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Review

Role of the Gut Bacteria-Derived Metabolite Phenylacetylglutamine in Health and Diseases

Naveen Kumar Krishnamoorthy, Manjunath Kalyan, Tousif Ahmed Hediyal, Nikhilesh Anand, Pavan Heggadadevanakote Kendaganna, Gurudutt Pendyala, Sowmya V. Yelamanchili, Jian Yang, Saravana Babu Chidambaram, Meena Kishore Sakharkar,* and Arehally M. Mahalakshmi*

Cite This: *ACS Omega* 2024, 9, 3164−3172

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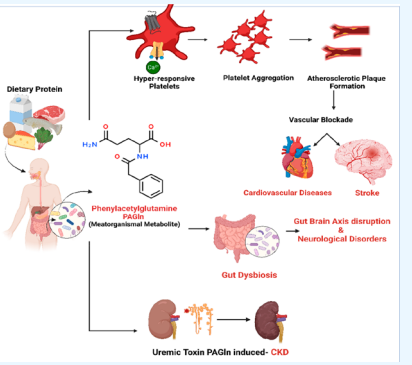
ABSTRACT: Over the past few decades, it has been well established that gut microbiota-derived metabolites can disrupt gut function, thus resulting in an array of diseases. Notably, phenylacetylglutamine (PAGln), a bacterial derived metabolite, has recently gained attention due to its role in the initiation and progression of cardiovascular and cerebrovascular diseases. This meta-organismal metabolite PAGln is a byproduct of amino acid acetylation of its precursor phenylacetic acid (PAA) from a range of dietary sources like egg, meat, dairy products, etc. The microbiota-dependent metabolism of phenylalanine produces PAA, which is a crucial intermediate that is catalyzed by diverse microbial catalytic pathways. PAA conjugates with glutamine and glycine in the liver and kidney to predominantly form phenylacetylglutamine in humans and phenylacetylglycine in rodents. PAGln is associated with thrombosis as it enhances platelet activation mediated through the GPCRs receptors α2A, α2B, and β2 ADRs, thereby aggravating the pathological conditions. Clinical evidence suggests that elevated levels of PAGln are associated with pathology of cardiovascular, cerebrovascular, and neurological diseases. This Review further consolidates the microbial/biochemical synthesis of PAGln and discusses its role in the above pathophysiologies.



INTRODUCTION

Phenylacetylglutamine (PAGln) is a highly nitrogenous and meta-organismal metabolite produced by the conjugation of phenylacetate (from microbial-dependent production) with glutamine in the liver and kidneys. It is a common constituent in the urine of human, cat, dog, sheep, and monkey, among several other mammals. *Christensenellaceae*, *Ruminococcaceae*, and *Lachnospiraceae*, *Proteus mirabilis*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae Bacteroidetes*,[1,2] *Firmicutes*,[3] *Proteobacteria*, and *Staphylococcus aureus*[4] are some of the major microorganisms involved in production of phenylacetylglutamine. Microbial transformation of dietary phenylalanine into phenylacetic acid occurs in the gut and is catalyzed by phenylpyruvate ferredoxin-oxidoreductase (PPFOR) and phenylpyruvate decarboxylase (PPDC) followed by conjugation in the liver to produce PAGln.[4]

PAGln production by gut microbiota requires phenylalanine as a precursor. Phenylalanine is a naturally occurring amino acid found in dietary sources like egg, milk, and meat and is also sold as a dietary supplement. Since PAGln is present in organs like the kidney and liver and in systemic circulation, the increased levels of this metabolite have been linked to the initiation and progression of diseases involving renal, hepatic, cardiovascular, and cerebrovascular systems. Translocation of the microbes or the compound PAGln from the gut to other organs has also been linked to cerebrovascular, cardiovascular, and liver damage, chronic kidney disease, and inflammation-activated pathways.[5−8] Also, the leakage of PAGln from the gut to systemic circulation, in the case of gut dysbiosis, has been reported to account for a multitude of diseases. These diseases have been suggested to be associated with alterations in the gut−brain axis, gut−heart axis, gut−liver axis, and gut−kidney axis.[5−8] This Review summarizes the impact of elevated levels of PAGln in cardiovascular, cerebrovascular, and neurological diseases.

