. Screening kits for metabolites including glutamine and some amino acid ratios (e.g. glutamine/citrulline) have been proposed for the diagnosis of OTCD [84].

Since the time window to identify newborns before the onset of first symptoms is often narrow, the impact of NBS on UCDs is highly controversial. In Europe, UCDs are rarely included into national NBS programs, with the exception of ASS and ASL. Instead it is a common practice in the USA.

The clinical benefit of UCD diagnosis by neonatal screening is still unclear. However, NBS has been recently associated to a trend toward neurological long-term improvement in patients with ASSD, ASLD, and ARG1D [85,86]. Moreover, a recent cross-border surveillance in Germany, Austria, and Switzerland revealed a positive impact of NBS on patients with UCDs, in particular cytosolic enzymatic defects. Ten out of eleven patients promptly identified by NBS (1 OTCD, 4 ASSD, 3 ASLD, 1 ARG1D, 1 citrinD) remained asymptomatic during a short observation [87]. Nevertheless, NBS may also identify benign phenotypes such as untreated ASL individuals with normal intellectual and psychomotor development without any intervention [88].

Due to a significant disparity among NBS programs in many countries [88], the pediatricians should become familiar with the capabilities and limitations of MS/MS testing performed in their pediatric population.

A false negative result is always possible due to the nature of newborn screening as a large-scale screening test. As previously pointed up, Cit as a primary marker may not be able to detect all the UCD cases. A missed case of ASLD has been recently reported because subtle elevation of this marker may overlap with the normal range [89].

In the case of UCD, clinicians should maintain a high degree of clinical suspicion when a dietary history and other clinical features may suggest a hyperammonemic disorder.

4.2. Diagnosis of late-onset inherited hyperammonemias in adults

Late-onset forms of inherited hyperammonemias have increasingly been reported in adults. They can present with acute hyperammonemic coma or milder chronic or recurrent hyperammonemic episodes [90].

OTCD, which is inherited as an X-linked disorder, is the most well-described UCD in adulthood.

The acute manifestations are usually precipitated by ammonia overproduction, that overcomes liver clearance capacity. The most common triggers are exogenous protein load, hypercatabolic states such as infections, fever, fasting, steroid therapy, trauma, pregnancy, surgery, gastrointestinal or internal bleeding [4], infections by urea-splitting bacteria [91], and pharmacological treatments, in particular chemotherapy protocols with L-asparaginase/pegaspargase [92] and sodium valproate administration [93].

Box 1. Drugs summary.

When ammonia should be measured
In patients at any age presenting:
  (1) an unexplained change in consciousness
  (2) unusual or unexplained neurological illness
  (3) cerebral edema
  (4) acute liver failure
  (5) suspected intoxication
  (6) recurrent vomiting and protein aversion or self-selected low-protein diet
In newborns with:
  (1) respiratory alkalosis (when metabolic alkalosis or metabolic acidosis are suspected)
In adolescents and adults with:
  (1) psychiatric symptoms
  (2) acute loss of vision
  (3) recurrent migraine-like headaches
  (4) stroke-like episodes
Selective investigations to perform in case of hyperammonemia
  (1) Plasma and urine amino acids
  (2) Blood or plasma acylcarnitines
  (3) Urinary organic acids
  (4) Urinary orotic acid
  (5) Perimortem: DNA to store, storage of frozen aliquots of all samples obtained of plasma and urine, consider liver biopsy for enzymatic studies and skin biopsy for fibroblast culture.

Most symptoms of inherited hyperammonemias in adults are neurological but nonspecific (Box 1). Hepatic-gastrointestinal and psychiatric manifestations are second in frequency [4,90].

Variability in disease severity is characteristic for OTCD heterozygous females (due to lyonization), but is also found in all UCDs, being mainly attributable to differences in the type and in the severity of the genetic change [4].

Recognizing UCDs in adults can be challenging due to milder or atypical symptoms compared to classic UCD presentations. Often the diagnosis in adulthood is delayed by comorbid conditions and ammonia levels are performed too late for starting the emergency therapy. The crucial first step is to consider ammonia at presentation of all encephalopathy or when recurrent symptoms are present.

Selective investigations should be performed in any patients who present hyperammonemia (Box 1). In particular, plasma and urine should be collected for measuring plasma amino acids and urinary orotic acid. Plasma Gln, Cit and ASA levels, and urinary orotic acid are easily detectable during acute phase and may suggest the diagnosis.

