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The Role of the Urea Cycle in the Alzheimer's Disease Brain

Najlaa A. Al-Thani[1,2] | Gavin S. Stewart[3] | Derek A. Costello[1,2]

[1]UCD School of Biomolecular and Biomedical Science, University College Dublin, Dublin, Ireland | [2]UCD Conway Institute, University College Dublin, Dublin, Ireland | [3]UCD School of Biology and Environmental Science, University College Dublin, Dublin, Ireland

Correspondence: Derek A. Costello (derek.costello@ucd.ie)

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ABSTRACT

Alzheimer's Disease (AD) is a neurodegenerative disorder classified as the leading form of dementia in the elderly. Classical hallmarks of AD pathology believed to cause AD include Amyloid-beta (Aβ) plaques as well as neurofibrillary tau tangles (NTT). However, research into these classical hallmarks has failed to account for a causative link or therapeutic success. More recently, metabolic hallmarks of AD pathology have become a popular avenue of research. Elevated urea and ammonia detected in cases of AD point towards a dysfunctional urea cycle involved in AD. This review covers the expansive body of literature surrounding the work of researchers deciphering the role of the urea cycle in AD pathology through the study of urea cycle enzymes, metabolites, and transporters in the AD brain. Urea cycle enzymes of interest in AD pathology include OTC, NOS isoforms, ARG1, ARG2, MAOB, and ODC, which all present as promising therapeutic targets. Urea metabolites indicated in AD pathology have varying concentrations across the regions of the brain and the different cell types (neurons, microglia, astrocytes). Finally, the role of UT-B as a clearance modulator presents this protein as a key target for research in the role of the urea cycle in the AD brain. In the future, these key enzymes, pathways, and proteins relating to the urea cycle in AD should be further investigated to better understand the cell-specific urea cycle profiles in the AD brain and uncover their therapeutic potential.

1 | Introduction

Alzheimer's Disease (AD) is a neurodegenerative disorder and the leading form of dementia in the elderly worldwide, accounting for 60%–70% of cases (World Health Organization 2020). AD typically progresses through different stages, initiating with mild forgetfulness and confusion and advancing to severe cognitive impairment and memory loss (Knopman et al. 2021). Early and late stages of AD correlate to gradual brain atrophy and neuronal loss primarily in the hippocampus and cerebral cortex responsible for memory formation and cognition, respectively (Calabrò et al. 2021; Tahami Monfared et al. 2022; Töpperwien et al. 2020). The rate of progression of AD varies among individuals affected and can be attributed to the presence of several

Abbreviations: 3′-UTRs, 3′-untranslated regions; 6-OHDA, 6-hydroxydopamine; Acetyl-CoA, acetyl coenzyme A; AD, Alzheimer's disease; ADC, arginine decarboxylase; AGMAT, agmatinase; ALS, amyotrophic lateral sclerosis; AOO, age at onset; APOE ε4, apolipoprotein-E ε4 allele; APP, amyloid precursor protein; ARG1, arginase 1; ARG2, arginase 2; ASL, argininosuccinate-lyase; ASS, argininosuccinate synthetase; Aβ, amyloid-beta; Bdnf, brain derived neurotrophic factor; CB, cerebellum; CE-HR MS, capillary electrophoresis coupled to high-resolution mass spectrometry; CE-MS, capillary electrophoresis-mass spectrometry; CG, cingulate gyrus; CO_2, carbon dioxide; CSF, cerebral spinal fluid; DFMO, difluoromethylornithine; eNOS, endothelial nitrogen oxide synthase; ENT, entorhinal cortex; FC, frontal cortex; Fizz1, inflammatory zone 1; GABA, γ-aminobutyric acid; GABR, global arginine bioavailability ratio; GC-MS, gas-chromatography mass-spectrometry; GDC, glutamate decarboxylase; GLS2, glutamine transferase 2; GO, gene ontology; GS, glutamine synthase; GWAS, genome-wide association studies; H_2O, water; H_2O_2, hydrogen peroxide; HD, Huntington's disease; HP, hippocampus; IFNγ, interferon gamma; Il1rn, interleukin 1 receptor antagonist; IL-1β, interleukin-1β; IL-6, interleukin-6; iNOS, inducible nitrogen oxide synthase; ITG, inferior-temporal gyrus; KD, knockdown; LPS, lipopolysaccharides; LTA, lipoteichoic acid; MAOB, monoamine oxidase-B; MCX, motor cortex; mNOS2, nitric oxide synthase 2, inducible; mRNA, messenger RNA; MTG, middle-temporal gyrus; MTs, microtubules; NAGS, *N*-acetyl glutamine synthase; NGS, next generation sequencing; NH_3, ammonia; nNOS, neuronal nitrogen oxide synthase; NO, nitric oxide; NTT, neurofibrillary tau tangles; OAT, ornithine aminotransferase; ODC, ornithine decarboxylase; OTC, ornithine transcarbamoylase; PC12, rat pheochromocytoma cells; PD, Parkinson's disease; PFC, pre-frontal cortex; proBDNF, pro-brain-derived neurotrophic factor; PSEN1, presenilin 1; PSEN2, presenilin 2; RNS, reactive nitrogen species; SCX, sensory cortex; SFG, superior frontal gyrus; sMRI, structural magnetic resonance imaging; T2DM, type 2 diabetes mellitus; TBE, traumatic brain edema; t-BHP, tert-butyl hydroperoxide; TNFα, tumor necrosis factor-α; UCD, urea cycle disorders; UT-B, urea transporter B; WT, wildtype.

heritable and modifiable risk factors. Arguably the main risk factor of AD is age, which can be further attributed to genetic risk factors associated with age at onset (AAO) (Guerreiro and Bras 2015). Early AAO has been linked to mutations in several AD-associated genes encoding the amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2), as well as the presence of the apolipoprotein-E ε4 (APOE ε4) allele (Guerreiro and Bras 2015; Knopman et al. 2021; Thinakaran and Koo 2008). Although these genetic risk factors have been heavily studied in AD research, they do not account for the variance observed in AAO, indicating the involvement of other genetic components (Thambisetty et al. 2013). Modifiable risk factors include the presence of comorbidities such as type 2 diabetes mellitus (T2DM), hypertension, obesity, and low HDL cholesterol, as well as disruptive behaviors or events such as chronic sleep disorder, hearing loss, traumatic brain injury, and alcohol abuse (Knopman et al. 2021; Shokri-Kojori et al. 2018; Sun et al. 2020). Based on the localization of neuronal loss and risk factors attributed to AD, several theories have been put forward to explain the neuropathology of AD based on identified protein and metabolic hallmarks attributing to microglial activation, reactive astrocytes, and synaptic disorder.