BIOCHEMICAL AND MICROBIAL SYNTHESIS OF PHENYLACETYLGLUTAMINE

Major sources for phenylacetylglutamine are meat and dietary products. Synthesis of phenylacetylglutamine occurs in two steps. In the first step, the dietary protein phenylalanine (Phe) gets converted into phenylacetic acid (PAA) by gut microbes

Received: October 18, 2023
Revised: December 19, 2023
Accepted: December 21, 2023
Published: January 8, 2024

https://doi.org/10.1021/acsomega.3c08184
ACS Omega 2024, 9, 3164−3172

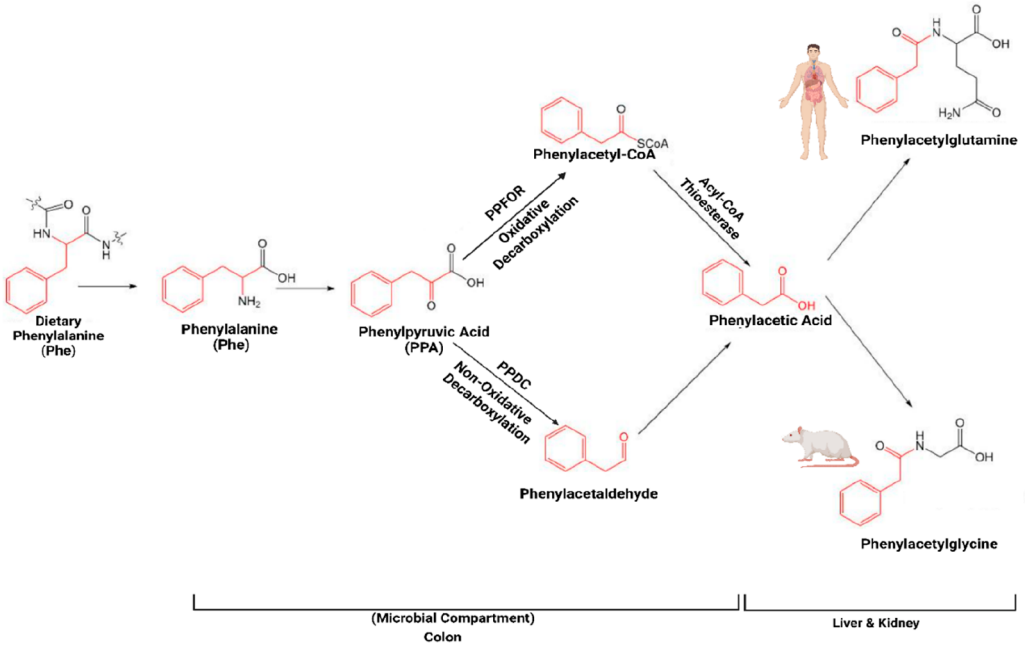


Figure 1. Biosynthesis of phenylacetylglutamine and phenylacetylglycine. PPFOR, phenylpyruvate ferredoxin oxidoreductase; PPDC, phenylpyruvate decarboxylase.

in the colon through two distinct thiamine pyrophosphate (TPP)-dependent pathways.

In the colon, gut bacteria transform the dietary phenylalanine into phenyl pyruvic acid (PPY) by deamination of phenylalanine. This is carried out by numerous microbial enzymes including Phe dehydrogenase (EC: 1.4.1.20)[9] and aromatic amino acid aminotransferase (EC: 2.6.1.57)[10] followed by two microbial pathways catalyzed by phenylpyruvate ferredoxin oxidoreductase (PPFOR) and phenylpyruvate decarboxylase (PPDC),[4] respectively. PPFOR transforms the PPY to an intermediate phenylacetyl-CoA through oxidative decarboxylation. Phenylacetyl-CoA further transforms into PAA by acyl-CoA thioesterase.[11] Nonoxidative decarboxylation of PAA is carried out by PPDC to transform PPY into phenylacetaldehyde by phenylacetaldehyde dehydrogenase or phenylacetaldehyde:ferredoxin oxidoreductase.[12,13]