Nevertheless, in some cases, establishing the specific enzyme defect may be challenging. Females with OTCD may only have slight elevations in orotic acid. Moreover, patients suspected of having NAGSD and CPS1D have similar plasma amino acid profiles.

For these reasons, molecular genetic testing is now a useful diagnostic tool.

NGS panels of genes associated with UCDs/hyperammonemia are now available and are becoming increasingly inexpensive and faster [94,95]. They may change the diagnostic paradigm for inherited hyperammonemias improving the accuracy of the diagnosis and potentially avoiding the need of invasive or expensive functional tests [94].

NGS technology seems to be particularly promising in patients with both consistent clinical and biochemical suspicion of an inherited cause of hyperammonemia [95].

5. Conclusions

Understanding the mechanisms of ammonia neurotoxicity is important for optimizing the treatment of hyperammonemic crisis and for identifying new therapeutic strategies. The number of hypotheses indicates that a major cause has not been identified and that several mechanisms may contribute.

Due to the deleterious effects on CNS of increased ammonia concentrations, a prompt ascertainment of hyperammonemia is crucial at any age for a good prognosis.

Because of the unspecific clinical presentation of hyperammonemia, alternative measures to clinical diagnosis are needed. Some IMDs presenting with hyperammonemia are now part of the recommended uniform screening panel for NBS in many countries. Nevertheless, some inherited hyperammonemias do not have reliable markers for NBS or present before the NBS results are available, making still the diagnosis a challenge for the clinicians.

Moreover, diagnosis of late-onset forms of hyperammonemia in adults can be challenging due to milder or atypical symptoms compared to classic UCD presentations. In these cases, NGS panels of genes associated with UCDs/hyperammonemia are a promising diagnostic tool.

6. Expert opinion

Inherited hyperammonemias include primary hyperammonemias due to loss-of-function defects of any of the urea cycle enzymes and secondary hyperammonemias due to a secondary low activity of the urea cycle pathway. All these disorders are characterized by elevated concentrations of ammonia that are toxic to both neuronal and astrocytic elements in the CNS by several pathogenetic mechanisms.

In the light of the recent discovery of the role of the amino acid arginine, UCDs may reveal new insights [96].

L-arginine is the substrate for four enzymes, some of which exist as multiple isoforms: the arginase, converting L-arginine in L-ornithine in the urea cycle; the nitric oxide synthase (NOS), producing NO; the arginine:glycine amidinotransferase (AGAT), forming guanidinoacetate (GAA), which is then converted to creatine; and the arginine decarboxylase, producing agmatine [97].

Intriguingly, some of these enzymes have a unique and not overlapping distribution in the different brain areas, as previously demonstrated in the rat [98]. A similar area-specific distribution in the rat brain is also observed for ASS and ASL, which in concert with L-arginine constitute the citrulline-NO cycle [48].

It is known that NH_4^+ exposure of brain cells leads to very different outcomes on NO synthesis, depending on whether brain cells are or are not in shortage of Arg [3]. Arg deficiency promotes the uncoupling of NOS and eventually the production of superoxide rather than NO. Moreover, it compromises the synthesis of Cit, Orn, creatine and agmatine [97].

Patients with UCDs (except ARG1D) are usually treated with a supplement of L-arginine, because in these disorders Arg becomes an essential amino acid. Nevertheless, concern has been recently raised about the safety of high L-arginine doses in UCD patients [97,99], in particular in those affected by ASLD. In theory, L-arginine supplementation increases not only NO production and Cit, Orn, creatine and agmatine levels, but also those of ASA. The trapping of ASA in the brain could be the pathological mechanism responsible for the poor cognitive outcome of patients with ASLD, either directly or via the formation of guanidino compounds [52].

The detrimental effect of high levels of L-arginine has also been postulated in LPI. In that disorder, NO overproduction secondary to Arg intracellular trapping due to a defective efflux from the cell may explain some long-term complications, such as immune dysfunction [100].

A natural model of the possible toxic effects of high Arg concentrations is ARG1D. Individuals with ARG1D typically present with highly elevated Arg levels and mildly or moderately increased ammonia in plasma, a biochemical pattern strikingly different from those of other UCDs. In particular, plasma Arg concentrations often remain significantly elevated even in patients strictly adhering to dietary protein restriction.

High Arg levels may be responsible of the peculiar clinical phenotype of ARG1D patients characterized by variable degrees of cognitive impairment, epilepsy, and progressive spastic diplegia or paraparesis [82].

In the absence of a method to achieve the genetic correction of the defective metabolic pathways, new therapeutic strategies for the neurological complications of hyperammonemia are an interesting area of research and deserve further investigation.