The most notable theories elucidating AD neuropathology are the amyloid-beta (Aβ) plaque theory and the neurofibrillary tau tangles (NTT) theory (Calabrò et al. 2021; Knopman et al. 2021). Aβ plaques are produced during the dysregulated cleavage of APP by β- and γ-secretases (Holtzman et al. 2011; Knopman et al. 2021; Thinakaran and Koo 2008). This results in the formation of large Aβ fragments that aggregate, creating the characteristic Aβ plaques (Holtzman et al. 2011; Knopman et al. 2021; Thinakaran and Koo 2008). Accumulation of Aβ plaques was found to occur 15–20 years before the appearance of clinical symptoms of AD, providing a promising link between Aβ plaque accumulation and the onset of AD (Knopman et al. 2021; Thinakaran and Koo 2008). However, after more than 20 years of Aβ plaque research and targeted therapy since its discovery in the 1990s, sustainable evidence of clinical relevance remains limited (Herrup 2015; Panza et al. 2019). Alternatively, the NTT theory presents as a more convincing indicator of AD as it is also found in the brains of patients with other forms of dementia (Giacobini and Gold 2013). Tau proteins typically bind to microtubules (MTs) within neurons, resulting in their subsequent stabilization and ensuring fast axonal transportation along MT tracks (Gallardo and Holtzman 2019; Pooler et al. 2014). Dephosphorylation of Tau proteins by phosphatase enzymes strengthens the affinity of Tau to MTs in normal brains (Wang et al. 2007). In dementia and AD patients, constitutive phosphorylation of Tau proteins by kinases decreases Tau binding affinity to MTs, leading to the destabilization and depolymerization of MTs (Dujardin et al. 2020; Wang et al. 2007). Unbound, hyperphosphorylated Tau proteins accumulate and aggregate together to form helical filaments known as NTT that are typically seen in dementia and AD patients (Knopman et al. 2021; Pooler et al. 2014; Wang et al. 2007). Although the NTT theory serves as a popular biomarker for AD pathology, the presence of hyperphosphorylated Tau proteins in other diverse and distinct neurodegenerative diseases known as tauopathies makes NTT an unspecific AD hallmark (Behl 2023). Over the years, researchers have theorized that both the Aβ plaque and NTT theories may be contributing to AD pathology but at different stages, with Aβ plaques appearing early on in the cascade of pathological events while NTT functions as a downstream effector causing nerve cell death (Knopman et al. 2021). However, the definitive causative factors specific to AD leading to the formation of both Aβ plaques and NTT remain unknown.

In more recent years, AD research has focused on understanding the role of metabolic disorders and dysregulation in the development and progression of AD (Poddar et al. 2021; Yan et al. 2020). This shift in the scope of AD research has altered our understanding of AD from being a purely neurological disorder to a systemic metabolic disorder expressed in the brain. Based on metabolic epidemiological studies, the risk of AD is increased in individuals with T2DM through impaired glucose metabolism and insulin secretion (Sun et al. 2020). Furthermore, animal model-based experiments have indicated a relationship between T2DM and tau hyperphosphorylation (Kim et al. 2009). Impaired glucose metabolism can affect microglial cell clearance of Aβ plaque buildup through metabolic glucose reprogramming from the oxidative phosphorylation pathway to the glycolysis pathway (Dewanjee et al. 2022; Fairley et al. 2021). Moreover, impaired insulin activity has been linked to inflammation and hyperactivity in Aβ plaque-induced astrocytes (González-Casimiro et al. 2021). In addition to impaired glucose metabolism, dysregulation of the urea cycle and urea trafficking has also been implicated in several neurodegenerative disorders such as Huntington's Disease (HD), Parkinson's disease (PD) and AD (Bergen et al. 2015; Hansmannel et al. 2010; Patassini et al. 2015; Scholefield et al. 2021). In a healthy non-diseased brain, the urea cycle is able to metabolize highly toxic ammonia into less toxic urea through the activity of several key enzymes, primarily eliminating key substrates, citrulline and arginine (Figure 1) (Buniatian and Davtian 1966; Kemp and Woodbury 1965; Sadasivudu et al. 1985; Sporn et al. 1959). In AD, excessive levels of toxic ammonia have been detected in CSF analysis of patients (Kaiser et al. 2010). Correspondingly, high levels of urea have also been detected in cases of AD, indicating alterations in the urea cycle in AD pathology (Adlimoghaddam et al. 2016; Seiler 2002; Xu et al. 2016). Over the last 10 years of AD research, the role of the urea cycle has become an increasingly popular and ever-evolving field of study to understand whether urea accumulation is a causative or after-effect of AD pathology. The aim of this review is to discuss the role of urea cycle enzymes, metabolites, and transporters in the AD brain as reported in the scientific literature, covering the extensive timeline of research into urea cycle involvement in AD pathology and identifying key candidates for further research.

2 | The Conventional Urea Cycle in the Brain

The urea cycle, also known as the Krebs–Henseleit cycle, plays an important role in cellular metabolism via the regulation of toxic ammonia (NH_3) produced from the degradation of peptides and amino acids (Yudkoff 1999). This toxic waste conversion and removal process was classically believed to primarily occur in major waste removal organs such as the liver and to a lesser extent, the kidneys (Morris 2002; Sands 2003; Yudkoff 1999). However, studies demonstrating the detection of urea in the brain as well as key urea cycle enzymes, metabolites, and transporters have proven the presence of a functional

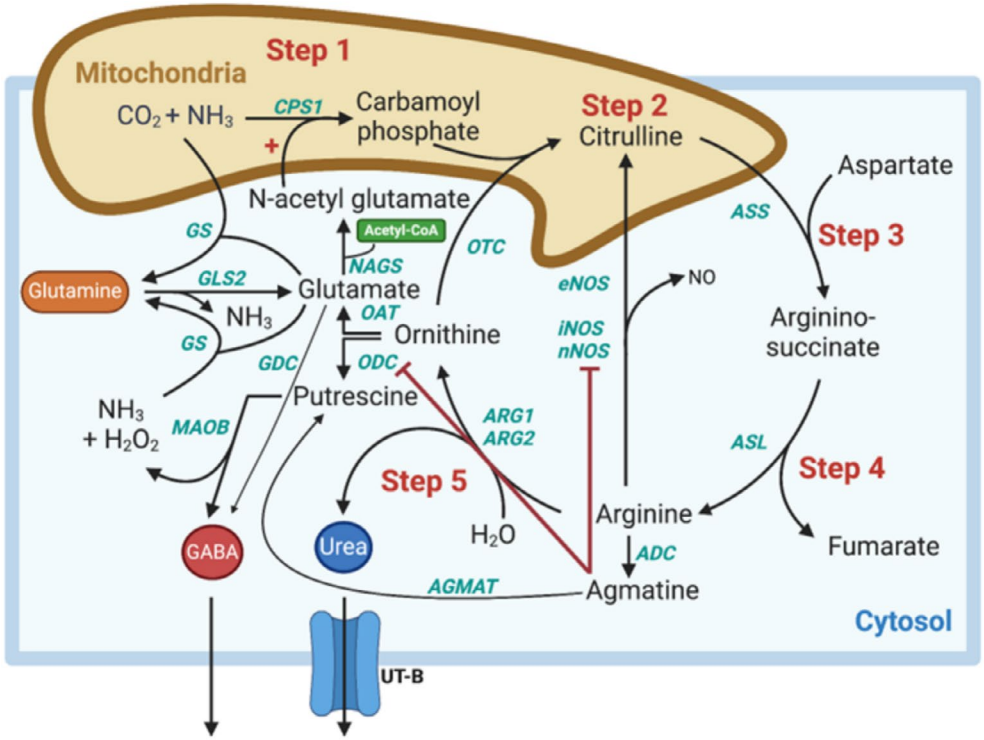

FIGURE 1 | Conventional urea cycle in the brain. Created with BioRender.com. Acetyl coenzyme A (Acetyl-CoA); agmatinase (AGMAT); ammonia (NH_3); arginase 1 (ARG1); arginase 2 (ARG2); arginine decarboxylase (ADC); argininosuccinate synthetase (ASS); argininosuccinate-lyase (ASL); carbamoyl phosphate synthase 1 (CPS1); carbon dioxide (CO_2); endothelial nitrogen oxide synthase (eNOS); glutamate decarboxylase (GDC); glutamine synthase (GS); glutamine transferase 2 (GLS2); hydrogen peroxide (H_2O_2); inducible nitrogen oxide synthase (iNOS); monoamine oxidase-B (MAOB); *N*-acetyl glutamine synthase (NAGS); neuronal nitrogen oxide synthase (nNOS); nitric oxide (NO); ornithine aminotransferase (OAT); ornithine decarboxylase (ODC); ornithine transcarbamoylase (OTC); urea transporter B (UT-B); water (H_2O); γ-aminobutyric acid (GABA).