In vivo and in vitro assays suggest that PAA is produced through oxidative and nonoxidative catalytic decarboxylation of PPY. However, the microbial enzymes involved in these processes still remain unclear. Following the microbial generation, PAA enters the portal circulation and reaches hepatic and renal systems. In the liver, it conjugates with glutamine and glycine through amino acid acetylation mediated by the enzyme phenylacetyltransferase and glycine *N*-phenylacetyltransferase to complete the meta-organismal transformation of phenylacetylglutamine and phenylacetylglycine.[14] Meta-organismal production of phenylacetylglutamine is predominantly found in humans whereas phenylacetylglycine is more common in rodents[5,15−17] (Figure 1).

Bacterial isolates from three major phyla: *Bacteroidetes*,[1,2] *Firmicutes*,[3] and *Proteobacteria* are involved in PAA formation in humans and other vertebrates. Studies reported that the microbial porA(ClOSPO_00147) gene is responsible for the conversion of Phe into PAA by the human commensal *Clostridium sporogenes* in the majority of reported instances.[3,5] Also PPFOR is seen as the major contributor for the generation of PAA by *Bacteroides thetaiotaomicron*.[4] The α-ketoisovalerate:ferredoxin oxidoreductase (VOR) (EC 1.2.7.7), a member of the oxoacid:ferredoxin oxidoreductase superfamily, has a structure similar to that of PPFOR which is required for oxidation of PPY to phenylacetyl-CoA in *Hyperthermophilic archaea*.[18−20] In addition to these super/key producers, gene clusters coding for indolepyruvate:ferredoxin oxidoreductase (IOR) (EC:1.2.7.8)[21] which oxidatively decarboxylates PPY into phenylacetyl-CoA have been seen in archaea.[22] It is also reported that inhibiting the PPFOR/VOR is not enough to inhibit the gut microbial PAA production,[4] indicating that there are other pathways/means to generate PAA in the gut. Interestingly, microorganisms like *Proteus mirabilis*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* are also reported to produce PAA. These bacteria do not have a PorA protein homologue or 2-oxoacid:ferredoxin oxidoreductase but have a PPDC homologue. Here it is important to report that a search for the PPFOR gene in the reference database the Human Genome Project (HMP) revealed that it is primarily present in obligate anaerobes *Bacteroides* and *Firmicutes* (200 out of 1585 genomes). Besides the *Bacteroides* and *Firmicutes*, the putative VOR homologue is found to be present in the microaerophilic bacterium *Helicobacter pylori* belonging to proteobacteria (359 out of 1585 genomes). PPDC homologues were seen in aerobes and anaerobes of *Proteobacteria*, *Firmicutes*, and *Actinobacteria* (126 of 1585 genomes). They were also identified in *A. baumannii*, *K. pneumoniae*, and *S. aureus*.[4] However, it must be noted that the use of antibiotics is shown to alter gut microbial composition and also lower the phenylacetylglutamine levels.[4]

EXCRETION OF PHENYLACETYLGLUTAMINE

It is well established that kidneys clear numerous solutes from the blood and also protein-bound solutes like PAGln (produced by conjugating with glutamine).[23] PAGln can replace urea in the urea cycle and help excrete nitrogen waste from the body.[24] In the case of liver failure and urea cycle disorders, elevated levels of glutamine are observed. Upon