Several potential therapeutic agents, such as NMDA receptor antagonists, nitric oxide inhibitors, and creatine and acetyl-L-carnitine supplementation, have been recently proposed as possible neuroprotective treatments. Clinical trials to evaluate their safety and efficacy are necessary for extensive application to UCDs patients.

Declaration of Interest

A Burlina has received speaker honoraria and travel support from Sanofi Genzyme, Biomarin, Actelion and Nutricia. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. A reviewer discloses that they were Chief Medical and Development officer at Hyperion Therapeutics, which was responsible for development of glycerolphenylbutyrate for urea cycle disorders.

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Cooper AJ, Plum F. Biochemistry and physiology of brain ammonia. Physiol Rev. 1987 Apr;67(2):440–519. [PubMed: 2882529].

2. Adeva MM, Souto G, Blanco N, et al. Ammonium metabolism in humans. Metabolism. 2012 Nov;61(11):1495–1511. [PubMed: 22921946].

   • **This review summarizes relevant articles concerning the enzymes involved in human ammonium metabolism, and the main causes of hyperammonemia in humans.**

3. Braissant O, McLin VA, Cudalbu C. Ammonia toxicity to the brain. J Inherit Metab Dis. 2013 Jul;36(4):595–612. [PubMed: 3109059].

   •• **Dr. Braissant is one of the most important experts in the field of hyperammonemia. This review illustrates the most important mechanisms of CNS ammonium toxicity.**

4. Häberle J, Boddaert N, Burlina A, et al. Suggested guidelines for the diagnosis and management of urea cycle disorders. Orphanet J Rare Dis. 2012 May 29;7:32.

   •• **This review illustrates the suggested guidelines for the diagnosis and management of urea cycle disorders. Recommendations given in the guidelines are graded depending on evidence levels.**

5. Häberle J, Rubio V. Hyperammonemias and related disorders. In: Blau N, Duran M, Gibson KM, Dionisi-Vici C, eds. Physician's guide to the diagnosis, treatment, and follow-up of inherited metabolic diseases. Berlin Heidelberg: Springer-Verlag; 2014. p. 47–62.

   •• **Dr. Häberle is one of the most important experts in the field of urea cycle disorders.**

6. Baumgartner MR, Rabier D, Nassogne MC, et al. Delta1-pyrroline-5-carboxylate synthase deficiency: neurodegeneration, cataracts and connective tissue manifestations combined with hyperammonaemia and reduced ornithine, citrulline, arginine and proline. Eur J Pediatr. 2005 Jan;164(1):31–36. [PubMed: 15517380].

7. Van Karnebeek C, Häberle J. Carbonic Anhydrase VA Deficiency. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2017. [PubMed: 25834911].

8. de Koning TJ. Amino acid synthesis deficiencies. J Inherit Metab Dis. 2017 Jul;40(4):609–620. [PubMed: 28653176].

9. Rabier D. Ammonia metabolism in humans: production and removal. In: Häberle J, ed. Current approach to hyperammonemia. Future Medicine Ltd Unitec House, 2 Albert Place, London N3 1QB, UK, March 2015: p. 7–23.

10. Amino acid oxidation and the production of urea. In: Nelson D, Cox M, eds. Lehninger Principles of Biochemistri. 4th Edition. Chapter 18. New York: W.H. Freeman and Company; 2005. p. 656–689.

11. Cooper AJ. The role of glutamine synthetase and glutamate dehydrogenase in cerebral ammonia homeostasis. Neurochem Res. 2012 Nov;37(11):2439–2455. [PubMed: 22618691].

12. Lowenstein JM. Ammonia production in muscle and other tissues: the purine nucleotide cycle. Physiol Rev. 1972 Apr;52(2):382–414. [PubMed: 4260884].

13. Tapper EB, Jiang GZ, Patwardhan VR. Refining the ammonia hypothesis: a physiology-driven approach to the treatment of hepatic encephalopathy. Mayo Clin Proc. 2015 May;90(5):646–658. [PubMed: 25865476].

14. Atkinson DE, Bourke E. Metabolic aspects of the regulation of systemic pH. Am J Physiol. 1987 Jun;252(6 Pt 2):F947–56. [PubMed: 3296786].

15. Häussinger D. Hepatocyte heterogeneity in glutamine and ammonia metabolism and the role of an intercellular glutamine cycle during ureogenesis in perfused rat liver. Eur J Biochem. 1983 Jun 15;133(2):269–275. [PubMed: 6852039].