urea cycle in the brain that caters to neuronal homeostasis (Buniatian and Davtian 1966; Davies et al. 1961; Huang et al. 2021). The urea cycle consists of five primary steps catalyzed by 6 key enzymes (Morris 2002; Yudkoff 1999). The first two steps take place in the mitochondria, and the rest of the cycle occurs in the cytosol (Morris 2002; Yudkoff 1999). Initiation of the urea cycle occurs via the mitochondrial production of NH_3 and carbon dioxide (CO_2) through the degradation of polypeptides (Morris 2002; Yudkoff 1999). In the first step, NH_3 and CO_2 produced in the mitochondria are converted into carbamoyl phosphate through an ATP-driven reaction by carbamoyl phosphate synthase 1 (CPS1) and allosteric activator, *N*-acetyl glutamate (Ah Mew and Caldovic 2011). The second step of the urea cycle involves the conversion of ornithine to citrulline via the activity of transcarbamoylase (OTC) (Morris 2002; Yudkoff 1999). The third step relates to citrulline exiting the mitochondria and reacting with argininosuccinate synthetase (ASS) and aspartate in an ATP-driven reaction to produce argininosuccinate (Morris 2002; Yudkoff 1999). The fourth step involves the cleavage of argininosuccinate by argininosuccinate lyase (ASL) to form fumarate and arginine (Morris 2002; Yudkoff 1999). The fifth step comprises the hydrolyzation of arginine by either arginase 1 (ARG1) or arginase 2 (ARG2) to form urea and ornithine (Morris 2002; Yudkoff 1999). Urea is cleared out of the cell through urea transporter B (UT-B) which has been identified as the primary urea transporter in the brain (Sands 2003). No region-specific differences in UT-B expression have been reported in the brain under normal conditions, with the majority of expression being in astrocytes as well as in some neuronal populations and lower levels of expression in microglia (Berger et al. 1998; Huang et al. 2021; Li et al. 2012; Pinki et al. 2023; Trinh-Trang-Tan et al. 2005). As for ornithine, it reenters the urea cycle through step 2 involving OTC and carbamoyl phosphate. Ornithine is also able to undergo a secondary step catalyzed by ornithine decarboxylase (ODC) to produce polyamine putrescine, which is essential for optimal cell growth and function (Alm and Oredsson 2009; Igarashi and Kashiwagi 2010; Wallace et al. 2003; Yudkoff 1999). Putrescine can also be produced through an alternate pathway involving catalysis of agmatine by agmatinase (AGMAT) (Morris 2002; Yudkoff 1999). Agmatine, produced from arginine by arginine decarboxylase (ADC), is considered a widespread putative neuromodulator that interacts with multiple receptors and is involved in a plethora of neuronal pathways linked to central and peripheral functions related to learning and memory processes (Halaris and Plietz 2007; Leitch et al. 2011; Li et al. 2006, 2003; Liu et al. 2008; Reis and Regunathan 2000; Rushaidhi et al. 2012; Seo et al. 2011; Wu and Morris 1998). Moreover, agmatine is able to induce the production of antizyme, a small regulatory protein, which inhibits ODC and stops the production of polyamines such as putrescine and other downstream compounds (Satriano 2003).

The urea cycle is also known to produce essential neurotransmitters, γ-aminobutyric acid (GABA) and Glutamate. GABA is a chief inhibitory neurotransmitter in the brain associated with synaptic inhibition, cognitive control, emotional processing, and the immune system (Jin et al. 2013; Kiemes et al. 2021; Stein and

Nicoll 2003). GABA is produced by the urea cycle through further processing of putrescine by monoamine oxidase-B (MAOB), additionally yielding toxic NH_3 and hydrogen peroxide (H_2O_2) as byproducts. Glutamate is the major excitatory neurotransmitter in the human brain known to cause excitotoxicity in neurons, an event that is widely modulated by glutamate transporters (Pol et al. 1990; Zhou and Danbolt 2014). Glutamate is produced within the urea cycle from ornithine by ornithine aminotransferase (OAT) (Morris 2002; Yudkoff 1999). *N*-acetyl glutamate synthase (NAGS) can further metabolize glutamate with the help of acetyl coenzyme A (Acetyl-CoA) to produce *N*-acetyl glutamate, which reenters the urea cycle via step 1 (Morris 2002; Yudkoff 1999). Glutamate can also be further processed to generate GABA by glutamic acid decarboxylase (GDC) (Wu and Morris 1998). Glutamine synthetase (GS) can further metabolize glutamate by using available cytosolic NH_3 to produce the amino acid glutamine (Albrecht et al. 2010; Tapiero et al. 2002). The reverse can also occur, and glutamine can convert back to glutamate by glutamine transferase 2 (GLS2), producing byproduct NH_3 (Albrecht et al. 2010; Tapiero et al. 2002). The several reactions surrounding glutamate are heavily influenced by NH_3 levels and are regulated to maintain NH_3 homeostasis (Albrecht et al. 2010).

Central to the urea cycle is the production of nitric oxide (NO) and citrulline by arginine and nitric oxide synthase (NOS) isoforms. NO derived from neuronal NOS (nNOS) has an essential role in synaptic plasticity, learning, memory, and emotional processing (Feil and Kleppisch 2008; Susswein et al. 2004; Zhou and Zhu 2009; Zhou et al. 2011). NO derived from endothelial NOS (eNOS) is known to be involved in the regulation and stabilization of the vascular microenvironment (Torre and Aliev 2005; Förstermann and Sessa 2012). NO derived from inducible NOS (iNOS) is typically detected in the normal aging brain and plays a major role in the brain's defense against infectious, inflammatory, or traumatic episodes (Licinio et al. 1999). Remarkably, agmatine can regulate the production of NO by inhibiting nNOS and iNOS but instead stimulates eNOS (Halaris and Plietz 2007; Joshi et al. 2007; Santhanam et al. 2007; Satriano 2003).

3 | Urea Cycle Alterations in the AD Brain

3.1 | Alterations in Urea Cycle Enzyme Levels and Expression in the AD Brain

Research into the alterations in the activity and expression levels of urea cycle enzymes in AD has gained traction over the years. Different models have been employed to study urea cycle enzymes under AD-like conditions, most notably human AD brain tissue, along with rodent-derived cell lines representing neuronal, astrocytic, and microglial cells. Although changes in the expression of several urea cycle enzymes have been implicated in AD pathology, a few key enzymes have been identified as potential therapeutic targets.

OTC is the urea cycle enzyme that has been associated most prominently with AD pathology. Bensemain et al. (2009) first identified OTC as an AD gene marker using a customized microarray to detect candidate genes present in chromosomal regions of interest from brain tissue samples of control and AD patients (Bensemain et al. 2009). Their findings indicated increased expression of OTC and other key urea cycle enzymes in AD patients compared to controls, with up to an approximate 9-fold increase in OTC activity (Bensemain et al. 2009). Following the discovery, further microarray analysis confirmed that all the urea cycle enzyme genes are expressed in the AD brain (Hansmannel et al. 2010). Moreover, genome-wide association studies (GWAS) of select populations in France, the United Kingdom, and Italy reported a weak but significant association between a point mutation in the promoter region of the OTC gene, −389 G/A [rs5963409] and risk of AD (Bensemain et al. 2009; Hansmannel et al. 2009). Interestingly, this association was independent of APOE ε4 allele status and age, suggesting that mutations in the OTC gene may be a primary genetic determinant of AD (Bensemain et al. 2009; Hansmannel et al. 2009).