https://doi.org/10.1021/acsomega.3c08184
ACS Omega 2024, 9, 3164−3172

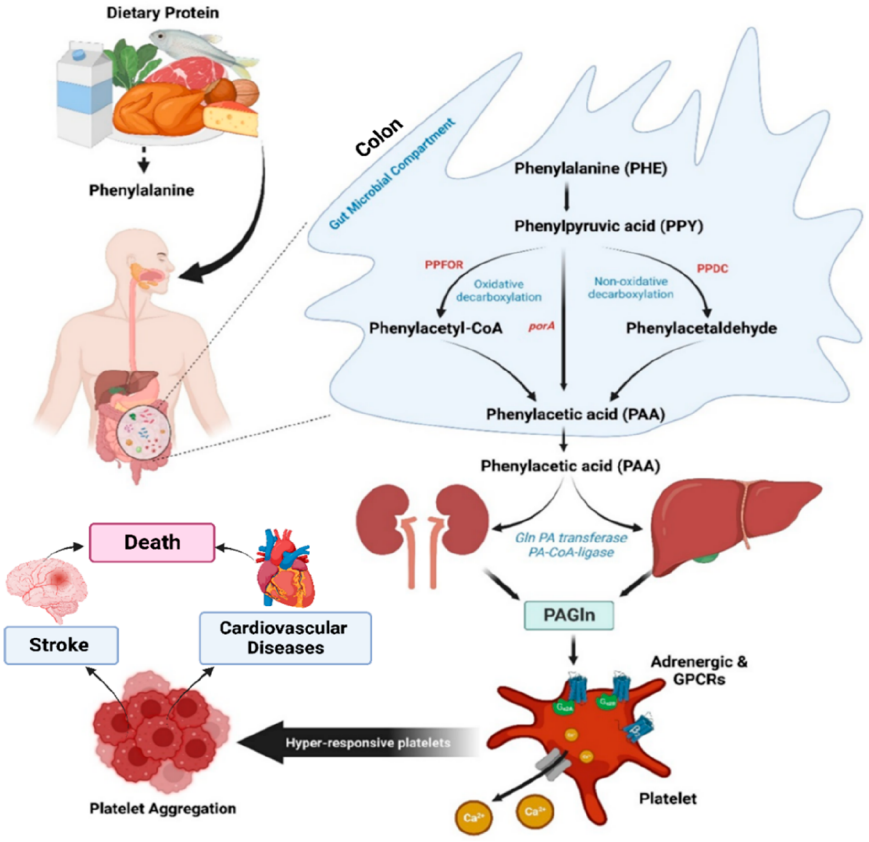


Figure 2. Diagram depicting the effects of phenylacetylglutamine in stroke. Microbial generation of phenylacetic acid (PAA) is followed by conversion of dietary phenylalanine into phenylpyruvic acid (PPY). Further PPY is converted into phenylacetic acid through two distinct thiamine pyrophosphate-dependent pathways (i.e, PPFOR—phenylpyruvate ferredoxin oxidoreductase and PPDC—phenylpyruvate decarboxylase) and a porA-dependent pathway. PAA will reach the systemic circulation and get converted into phenylacetylglutamine (PAGln) through a meta-organismal pathway. PAGln with its ability to act on adrenergic receptors will lead to hyperactivation of platelets causing platelet aggregation. Hence, through enhancing the hyper-responsive platelets and thrombosis, PAGln can cause or increase the severity of cardiovascular diseases and stroke.

administration of phenylbutyrate[25] and L-ornithine phenylacetate, it reduced the elevated ammonia levels in the liver[26] by conjugating with glutamine to produce PAGln, which is excreted through the kidneys (acts as an alternate nitrogen waste removal pathway in the urea cycle). This renal clearance of phenylacetylglutamine is inversely related to the plasma levels of PAGln and is stronger among patients with normal renal function than in patients with abnormal renal function.[27] Accumulation of these gut-derived uremic toxins—PAGln is observed among uremic patients.[28−30] The glomerular filtration rate (GFR) is an accurate estimate of kidney function, and its alteration is observed in the case of kidney diseases.[31] Some investigations have shown a direct correlation between chronic kidney disease (CKD) and cardiovascular disease (CVD).[32] In a study that correlates urine metabolites to nephropathy, it was reported that glutamine levels were lower in Type-2 DM patients in comparison to controls with albuminuria. Among these patients, few (n = 42) developed diabetic nephropathy or kidney diseases in later stages.[33] The gut−kidney axis is also known to be bidirectional, i.e., increase in the uremic toxins like phenylacetylglutamine and indoxyl sulfate has been known to induce changes in the gut microbiota.[34] Thus, it can be inferred from the gut−kidney axis that alterations in either the uremic environment in the kidney or the gut dysbiosis result in renal injury and progressive decline in kidney function culminating in CKD.[35]