   • **This paper provided the first evidence supporting the perivenous localization of GS and the predominantly periportal localization of GA and urea synthesis.**

16. Filipowicz HR, Ernst SL, Ashurst CL, et al. Metabolic changes associated with hyperammonemia in patients with propionic acidemia. Mol Genet Metab. 2006 Jun;88(2):123–130. [PubMed: 16406646].

   • **This paper highlights the mechanisms of low or normal GLN levels typically found in hyperammonemia due to organic acidurias.**

17. Owen EE, Johnson JH, Tyor MP. The effect of induced hyperammonemia on renal ammonia metabolism. J Clin Invest. 1961 Feb;40:215–221. [PubMed: 13731806]

18. Lockwood AH, McDonald JM, Reiman RE, et al. The dynamics of ammonia metabolism in man. Effects of liver disease and hyperammonemia. J Clin Invest. 1979 Mar;63(3):449–460. [PubMed: 429564].

19. Nose K, Mizuno T, Yamane N, et al. Identification of ammonia in gas emanated from human skin and its correlation with that in blood. Anal Sci. 2005 Dec;21(12):1471–1474. [PubMed: 16379388].

20. Hunt JF, Erwin E, Palmer L, et al. Expression and activity of ph-regulatory glutaminase in the human airway epithelium. Am J Respir Crit Care Med. 2002 Jan 1;165(1):101–107. [PubMed: 11779738].

21. Cooper AJ, Freed BR. Metabolism of [13N]ammonia in rat lung. Neurochem Int. 2005 Jul;47(1–2):103–118. [PubMed: 15923062].

22. Dasarathy S, Mookerjee RP, Rackayova V, et al. Ammonia toxicity: from head to toe? Metab Brain Dis. 2017 Apr;32(2):529–538. [PubMed: 28012068].

23. Braissant O. Current concepts in the pathogenesis of urea cycle disorders. Mol Genet Metab. 2010;100 Suppl 1:S3–S12. [PubMed: 20227314].

   •• **This review highlights the most important concepts in the pathogenesis of urea cycle disorders and related ammonia toxicity.**

24. Msall M, Batshaw ML, Suss R, et al. Neurologic outcome in children with inborn errors of urea synthesis. Outcome of urea-cycle enzymopathies. N Engl J Med. 1984 Jun 7;310(23):1500–1505. [PubMed: 6717540].

   • **This study demonstrated for the first time that the severity of brain damage in hyperammonemic children depends on both the duration of the insult and the degree of hyperammonemia.**

25. Bireley WR, van Hove JL, Gallagher RC, et al. Urea cycle disorders: brain MRI and neurological outcome. Pediatr Radiol. 2012 Apr;42(4):455–462. [PubMed: 21989980].

26. Barkovich E, Robinson C, Gropman A. Brain biomarkers and neuroimaging to diagnose urea cycle disorders and assess prognosis. Expert Opinion on Orphan Drugs. 2016;4(11):1123–1132.

   • **This review illustrates the role of brain biomarkers and neuroimaging in the management of UCDs.**

27. Waisbren SE, Gropman AL, Batshaw ML, et al. Improving long term outcomes in urea cycle disorders-report from the urea cycle disorders consortium. J Inherit Metab Dis. 2016 Jul 39(4):573–584. [PubMed: 27215558].

28. Lichter-Konecki U. Profiling of astrocyte properties in the hyperammonaemic brain: shedding new light on the pathophysiology of the brain damage in hyperammonaemia. J Inherit Metab Dis. 2008 Aug;31(4):492–502. [PubMed: 18683079].

29. Brusilow SW, Koehler RC, Traystman RJ, et al. Astrocyte glutamine synthetase: importance in hyperammonemic syndromes and potential target for therapy. Neurotherapeutics. 2010 Oct;7(4):452–470. [PubMed: 20880508].

30. Albrecht J, Norenberg MD. Glutamine: a Trojan horse in ammonia neurotoxicity. Hepatology. 2006 Oct;44(4):788–794. [PubMed: 17006913].

   •• **Trojan horse hypothesis for ammonia neurotoxicity.**

31. Reinehr R, Görg B, Becker S, et al. Hypoosmotic swelling and ammonia increase oxidative stress by NADPH oxidase in cultured astrocytes and vital brain slices. Glia. 2007 May;55(7):758–771. [PubMed: 17352382].

32. Rama Rao KV, Reddy PV, Tong X, et al. Brain edema in acute liver failure: inhibition by L-histidine. Am J Pathol. 2010 Mar;176(3):1400–1408. [PubMed: 20075201].