Arginase isoforms have also been implicated in AD pathology. Detection of a rare ARG2 allele [rs742869] was associated with an increased risk of AD in males and with an earlier AAO overall (Hansmannel et al. 2010). This suggests that mutations in the ARG2 gene may be another primary genetic determinant of AD. Increased total arginase activity detected in the superior frontal gyrus (SFG) and hippocampus (HP) of AD patients compared to controls was largely attributed to increased ARG2 expression (Liu et al. 2014). Moreover, while ARG1 is reported to be distinctly expressed in regions with pronounced β-amyloidosis in an AD mouse model, ARG2 is found primarily in the cytosol of HP cells (Baruh Polis et al. 2018). The potential of ARG2 as a therapeutic target was deliberated by Polis et al. (2018, 2019) through the administration of the ARG2 inhibitor L-norvaline, a non-proteinogenic amino acid (Polis et al. 2018; Polis et al. 2019). L-norvaline was administered to a triple transgenic AD mouse, exhibiting synaptic deficiency and both Aβ plaque and NTT pathology (Polis et al. 2018; Polis et al. 2019). The results indicated improved memory acquisition associated with increased dendritic spine density in the HP, as well as a reduction in Aβ plaques in the HP and prefrontal cortex (PFC) (Polis et al. 2018; Polis et al. 2019). L-norvaline reduced microgliosis with evidence of a shift in microglial activation from an active to a resting state (Polis et al. 2018). Astrocytic degeneration was also reversed in brain regions with evident β-amyloidosis, coupled with reduced expression of APP and tumor necrosis factor-α (TNFα) (Polis et al. 2018). L-norvaline was further shown to enhance the expression of proteins related to synaptic plasticity such as vesicular glutamate transporters 1 and 3 and postsynaptic density protein 95, along with several different cellular pathways related to neuroplasticity and oxidative stress protection (Polis et al. 2018; Polis et al. 2019).

Conversely, OTC and arginase deficiencies are reportedly involved in the progression of urea cycle disorders (UCD). UCD has been known to cause neurobehavioral disorders affecting cognitive function and working memory, impacting the PFC (Gropman et al. 2007; Sen et al. 2021). In both 2006 and 2017, two separate case studies were published reporting early and late-onset OTC deficiency in infantile and juvenile male patients, respectively, resulting in fatalities due to hyperammonemia (López-Corella et al. 2017; Thakur et al. 2006). Both cases reported severe brain swelling as well as the presence of Alzheimer's type II astrocytes, immune cell recruitment,

and neuronal degeneration (López-Corella et al. 2017; Thakur et al. 2006).

Alterations in the activity and expression of NOS isoforms (nNOS, eNOS, and iNOS) and corresponding NO levels have been widely reported in AD pathology; however, published data on NOS and NO levels in AD are highly inconsistent (Balez and Ooi 2016; Benzing and Mufson 1995; Dorheim et al. 1994; Gargiulo et al. 2000; Hyman et al. 1992; Norris et al. 1996; Tao et al. 1999; Thorns et al. 1998; Tohgi et al. 1998; Yew et al. 1999). Regional brain studies of AD cases compared to age-matched control cases found a significant decrease in total NOS activity and protein expression levels accounting for nNOS and eNOs in the SFG, HP, and cerebellum (CB) (Liu et al. 2014, 2011). iNOS activity was undetectable in any of the brain regions of AD patients investigated, although iNOS protein expression was significantly decreased in the SFG with markedly intense iNOS-immunoreactive neurons (Liu et al. 2014). These results coincide with studies examining total NOS activity in AD-conditioned rats that also reported decreased total NOS activity accounting for only eNOS and not nNOS in the HP and no detectable iNOS (Liu et al. 2011). However, mRNA expression of iNOS has been reported to increase post-Aβ injection at 2 h to 5 days in mice HP and at 24 h post-injection in mice microglia co-treated with interferon gamma (IFNγ) (Alkam et al. 2008; McIntosh et al. 2019). Moreover, eNOS capillary expression has been shown to be attenuated by Aβ plaques and NTT found in the AD brain (Jeynes and Provias 2009; Provias and Jeynes 2008). Importantly, NO is known to convey both neuroprotective and neurotoxic effects as part of its role as an immunotoxin as well as an immunomodulator (Wink et al. 2011). eNOS-derived NO has been shown to provide a neuroprotective effect by directly regulating and preventing the increase of Aβ production (Malinski 2007). Conversely, reactive nitrogen species (RNS) and excessive levels of NO, particularly from proinflammatory microglial cells, have been shown to have a neurotoxic effect resulting in neurodegeneration (Boje and Arora 1992; Malinski 2007). Additionally, Aβ triggered NO production can cause mitochondrial fission leading to synaptic loss and neuronal damage (Cho et al. 2009). Although studies reporting NOS and NO levels detected in AD are relatively varied in their outcomes, they still indicate a pronounced effect on NO pathways in AD, which are known to be vital for vascular regulation, immune modulation, and neuronal function in the brain.

MAOB has been shown to be upregulated in AD patients, and evidence points towards its potential as a therapeutic target (Mahajan et al. 2020; Riederer et al. 2004; Saura et al. 1994; Thomas 2000). Increased expression of MAOB has been reported in the PFC of AD brains, with a 16% increase compared to controls (Kennedy et al. 2003). Elevated levels of MAOB were identified in astrocytes and neurons obtained from the frontal cortex (FC), entorhinal cortex (ENT), and HP of postmortem AD patients (Schedin-Weiss et al. 2017). In addition, it has been reported that MAOB is a γ-secretase-associated protein, as increased levels of MAOB correlate to higher intraneuronal Aβ levels (Schedin-Weiss et al. 2017). Overexpression of MAOB increased Aβ production, and gene silencing of *MAOB* significantly reduced levels of intraneuronal Aβ (Schedin-Weiss et al. 2017). Due to the clear link between MAOB and Aβ pathology, irreversible MAOB inhibitors such as Rasagiline and L-Deprenyl have been studied and shown to have neuroprotective effects in AD cases (Jossan et al. 1991; Mangoni et al. 2008; Song et al. 2021; Tariot et al. 1987). However, the undesirable side effects and unsustainable long-term activity of irreversible MAOB inhibitors make them unsuitable for AD treatment (Wilcock et al. 2002). Recently, a reversible MAOB inhibitor, KDS2010, has shown promising results in astrocytes of AD mouse models containing the APP and PS1 gene mutations [APP/PS1] (Park et al. 2019). KDS2010 was able to restore learning and memory in AD mice and maintain lower levels of GABA production compared to L-Deprenyl, for short-term and long-term treatment (Park et al. 2019).

Studies employing different cellular models with mutations in the expression of APP have investigated the effect of Aβ pathology on the expression of various urea cycle enzymes in vitro. In two separate studies published by Jęśko et al. (2016, 2018), expression and activity levels of urea cycle enzymes were studied using rat pheochromocytoma cells (PC12) to model neuronal cells overexpressing APP [APPwt] or additionally bearing the double 'Swedish' APP mutation [APPsw, K670M/N671L] to intensify Aβ production and AD-like pathology leading to dominant early AD onset (Jęśko et al. 2018, 2016). Both models revealed a significant reduction in the gene expression and activity levels of ARG1 and ARG2 as well as expression of ASS, whereas ASL appeared upregulated in APPsw cells alone (Jęśko et al. 2018, 2016). iNOS expression was not detectable in either model, while nNOS mRNA was elevated in both cells (Jęśko et al. 2018, 2016). Elevated levels of micro RNA 9 and 128a detected in clinical AD brain tissue samples are believed to modulate the expression of ASS and NOS through interactions via the 3′-untranslated regions (UTRs) of ASS and NOS messenger RNA (mRNA), respectively (Jęśko et al. 2016). Enzyme expression and activity levels of ASS and NOS observed in APPsw cells were found to correspond with changes observed in the HP of AD patients (Jęśko et al. 2018). Lastly, the expression of ODC was found to be downregulated in APPwt cells (Jęśko et al. 2016). These findings showcasing the enzymatic urea cycle profiles of AD-like neurons differ from other studies documenting urea cycle enzyme activity and expression levels, especially ODC, in reactive AD astrocytes and activated AD microglia.