PHENYLACETYLGLUTAMINE IN CARDIOVASCULAR DISEASES

Increased PAGln level is associated with a high risk of coronary artery disease, coronary atherosclerotic burden, and heart failure.[36−38] Increased plasma levels of phenylacetylglutamine are also reported to correlate with coronary artery diseases in patients with stent stenosis.[39] Gut microbiota play a key role in the development of atherosclerosis and associated cardiovascular complications.[40] A nontargeted metabolomics study conducted by Hazen et al. was the first to reveal a positive correlation between the gut-derived metabolite PAGln and thrombosis and platelet functions. Furthermore, PAGln levels were also found to be associated with a risk for cardiovascular dysfunction in Type-2 DM subjects.[5] In addition, they also demonstrated that PAGln enhances the platelet activation mediated through GPCRs receptors α2A, α2B, and β2 ADRs.[5,41] High plasma levels of PAGln are also reported to be a significant independent predictor for carotid plaque burden[42] suggesting its role in atherosclerosis. Heart failure patients with high plasma phenylalanine levels also had high levels of C-reactive proteins (CRP) and inflammatory cytokines (IL-8, IL-10), which is also reported to correlate with mortality among the critically ill patients.[43] Further research suggests that a direct correlation also exists between the levels of phenylacetylglutamine and NT-proBNP in patients with heart failure.[5,36] A metabolomic profiling study

https://doi.org/10.1021/acsomega.3c08184
ACS Omega 2024, 9, 3164−3172

in patients with cardiac fibrosis and steatosis with risk for HIV[44] and patients with HIV and CVD[45] showed high levels of PAGln.

Laboratory investigations using heart failure mice show an enhanced activation of the TLR4/AKT/mTOR signaling pathway by PAGln in in vitro and in vivo study. Increased levels of PAGln were reported to cause cardiac fibrosis and inflammation and worsen cardiac function. Thus, PAGln has multiple potential applications in cardiovascular medicine where it can be used as a diagnostic marker including its role as a predictor for β-blocker therapy response in cardiovascular disease.[5] However, further research is required to understand the role of PAGln in inflammation and cardiac failure clearly.[46]

PHENYLACETYLGLUTAMINE IN CEREBROVASCULAR DISORDERS

PAGln and phenylacetic acid are found in the cerebrospinal fluid of humans and monkeys indicating that PAGln can cross the blood−brain barrier (BBB). However, the mechanism of PAGln crossing the BBB is elusive, and further investigations are required.[17,47] Stroke, a cerebrovascular disease, is the second leading cause of death and fifth leading cause of disability across the globe. Disruption of brain−blood flow via blockage (ischemic stroke) or blood vessel rupturing (hemorrhagic stroke) causes excitotoxicity and increases intracellular calcium load causing oxidative stress and neuronal cell death.[48,49] Several studies indicate the role of gut dysbiosis in stroke patients and animal models.[50,51] Also multiple reports suggest association between the gut microbiome and metabolite concentrations as risk factors of stroke.[51,52] PAGln is shown to have a role in thrombosis formation through enhancing the platelet function which is well demonstrated in ex vivo studies (whole blood, PRPs, isolated platelets). Also, an in vivo study has shown thrombus enhancement by PAGln in an arterial injury model using direct administration of PAGln and microbial transplantation.[5,53] PAGln was also reported to interact with GPCRs as well as adrenergic receptors—α2A, α2B, and β2 (Figure 2), and a β-blocker was shown to reverse the prothromobotic effect in mice with arterial injury. Additional research has demonstrated that specific ADR-siRNA knockdown inhibits prothrombotic phenotypes induced by PAGln.[5,54] Thus, it is evident that PAGln elicits prothrombotic effects which could potentially play a role in acute ischemic stroke.