33. Hertz L, Song D, Peng L, et al. Multifactorial effects on different types of brain cells contribute to ammonia toxicity. Neurochem Res. 2017 Mar;42(3):721–736. [PubMed: 27286679].

34. Jafari P, Braissant O, Zavadakova P, et al. Ammonium accumulation and cell death in a rat 3D brain cell model of glutaric aciduria type I. PLoS One. 2013;8(1): e53735. [PubMed: 23326493].

35. Jafari P, Braissant O, Zavadakova P, et al. Brain damage in methylmalonic aciduria: 2-methylcitrate induces cerebral ammonium accumulation and apoptosis in 3D organotypic brain cell cultures. Orphanet J Rare Dis. 2013 Jan 8;8:4. DOI:10.1186/1750-1172-8-4

36. Natesan V, Mani R, Arumugam R. Clinical aspects of urea cycle dysfunction and altered brain energy metabolism on modulation of glutamate receptors and transporters in acute and chronic hyperammonemia. Biomed Pharmacother. 2016 Jul;81:192–202. [PubMed: 27261594].

37. Hermenegildo C, Monfort P, Felipo V. Activation of N-methyl-D-aspartate receptors in rat brain in vivo following acute ammonia intoxication: characterization by in vivo brain microdialysis. Hepatology. 2000 Mar;31(3):709–715. [PubMed: 10706562].

38. Kosenko E, Kaminsky Y, Grau E, et al. Brain ATP depletion induced by acute ammonia intoxication in rats is mediated by activation of the NMDA receptor and Na+, K(+)-ATPase. J Neurochem. 1994 Dec;63(6):2172–2178. [PubMed: 7964737].

39. Kosenko E, Kaminski Y, Lopata O, et al. Blocking NMDA receptors prevents the oxidative stress induced by acute ammonia intoxication. Free Radic Biol Med. 1999 Jun;26(11–12):1369–1374. [PubMed: 10401599]

40. Rao KV, Qureshi IA. Reduction in the MK-801 binding sites of the NMDA sub-type of glutamate receptor in a mouse model of congenital hyperammonemia: prevention by acetyl-L-carnitine. Neuropharmacology. 1999 Mar;38(3):383–394. [PubMed: 10219976].

41. Cauli O, Rodrigo R, Llansola M, et al. Glutamatergic and gabaergic neurotransmission and neuronal circuits in hepatic encephalopathy. Metab Brain Dis. 2009 Mar;24(1):69–80. [PubMed: 19085094]

42. Stepien KM, Heaton R, Rankin S, et al. Evidence of oxidative stress and secondary mitochondrial dysfunction in metabolic and nonmetabolic disorders. J Clin Med. 2017 Jul 19;6(7), [PubMed: 28753922].

43. Mousseau DD, Butterworth RF. Current theories on the pathogenesis of hepatic encephalopathy. Proc Soc Exp Biol Med. 1994 Sep;206(4):329–344. [PubMed: 7915420].

44. Garthwaite J. Concepts of neural nitric oxide-mediated transmission. Eur J Neurosci. 2008 Jun;27(11):2783–2802. [PubMed: 18588525].

45. Peunova N, Enikolopov G. Nitric oxide triggers a switch to growth arrest during differentiation of neuronal cells. Nature. 1995 May 4;375(6526):68–73. [PubMed: 7536899].

46. Nott A, Riccio A. Nitric oxide-mediated epigenetic mechanisms in developing neurons. Cell Cycle. 2009 Mar 1;8(5):725–730. [PubMed: 19221483].

47. Wiesinger H. Arginine metabolism and the synthesis of nitric oxide in the nervous system. Prog Neurobiol. 2001 Jul;64(4):365–391. [PubMed: 11275358].

48. Braissant O, Honegger P, Loup M, et al. Hyperammonemia: regulation of argininosuccinate synthetase and argininosuccinate lyase genes in aggregating cell cultures of fetal rat brain. Neurosci Lett. 1999 May 7;266(2):89–92. [PubMed: 10353334].

49. Zielińska M, Ruszkiewicz J, Hilgier W, et al. Hyperammonemia increases the expression and activity of the glutamine/arginine transporter y+ LAT2 in rat cerebral cortex: implications for the nitric oxide/cGMP pathway. Neurochem Int. 2011 Feb;58(2):190–195. [PubMed: 21115085].

50. Skowrońska M, Zielińska M, Wójcik-Stanaszek L, et al. Ammonia increases paracellular permeability of rat brain endothelial cells by a mechanism encompassing oxidative/nitrosative stress and activation of matrix metalloproteinases. J Neurochem. 2012 Apr;121(1):125–134. [PubMed: 22260250].