Current research examining the role of urea cycle enzymes in AD pathology has focused on investigating the activity of ODC in AD-reactive astrocytes and its potential therapeutic impact. Ju et al. (2022) demonstrated a shift in urea metabolism in primary cortical astrocytes prepared from 1-day postnatal C57BL/6 mice pre- and post-treatment with Aβ oligomers, mimicking AD-like conditions (Ju et al. 2022). Analysis of urea cycle mRNA levels in untreated primary astrocyte cultures using qt-PCR revealed that under normal conditions, astrocytes have a specialized, linear urea "cycle" that follows the ARG1 to ODC pathway for putrescine production due to lower expression levels of CPS1, OTC, ADC, and AGMAT (Ju et al. 2022). In astrocytes treated with Aβ oligomers, elevated mRNA expression levels of CSP1, OTC, ASL, ARG1, and ODC indicate a shift of urea metabolism into the conventional urea cycle in order to detoxify accumulated ammonia and aspartate in astrocytic cells due to Aβ uptake and autophagic

degradation (Ju et al. 2022). In addition, the level of GS was significantly lower in astrocytes under AD-like conditions (Ju et al. 2022). Interestingly, this relates to previously published evidence of increased GS levels in lumbar cerebrospinal fluid (CSF) of AD patients (Tumani et al. 1999). Observations seen in primary cortical astrocytes treated with Aβ oligomers were further confirmed by the detection of increased OTC, ARG1, and ODC in the HP of AD patients, resulting in increased putrescine and GABA production, consequentially leading to memory impairment characteristic of AD (Ju et al. 2022). The condition is further intensified by excessive toxic ammonia produced via MAOB activity, recycling ammonia back into the urea cycle and amplifying the process. Following the discovery of the toxic urea cycle pathway present in AD-reactive astrocytes, ODC, ARG1, and MAOB were identified as key enzymes for inhibition to shift the toxic urea cycle (Ju et al. 2022). Enzyme inhibition of MAOB (by KDS2010, 100 nM), ARG1 (by 2[S]-amino-6-boronohexanoic acid—hydrochloride, 10 μM), and ODC (by difluoromethylornithine [DFMO], 50 μM) were all found to significantly attenuate toxic ammonia levels and prevent further activity of the toxic urea cycle in AD-reactive astrocytes (Ju et al. 2022). Gene silencing of *ODC* resulted in a significant 40% reduction in Aβ plaque formation (Ju et al. 2022). However, gene silencing of *ARG1* lacked any significant Aβ plaque reduction, indicating ODC as a more viable therapeutic target for inhibition (Ju et al. 2022). Bhalla and Lee (2024) extended this work on ODC inhibition, showing that long-term *ODC* gene silencing in astrocytes of AD APP/PS1 mouse models reduces Aβ plaques in the HP by 60% at 8 weeks post *ODC* gene knockdown (KD) and complete Aβ plaque clearance 12 weeks post *ODC* KD (Bhalla and Lee 2024). Examination of cognitive factors such as pro-brain-derived neurotrophic factor (proBDNF) levels, a growth factor indicating enhanced cognition and neuroplasticity, revealed elevated proBDNF levels in HP astrocytes of APP/PS1 mice due to *ODC* KD (Woo et al. 2018). Next generation sequencing (NGS) and gene ontology (GO) analysis of RNA isolated from Aβ-treated primary astrocyte cultures with and without ODC inhibitor DFMO (50 μM) for 5 days revealed a drastic change in the transcriptome of AD-like reactive astrocytes treated with DFMO (Bhalla and Lee 2024). NGS analysis indicated a significant upregulation of key neurodevelopmental and neuroprotective genes including Brain Derived Neurotrophic Factor (*Bdnf*), transcription regulatory genes Nrf2 (*Nfe2l2*) and *Gtf2a1* and to a lesser extent, Aquaporin 4 (*Aqp4*) indicating improved learning and memory support (Woo et al. 2018). Alternatively, astrocyte reactivity markers (*Gfap* and *Lcn2*), apoptosis and autophagy-associated genes (*Gabarap*, *Bax*, and *Foxo4*), and complement C1q C Chain (*C1QC*) genes associated with synaptic pruning were all found to be downregulated (Bhalla and Lee 2024). GO analysis revealed upregulation of histone modification-associated processes and post-translational protein modifications as well as downregulation of proteolysis-associated processes and apoptotic pathways affecting neurons in AD-reactive astrocytes treated with DFMO (Bhalla and Lee 2024). This further showcases the significant transcriptional changes in AD-reactive astrocytes after long-term ODC inhibition. Both studies by Ju et al. (2022) and Bhalla and Lee (2024) showcase the key capabilities of ODC inhibition to transform reactive astrocytes in AD into Aβ-detoxifying astrocytes (short-term ODC inhibition) and Aβ clearing neuro-supportive active astrocytes (long term ODC inhibition) (Bhalla and Lee 2024; Ju et al. 2022).

Activated AD microglia have been reported to have altered expression patterns of ARG1 and ODC; however, differing opinions are present regarding their therapeutic role (Chen et al. 2023; Cheng et al. 2019; Kan et al. 2015). To investigate the role of urea cycle enzymes ARG1 and ODC in AD microglia, Kan et al. (2015) utilized a transgenic CVN-AD mouse model which is deficient in mouse nitric oxide synthase 2, inducible (*mNOS2*) and contains the Swedish vasculotropic Dutch/Iowa mutant APP (APPSwDI, K670N/M671L, and E693Q/D694N) (Kan et al. 2015). Upregulation of genes associated with immune suppression, such as *ARG1*, inflammatory zone 1 (*Fizz1*), and interleukin 1 receptor antagonist (*Il1rn*) in whole brain lysates of CVN-AD mice prior to significant neuronal death (before ~36 weeks) revealed early immune suppression as a key stage of AD disease progression (Kan et al. 2015). These genes were only transiently upregulated between 6 and 12 weeks, exhibiting similar levels to wildtype (WT) C57BL/6 control mice by 24 weeks (Kan et al. 2015). Using immunohistochemical and flow cytometric analyses, increased levels of CD45+ reactive microglia were detected in CVN-AD mice and were distinguished from reactive microglia from NOS2-deficient and WT mice via the progressive expression of CD11c, showing progressive cellular inflammation specifically in areas of Aβ deposition (Kan et al. 2015). Analysis of mRNA expressed in CD11c+ microglia exhibited an immunosuppressive phenotype, with genes associated with immune suppression and increased arginase activity being upregulated (such as *Gp49a*, *Apoe*, and *Pdcd1*) and genes from proinflammatory pathways and negative immune suppression regulators being downregulated (such as *Apobec3*, *Ifngr*, *Siglech*, and *Klf6*) (Kan et al. 2015). Detection of increased levels of extracellular ARG1 displayed a temporal and spatial correlation with that of Aβ and CD11c expression, suggesting CD11c+ microglia to be the likely source of ARG1 production and linking ARG1 expression to Aβ deposition (Kan et al. 2015). Analysis of the global arginine bioavailability ratio (GABR), the ratio of arginine to ornithine and citrulline, in CVN-AD mice revealed significantly reduced GABR compared to controls, indicating increased arginine catabolism and lower bioavailable brain arginine levels in CVN-AD mice (Kan et al. 2015). Disruption of the arginine utilization pathway using ODC inhibitor DFMO rescued CVN-AD mice from AD-like pathology via reduction of Aβ, significant improvement of memory acquisition and recall, and significant decrease of CD11c expression and CD11c+ cells (Kan et al. 2015). This suggests an additional therapeutic mode of ODC inhibitors by acting on CD11c+ microglia present in AD models. Alternatively, in a separate study, Cheng et al. (2019) reported that Aβ suppresses ODC protein expression in BV2 microglia by stimulating a +1 ribosomal frameshift in the mRNA of antizyme (an intrinsic ODC inhibitor) (Cheng et al. 2019). Aβ-mediated ODC suppression also resulted in reduced cellular polyamine levels (putrescine, spermidine, spermine), a decrease in *ARG1* mRNA expression, and the acquisition of a proinflammatory M1-like phenotype in the BV2 microglia (Cheng et al. 2019). Transfection of an ODC overexpression vector into BV2 microglia was able to reverse Aβ-induced polyamine reduction and allowed BV2 microglia