51. Shu X, Keller TC 4th, Begandt D, et al. Endothelial nitric oxide synthase in the microcirculation. Cell Mol Life Sci. 2015 Dec;72 (23):4561–4575. [PubMed: 26390975].

52. Jaffrey SR, Erdjument-Bromage H, Ferris CD, et al. Protein S-nitrosylation: a physiological signal for neuronal nitric oxide. Nat Cell Biol. 2001 Feb;3(2):193–197. [PubMed: 11175752].

53. Baruteau J, Jameson E, Morris AA, et al. Expanding the phenotype in argininosuccinic aciduria: need for new therapies. J Inherit Metab Dis. 2017 May;40(3):357–368. [PubMed: 28251416].
•• ASLD is a model of systemic NO deficiency.

54. Erez A, Nagamani SC, Shchelochkov OA, et al. Requirement of argininosuccinate lyase for systemic nitric oxide production. Nat Med. 2011 Nov 13;17(12):1619–1626. [PubMed: 22081021].

55. Nagasaka H, Komatsu H, Ohura T, et al. Nitric oxide synthesis in ornithine transcarbamylase deficiency: possible involvement of low NO synthesis in clinical manifestations of urea cycle defect. J Pediatr. 2004 Aug;145(2):259–262. [PubMed: 15289781].

56. Nagamani SC, Campeau PM, Shchelochkov OA, et al. Nitric-oxide supplementation for treatment of long-term complications in argininosuccinic aciduria. Am J Hum Genet. 2012 May 4;90(5):836–846. [PubMed: 22541557].

57. Braissant O, Cagnon L, Monnet-Tschudi F, et al. Ammonium alters creatine transport and synthesis in a 3D-culture of developing brain cells, resulting in secondary cerebral creatine deficiency. Eur J Neurosci. 2008 Apr;27(7):1673–1685. [PubMed: 18380667].

58. Jayakumar AR, Rama Rao KV, Tong XY, et al. Calcium in the mechanism of ammonia-induced astrocyte swelling. J Neurochem. 2009 May;109 Suppl 1:252–257. [PubMed: 19393035].

59. Rose C, Kresse W, Kettenmann H. Acute insult of ammonia leads to calcium-dependent glutamate release form cultured astrocytes, an effect of pH. J Biol Chem. 2005 Jun 3;280(22):20937–20944. [PubMed: 15802262].

60. Cagnon L, Braissant O. Role of caspases, calpain and cdk5 in ammonia-induced cell death in developing brain cells. Neurobiol Dis. 2008 Nov;32(2):281–292. [PubMed: 18722528].

61. Attwell D, Buchan AM, Charpak S, et al. Glial and neuronal control of brain blood flow. Nature. 2010 Nov 11;468(7321):232–243. [PubMed: 2106883].

62. Hertz L, Kala G. Energy metabolism in brain cells: effects of elevated ammonia concentrations. Metab Brain Dis. 2007 Dec;22(3–4):199–218. [PubMed: 17882538].

63. Ott P, Clemmesen O, Larsen FS. Cerebral metabolic disturbances in the brain during acute liver failure: from hyperammonemia to energy failure and proteolysis. Neurochem Int. 2005 Jul;47(1–2):13–18. [PubMed: 15921824].

64. Fitzpatrick SM, Hetherington HP, Behar KL, et al. Effects of acute hyperammonemia on cerebral amino acid metabolism and pHi in vivo, measured by 1H and 31P nuclear magnetic resonance. J Neurochem. 1989 Mar;52(3):741–749. [PubMed: 2563756].

65. Cooper AJ, Jeitner TM. Central role of glutamate metabolism in the maintenance of nitrogen homeostasis in normal and hyperammonemic brain. Biomolecules. 2016 Mar 26;6(2), [PubMed: 27023624].

66. Rao KV, Mawal YR, Qureshi IA. Progressive decrease of cerebral cytochrome C oxidase activity in sparse-fur mice: role of acetyl-L-carnitine in restoring the ammonia-induced cerebral energy depletion. Neurosci Lett. 1997 Mar 14;224(2):83–86. [PubMed: 9086462].

67. Skowronska M, Albrecht J. Oxidative and nitrosative stress in ammonia neurotoxicity. In: Häberle J, ed, Current approach to hyperammonemia. 2 Albert Place, London N3 1QB, UK 9781780844831: Future Medicine Ltd Unitec House. March 2015; 55–65.
•• This chapter highlights the most important concepts of oxidative and nitrosative stress in ammonia toxicity.