to shift towards an anti-inflammatory M2-like phenotype via the detection of CD206+ cells, and reduced levels of pro-inflammatory cytokines Intereukin-1β (1L-1β), Interleukin-6 (IL-6), and TNFα (Cheng et al. 2019). Finally, it was shown that microglial ODC protein expression was suppressed by intracerebroventricular injection of Aβ in Wistar rats, proposing that ODC suppression by Aβ via post-translational antizyme mRNA modifications may be mechanistically involved in activating M1-like microglia as seen in AD pathology (Cheng et al. 2019). The discourse between the role of ARG1 and ODC in AD microglia brings to attention their involvement in microglial activation and the need to further study the role of ARG1, ODC, and possibly other urea cycle enzymes and proteins in more models of M1/M2 microglial activation mediated by Aβ.

3.2 | Alterations in Urea Metabolite Levels in the AD Brain

Along with altered urea cycle enzyme expression in AD, altered levels of urea cycle metabolites found in the AD brain provide another window into the involvement of the urea cycle in AD pathology. Several large-scale metabolomic studies on AD patients have identified altered levels of urea cycle metabolites, amino acids, and relevant neurotransmitters indicating metabolomic dysregulation in the AD brain. In a study conducted by Xu et al. (2016), gas-chromatography mass-spectrometry (GC–MS) based metabolite profiling was used to identify altered metabolites in tissue samples from 7 distinct brain regions of human AD and control patients (Xu et al. 2016). These brain regions included severely affected regions in AD such as the HP, ENT, and middle-temporal gyrus (MTG) as well as moderately affected regions such as the sensory cortex (SCX), motor cortex (MCX), and cingulate gyrus (CG) (Xu et al. 2016). CB samples were collected as a control since this region is relatively spared in AD (Braak and Braak 1991; Bradley et al. 2002). Levels of urea were considerably elevated in all regions of AD brains, whereas urea cycle metabolites ornithine and *N*-acetyl glutamate were reduced (Xu et al. 2016). In addition, amino acids presented as the largest metabolite group to be altered in AD brains, with more significant alterations corresponding with more severely affected brain regions (Xu et al. 2016). Tryptophan showed the most significant increase across various brain regions of AD patients, including the HP, ENT, MTG, MCX, and CB, corresponding with a 2.5, 2.2, 4.7, 3.2, and 4.0-fold increase, respectively, followed by phenylalanine (2-fold increase in the MTG and CB) and cystine (2.0 and 1.4-fold increase in the HP and CG) (Xu et al. 2016). Serine showed the most significant decrease across various brain regions of AD patients, including the HP, ENT, and CB, corresponding with a 0.7, 0.6, and 0.7-fold decrease, respectively, followed by proline (0.5 and 0.4-decrease in the HP and ENT), aspartic acid (0.6-fold decrease in the HP and ENT) and glycine (0.7 and 0.8-fold decrease in the ENT and MTG) (Xu et al. 2016). As for neurotransmitters, GABA was overall reduced, especially in the ENT, MTG, and SCX (Xu et al. 2016). This finding is in contrast to the previously presented finding by Ju et al. (2022) detecting elevated GABA levels in the HP of AD patients (Ju et al. 2022). Since different brain regions serve tailored functions, their cellular makeup greatly determines the levels of detectable compounds, resulting in these varying observations that are not contradictory but are a result of the cellular topography predominantly employed within each brain region.

In a similar study, Mahajan et al. (2020) performed quantitative and targeted metabolomic assays using capillary electrophoresis-mass spectrometry (CE-MS) on brain samples from AD, asymptomatic AD, and control patients (Mahajan et al. 2020). Results from the two primary regions of interest in AD brains, the inferior temporal gyrus (ITG) and MTG, revealed significant reductions in concentrations of metabolites involved in the urea cycle, including *N*-acetyl glutamate and GABA, which were coupled with greater neuritic plaque and neurofibrillary burden (Mahajan et al. 2020). Furthermore, quantitative transcriptomic analysis identified reduced mRNA expression of key enzymes ARG2, ASL, ASS, OAT, ODC, and GLS2 in the HP of AD brains compared to controls (Mahajan et al. 2020). As previously stated, neuronal APPwt cells and activated microglia reported a down-regulation in ODC expression correlating with quantitative transcriptome analysis of brain samples from AD patients, whereas reactive AD astrocytes revealed elevated levels of ODC (Cheng et al. 2019; Jęśko et al. 2016; Ju et al. 2022; Mahajan et al. 2020). These discrepancies in varying levels of ODC among various cell types again illustrate the cell-specific metabolic profiles that significantly impact findings from regional brain studies based on the cellular makeup of specific brain regions.

A subsequent investigation by Eldridge et al. (2022) interrogated data from structural magnetic resonance imaging (sMRI) of brains and metabolomic analysis of CSF from patients with mild cognitive impairment and early AD. This multiomics analysis indicated 7 distinctive clusters corresponding to 7 different brain regions: 3 distinct clusters in the HP/parahippocampal gyrus, 1 in the thalamus, 1 in the posterior thalamus, 1 in the parietal cortex, and 1 distinct cluster in the occipital lobe (Eldridge et al. 2022). Metabolic pathway analysis further highlighted dysregulated urea cycle metabolism, with altered amino acid metabolism associated with each region, most notably a cluster in the left HP/parahippocampal gyrus (Eldridge et al. 2022).

Coupled with the metabolomic studies outlined above, targeted research directly measuring urea cycle metabolites in AD brains and animal models has helped construct a metabolic profile of the urea cycle in AD pathology. Arginine levels were found to be significantly elevated in the SFG of AD patients compared to controls, while citrulline remained unchanged (Liu et al. 2014). In an AD rat model, arginine was not altered in Aβ-treated rats (Liu et al. 2011). Similarly, APPwt PC12 cells did not show altered levels of arginine, whereas APPsw PC12 cells were found to have elevated levels of arginine and citrulline, as well as ornithine (Jęśko et al. 2018, 2016). In a novel study tracing metabolic products of arginine over time using uniformly labeled arginine ($^{13}C_6{}^{15}N_4$) and capillary electrophoresis coupled to high-resolution mass spectrometry (CE-HR MS) for isotope tracing in a sporadic AD mouse model (APOE4/huNOS2), researchers discovered differences in arginine pathway activity between female and male mice (Adams et al. 2021). Results revealed higher incorporation of arginine metabolites in female mice, with female mice incorporating significantly more citrulline and arginine over the dosing time course compared to male mice (Adams et al. 2021). This suggests increased arginine metabolic activity

in females compared to males and describes a critical technique in studying urea cycle metabolites in vivo. Ornithine levels were also found to be lower in the SFG, HP, and CB regions of AD patients and the HP of AD-conditioned mice (Liu et al. 2014, 2011). On the other hand, agmatine levels differed in the SFG and CB regions but not in the HP and were found to be lower in 80-year-old AD and control groups compared to 60-year-old AD and control groups (Liu et al. 2014). This coincides with other studies that confirmed reduced agmatine levels in Aβ peptide-injected mice; however, reduced agmatine levels were found in the HP and PFC, not the SFG and CB (Kotagale et al. 2020; Liu et al. 2011). Interestingly, studies have reported on the protective effect of intra-hippocampal agmatine treatment on learning and memory impairment in AD-conditioned mice, promoting agmatine as a possible therapeutic candidate (Kotagale et al. 2020; Moosavi et al. 2014).

Glutamate and putrescine, both of which are produced from enzymatic reactions of ornithine with OAT and ODC, respectively, were found to be elevated in the HP of AD rats and AD-reactive astrocytes (Ju et al. 2022; Liu et al. 2011). Conversely, glutamate was significantly reduced in the HP and higher in the CB of 80-year-old AD patients compared to age-matched controls, whereas glutamine, the end product of glutamate reacting with GS and intracellular ammonia, was found to be significantly elevated in the SFG and CB of AD cases (Liu et al. 2014). Lower levels of putrescine were also detected in the SFG, HP, and CB of 80-year-old control subjects, compared with the 60-year-old cohort. Moreover, putrescine was further reduced in 80-year-old AD patients compared to age-matched controls (Liu et al. 2014). This highlights an AD-associated reduction in putrescine that is independent of age-related changes. In AD-reactive astrocytes, putrescine and aspartate levels were found to be elevated, whereas glutamate levels were unchanged compared to controls (Ju et al. 2022).

Early research examining amino acid neurotransmitters in AD and control brains post-mortem reported decreased GABA levels in AD brains compared to control (Ellison et al. 1986). In more recent regional brain studies of the AD brain, no significant changes were detected in the level of neurotransmitter GABA in any of the regions examined in either AD or control groups (Liu et al. 2014). In addition, the reduction in GABAergic neurons in the parafacial zone of AD rat models has been linked to sleep disturbances associated with AD (Shokri-Kojori et al. 2018; Song et al. 2018). In a mouse model of AD-conditioned reactive astrocytes, GABA was found to be elevated and linked to impaired memory function associated with AD (Jo et al. 2014; Ju et al. 2022).

3.3 | Alterations in Urea Transporter B (UT-B) Levels and Expression in the AD Brain

Urea transporter B (UT-B) is a membrane channel protein that facilitates the removal of accumulated urea within the cell (Yu et al. 2019). Expression of UT-B is known to be altered to accommodate the removal of excess urea (Inoue et al. 2005). In HD, neurodegeneration is accompanied by an upregulation in the expression of UT-B, along with elevated urea concentration in the brain (Handley et al. 2017). GWAS studies comparing gene expression profiles of APPsw/PS1 mouse models and genes expressed in human AD have revealed a similar outcome, with significant upregulation of the UT-B gene, *SLC14A1*, in the PFC, suggesting a role in mediating the pathology of β-amyloidosis (Wirz et al. 2013). This suggestion is further evidenced by a study published by Recabarren and Alarcon (2017) reporting alterations in the *SLC14A1* gene and association with neurodegenerative diseases, Amyotrophic lateral sclerosis (ALS), PD, and AD (Recabarren and Alarcon 2017). Gene alterations affecting the UT-B protein may impact its interaction with Caveolin-1, a transmembrane scaffolding protein also involved in ALS, PD, and AD and implicated in Aβ plaque formation and cellular apoptosis (Recabarren and Alarcon 2017). Additionally, Ju et al. (2022) reported upregulation and expression of UT-B in AD-reactive astrocytes (Ju et al. 2022). Huang et al. (2021) reported upregulation of UT-B in traumatic brain edema (TBE) rat models, regulating osmotic pressure in the brain (Huang et al. 2021). In addition, the number of neurons expressing UT-B increased in rats post-TBE, reaching a peak at 24 h post-trauma and then began decreasing, with the level of immunoreactivity experienced in the brain correlating to UT-B positive neurons (Huang et al. 2021). UT-B null mouse models revealed high urea concentrations in the PFC inhibiting mTORC1-S6K-dependent dendritic protein synthesis and impairing long-term potentiation typically associated with learning and memory (Li et al. 2012; Martinez and Derrick 1996). Additionally, neuronal morphology was altered, indicating nuclear dissolution and membrane degeneration. Ultimately, UT-B null mouse models revealed neurological impacts that were linked to depression-like behavior. In a study published by Jones et al. (2021), the expression of UT-B in BV2 microglial and N2a neuronal cell models was investigated following lipopolysaccharide (LPS)-induced inflammation and whether inhibition of UT-B would modulate this inflammatory response (Jones et al. 2021). UT-B protein expression was found to be evident in both microglial and neuronal cells; however, gene expression of *SLC14A1* was only detected by end-point PCR in microglial cells (Jones et al. 2021). Inhibition of UT-B was found to alleviate LPS-induced cytokine release from microglial and neuronal cells. Interestingly, however, while UT-B inhibition also reduced LPS-induced production of NO from microglia, this was in fact enhanced in neuronal cells. These findings highlight a key role for UT-B in regulating the brain's response to inflammatory challenges (Jones et al. 2021). More recently, Al-Thani et al. (2024) assessed the involvement of urea accumulation and urea transportation via UT-B inhibition in regulating neurotoxicity and neuroinflammation associated with AD in SH-SY5Y neurons and BV2 microglia, respectively (Al-Thani et al. 2024). Neither increased extracellular urea nor UT-B inhibition had any significant impact on the cell viability or cytotoxicity of SH-SY5Y neurons exposed to oxidative stressors tert-butyl hydroperoxide (t-BHP) and 6-hydroxydopamine (6-OHDA) simulating neurotoxicity (Al-Thani et al. 2024). However, in BV2 microglia stimulated by toll-like receptor (TLR) 2 agonist lipoteichoic acid (LTA), UT-B inhibition had a significant anti-inflammatory effect by reducing the formation of NO and the expression of TNFα and CCL2 along with the reduction of extracellular urea and upregulation of UT-B expression (Al-Thani et al. 2024). Exogenous urea was also shown to mediate the inflammatory profile of BV2 cells but had less of an impact on UT-B expression (Al-Thani et al. 2024). In addition, the metabolic profile of LTA-stimulated BV2 microglia treated

with UT-B inhibitor showed an upregulation of genes associated with both glycolysis and fatty acid oxidation (Al-Thani et al. 2024). These findings further exemplify the role of UT-B as a neuroinflammatory regulatory agent and provide an incentive for further research into the potential of UT-B as a therapeutic target.