68. Kowaltowski AJ, Castilho RF, Vercesi AE. Mitochondrial permeability transition and oxidative stress. FEBS Lett. 2001 Apr 20;495(1–2):12–15. [PubMed: 11322939].

69. Jayakumar AR, Norenberg MD. The Na-K-Cl Co-transporter in astrocyte swelling. Metab Brain Dis. 2010 Mar;25(1):31–38. [PubMed: 20336356].

70. Holm LM, Jahn TP, Møller AL, et al. NH3 and NH4+ permeability in aquaporin-expressing Xenopus oocytes. Pflugers Arch. 2005 Sep;450(6):415–428. [PubMed: 15988592].

71. Lockwood AH, Bolomey L, Napoleon F. Blood-brain barrier to ammonia in humans. J Cereb Blood Flow Metab. 1984 Dec;4 (4):516–522. [PubMed: 6334091].

72. Detry O, De Roover A, Honore P, et al. Brain edema and intracranial hypertension in fulminant hepatic failure: pathophysiology and management. World J Gastroenterol. 2006 Dec 14;12(46):7405–7412. [PubMed: 17167826].

73. Kölker S, Garcia-Cazorla A, Valayannopoulos V, et al. The phenotypic spectrum of organic acidurias and urea cycle disorders. Part 1: the initial presentation. J Inherit Metab Dis. 2015 Nov;38(6):1041–1057. Epub 2015 Apr 15. Erratum in: J Inherit Metab Dis. 2015 Nov;38(6):1155-6. Cazorla, Angeles Garcia [corrected to Garcia-Cazorla, Angeles. [PubMed: 25875215].

74. Knerr I, Vockley J, Gibson MK, et al. Disorders of leucine, isoleucine and valine metabolism. In: Blau Neds. Physician's guide to the diagnosis, treatment, and follow-up of inherited metabolic diseases. Berlin Heidelberg: Springer-Verlag; 2014. p. 103–141.

75. Baumgartner MR, Hörster F, Dionisi-Vici C, et al. Proposed guidelines for the diagnosis and management of methylmalonic and propionic acidemia. Orphanet J Rare Dis. 2014 Sep 2;9:130. [PubMed: 25205257].

76. Malvagia S, Haynes CA, Grisotto L, et al. Heptadecanoylcarnitine (C17) a novel candidate biomarker for newborn screening of propionic and methylmalonic acidemias. Clin Chim Acta. 2015 Oct 23;450:342–348. [PubMed: 26368264]

77. Ensenauer R, Fingerhut R, Maier EM, et al. Newborn screening for isovaleric acidemia using tandem mass spectrometry: data from 1.6 million newborns. Clin Chem. 2011 Apr;57(4):623–626. [PubMed: 21335445].

78. Couce ML, Ramos F, Bueno MA, et al. Evolution of maple syrup urine disease in patients diagnosed by newborn screening versus late diagnosis. Eur J Paediatr Neurol. 2015 Nov;19(6):652–659. [PubMed: 26232051].

79. Turgeon CT, Magera MJ, Cuthbert CD, et al. Determination of total homocysteine, methylmalonic acid, and 2-methylcitric acid in dried blood spots by tandem mass spectrometry. Clin Chem. 2010 Nov;56(11):1686–1695. [PubMed: 20807894].

80. Se O. Pathophysiology of fatty acid oxidation disorders and resultant phenotypic variability. J Inherit Metab Dis. 2013 Jul;36(4):645–658. [PubMed: 23674167].

81. Landau YE, Waisbren SE, Chan LM, et al. Long-term outcome of expanded newborn screening at Boston children's hospital: benefits and challenges in defining true disease. J Inherit Metab Dis. 2017 Mar;40(2):209–218. [PubMed: 28054209].

82. Huemer M, Carvalho DR, Brum JM, et al. Clinical phenotype, biochemical profile, and treatment in 19 patients with arginase 1 deficiency. J Inherit Metab Dis. 2016 May;39(3):331–340. [PubMed: 27038030].

•• ARGD1 is natural model of the possible toxic effects of high ARG concentrations.

83. Cavicchi C, Malvagia S, La Marca G, et al. Hypocitrullinemia in expanded newborn screening by LC-MS/MS is not a reliable marker for ornithine transcarbamylase deficiency. J Pharm Biomed Anal. 2009 Jul 12;49(5):1292–1295. [PubMed: 19359120].