4 | Future Directions: Therapeutic Targets and Other Potential Pathways

Based on the extensive research conducted on the role of the urea cycle in AD, several key enzymes, potential pathways, and proteins have been identified, but further research is required to clarify their involvement within the precise urea cycle profile related to AD pathology. ARG2, MAOB, and ODC have all presented as viable target enzymes for inhibition and have yielded promising results (Bhalla and Lee 2024; Ju et al. 2022; Kan et al. 2015; Park et al. 2019; Polis et al. 2018; Polis et al. 2019). However, DFMO presents as an aggressive ODC inhibitor with undesirable side effects; therefore, a more suitable ODC inhibitor is required for therapeutic purposes (Ju et al. 2022). Agmatine has the ability to naturally inhibit ODC by producing an antizyme which inhibits ODC and downregulates putrescine production, presenting itself as a natural supplement to ODC inhibition (Satriano 2003). Potential pathways of interest include the autophagy system involving the uptake of Aβ peptides in glial cells and reactive astrocytes, as this system has been shown to mediate the activation of the urea cycle in primary astrocytes exposed to Aβ, with inhibition of ODC using DFMO downregulating autophagy-associated genes in this AD-astrocytic model (Ju et al. 2022; Ries and Sastre 2016). This pathway presents as a promising mode of intervention for clinical trials in the near future, as the promotion of autophagy in AD patients can be induced using non-invasive methods such as diet via intermittent fasting (Shabkhizan et al. 2023). In addition, protein-restricted diets and ammonia-lowering scavengers could also present as promising therapeutic options related to urea cycle dysfunction in AD, as AD mouse models under protein-restricted diets showed improved cognition and protective metabolic outcomes (Cummings et al. 2018; Parrella et al. 2013), and ammonia removal by metabolic scavengers has been shown to prevent and

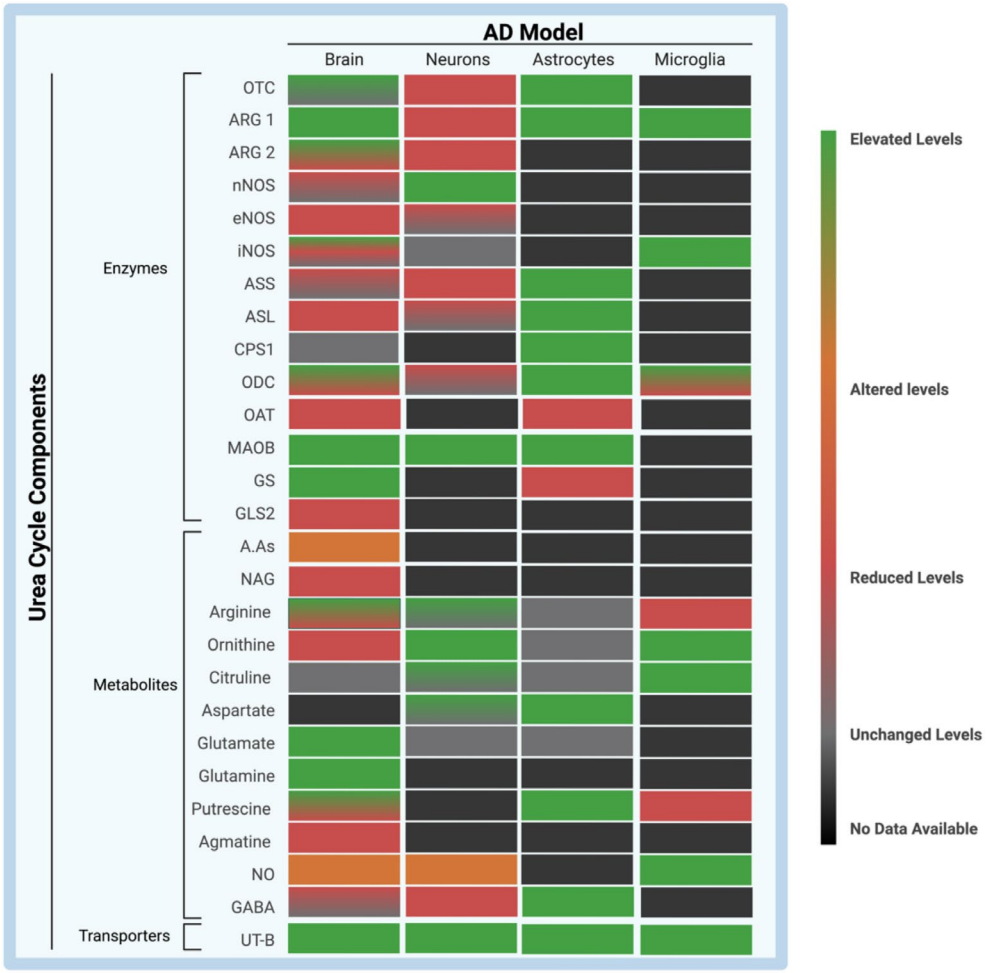

FIGURE 2 | Summary figure of Urea cycle metabolite, enzyme, and transporter levels in AD models. Created with Biorender.com. Alzheimer's Disease (AD); amino acids (AAs); arginase 1 (ARG1); arginase 2 (ARG2); argininosuccinate synthetase (ASS); argininosuccinate-lyase (ASL); carbamoyl phosphate synthase 1 (CPS1); endothelial nitrogen oxide synthase (eNOS); glutamine synthase (GS); glutamine transferase 2 (GLS2); inducible nitrogen oxide synthase (iNOS); monoamine oxidase B (MAOB); *N*-acetyl glutamate (NAG); neuronal nitrogen oxide synthase (nNOS); nitric oxide (NO); ornithine aminotransferase (OAT); ornithine decarboxylase (ODC); ornithine transcarboxylase (OTC); urea transporter B (UT-B); γ-aminobutyric acid (GABA).

treat other neurological conditions, such as hepatic encephalopathy (Butterworth 2021). Finally, mutations in UT-B have been implicated in AD progression and hinder the ability of cells to promote urea clearance (Recabarren and Alarcon 2017). Additionally, inhibition of UT-B has been shown to dampen inflammatory actions of microglia and neurons (Al-Thani et al. 2024; Jones et al. 2021). These findings suggest a possible therapeutic avenue of UT-B in which upregulation may facilitate accumulated urea clearance and proinflammatory activity under AD conditions. However, further studies on microglial and neuronal cellular models are required to understand the mechanism of action behind UT-B inhibition with regard to the urea cycle, urea accumulation, and amyloidosis.

Major discrepancies between levels of urea cycle metabolites and enzyme expressions occur among the studies mentioned in this review. This is largely due to the nature of the AD tissue samples (brain regions, age), specific animal models investigated, or cell types used in vitro (neurons, microglia, astrocytes). Therefore, to fully explore the therapeutic potential of targeting the urea pathway, it is important to better understand the cell- and region-specific alterations in the expression of urea cycle metabolites, enzymes, and transporters within the brain as different brain regions consist of varying cellular landscapes.

In conclusion, this review has covered the expansive body of literature surrounding the work of researchers deciphering the role of the urea cycle in AD pathology through the study of urea cycle metabolites, enzymes, and transporters in the AD Brain. Urea cycle enzymes of interest in AD pathology include OTC, NOS isoforms, ARG1, ARG2, MAOB, and ODC, which all present as promising therapeutic targets (Figure 2). Urea metabolites indicated in AD pathology differ in concentrations across brain regions and cells, with Agmatine presenting as a key supplementary treatment for ODC inhibition (Figure 2). Finally, the role of UT-B as a clearance modulator presents this protein as a key target for research into the role of the urea cycle in the AD brain (Figure 2). In the future, these key enzymes, pathways, and proteins relating to the urea cycle in AD should be further investigated to uncover their therapeutic potential and to better understand the cell-specific urea cycle profiles within AD.

Author Contributions

Najlaa A. Al-Thani: conceptualization, investigation, writing – original draft, writing – review and editing. **Gavin S. Stewart:** conceptualization, writing – review and editing, supervision. **Derek A. Costello:** conceptualization, writing – review and editing, supervision.

Consent

All authors consent to the publication of this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Peer Review

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