84. Trinh MU, Blake J, Harrison JR, et al. Quantification of glutamine in dried blood spots and plasma by tandem mass spectrometry for the biochemical diagnosis and monitoring of ornithine transcarbamylase deficiency. Clin Chem. 2003 Apr;49(4):681–684. [PubMed: 12651832].

85. Posset R, Garcia-Cazorla A, Valayannopoulos V, et al. Age at disease onset and peak ammonium level rather than interventional variables predict the neurological outcome in urea cycle disorders. J Inherit Metab Dis. 2016 Sep;39(5):661–672. [PubMed: 27106216].

• This study highlights the predictive variables for the neurological outcome of UCD patients.

86. Mercimek-Mahmutoglu S, Moeslinger D, Häberle J, et al. Long-term outcome of patients with argininosuccinate lyase deficiency diagnosed by newborn screening in Austria. Mol Genet Metab. 2010 May;100(1):24–28. [PubMed: 20236848].

87. Nettesheim S, Kölker S, Karall D, et al. Incidence, disease onset and short-term outcome in urea cycle disorders -cross-border surveillance in Germany, Austria and Switzerland. Orphanet J Rare Dis. 2017 Jun 15;12(1):111. [PubMed: 28619060].

88. Loeber JG, Burgard P, Cornel MC, et al. Newborn screening programmes in Europe; arguments and efforts regarding harmonization. Part 1. From blood spot to screening result. J Inherit Metab Dis. 2012;35:603–611, [PubMed: 22552820].

89. Ganetzky RD, Bedoukian E, Deardorff MA, et al. Argininosuccinic acid lyase deficiency missed by newborn screen. JIMD Rep. 2017;34: 43–47. [PubMed: 27515243].

90. Smith W, Ps K, Lee B, et al. Urea cycle disorders: clinical presentation outside the newborn period. Crit Care Clin. 2005 Oct;21(4 Suppl):S9–17. [PubMed: 16227115].

91. Sato S, Yokota C, Toyoda K, et al. Hyperammonemic encephalopathy caused by urinary tract infection with urinary retention. Eur J Intern Med. 2008 Dec;19(8):e78–9. [PubMed: 19046708].

92. Leonard JV, Kay JD. Acute encephalopathy and hyperammonaemia complicating treatment of acute lymphoblastic leukaemia with asparaginase. Lancet. 1986 Jan 18;1(8473):162–163. [PubMed: 2867384].

93. Mock CM, Schwetschenau KH. Levocarnitine for valproic-acid-induced hyperammonemic encephalopathy. Am J Health Syst Pharm. 2012 Jan 1;69(1):35–39. [PubMed: 22180549].

94. Ghosh A, Schlecht H, Heptinstall LE, et al. Diagnosing childhood-onset inborn errors of metabolism by next-generation sequencing. Arch Dis Child. 2017;102(11):1019–1029.

• This paper highlights that NGS-based testing can improve the accuracy of diagnosis of IMDs and potentially avoid the need for invasive or expensive functional testing.

95. Yubero D, Brandi N, Ormazabal A, et al. Targeted next generation sequencing in patients with inborn errors of metabolism. PLoS One. 2016 May 31;11(5):e0156359. [PubMed: 27243974].

• This study demonstrates that targeted NGS in patients with IMDs is highly productive and cost effective in the presence of a consistent clinical and biochemical suspicion.

96. Scaglia F, Brunetti-Pierri N, Kleppe S, et al. Clinical consequences of urea cycle enzyme deficiencies and potential links to arginine and nitric oxide metabolism. J Nutr. 2004 Oct;134(10 Suppl):2775S–2782S. discussion 2796S-2797S. [PubMed: 15465784].

97. Coman D, Yaplito-Lee J, Boneh A. New indications and controversies in arginine therapy. Clin Nutr. 2008 Aug;27(4):489–496. [PubMed: 18640748].

98. Nakamura H, Saheki T, Nakagawa S. Differential cellular localization of enzymes of L-arginine metabolism in the rat brain. Brain Res. 1990 Oct 15;530(1):108–112. [PubMed: 2271938]].

99. Dioguardi FS. To give or not to give? Lessons from the arginine paradox. J Nutrigenet Nutrigenomics. 2011;4(2): 90–98. [PubMed: 21625171].

100. Ogier de Baulny H, Schiff M, Dionisi-Vici C. Lysinuric protein intolerance (LPI): a multi organ disease by far more complex than a classic urea cycle disorder. Mol Genet Metab. 2012 May;106(1):12–17. [PubMed: 22402328].