As the levels of PAGln are associated with severity and outcomes in patients with ischemic stroke, PAGln may serve as a biomarker for ischemic stroke.[55] In an independent cohort study, PAGln was observed to be associated with major associated cardiac events (myocardial infraction, stroke, death) through enhanced platelet activation and thrombosis among subjects with Type-2 DM.[5] A cross-sectional study investigated the association between plasma PAGln and white matter hyperintensity (WMH) in subjects with acute ischemic stroke, where higher plasma levels of PAGln were observed among moderate to severe WMH in subjects with acute ischemic stroke.[56] In another study investigating the association between the gut microbiome and metabolome in cerebral ischemic stroke, the phenylacetic acid precursor of PAGln was observed to be upregulated in patients with ischemic stroke.[52] High levels of PAGln were also seen in ischemic stroke patients with Type-2 DM which has also been reported to be associated with gut microbiome dysbiosis.[57] When compared with the healthy controls, higher levels of PAGln were seen to be associated with onset and recurrence of stroke and its risk factors among patients with acute ischemic stroke.[7] A metabolomic study has shown an enriched phenylalanine (precursor of PAGln) metabolism which correlated with downregulation of L-abrine (free radicle scavenger) among acute ischemic stroke patients.[58]

PAGLN IN NEUROLOGICAL DISORDERS

The An association between gut dysbiosis and elevated deleterious microbial metabolites including PAGln in Parkinson's disease patients has been shown by Cirstea et al.[59] Phenylacetylglutamine levels are reported to be high in PD patients when compared to healthy individuals.[60] Metagenomics analysis of the samples collected from PD patients revealed an increased microbial ability to degrade mucin and glycan which influences the folate deficiency and hyper-homocysteinemia.[61] Similarly, increased proteolytic fermentation has been shown to be linked to GI dysfunction due to production of toxic amino acid metabolites like PAGln.[59] Gut dysbiosis-induced proinflammatory cascade is seen among patients with PD and is reported to have a potential effect on misfolding of α-Syn induced by inflammation.[62] Studies have reported significant decrease in phenylalanine concentrations among PD patients.[63,64] Furthermore, the production of phenylalanine (PAGln precursor) and phenylalanine metabolism is shown to be high in PD patients in comparison to healthy controls.[61,65] Elevated levels of salivary phenylalanine are seen in PD patients.[66] Also, longitudinal metabolomics revealed decreased sulfur metabolism and increased presence of amino acids like phenylalanine among PD patients.[67] Elevated phenylalanine levels are identified as a metabolic predictor for long-term mortality.[68]

It is observed that individuals with increased colonic transit time have phenylacetylglutamine in their urine.[69] Elevated levels of PAGln among healthy individuals are related with high bowel movement frequency, which might also drive long-term chronic disease risk.[70] Increased phenylalanine metabolism is also seen among patients with panic disorders.[71] Decreased plasma levels of phenylalanine are seen to be associated with a risk for mild cognitive impairment in Alzheimer's disease.[72,73] It was observed that phenylalanine and phenylalanine metabolic pathways are altered along with other pathways associated with AD in humans and mice.[74] Targeted and untargeted metabolomics performed in brains of postmortem humans revealed that the phenylalanine metabolism is dysregulated in the hippocampus of these AD subjects.[75] A recent observational study exploring urinary biomarkers for Alzheimer's disease has shown upregulated *N*-phenylacetylglutamic acid among AD patients.[76] In Alzheimer's disease, increased levels of PAGln are found to be associated with impaired phospholipid and sphingolipid metabolism.[77] In an untargeted metabolomics study among AD and mild cognitive impairment patients, high amounts of phenylacetic acid (PAA) are seen in saliva.[78]

Elevated levels of PAGln are also reported in cerebrospinal fluid in patients with multiple sclerosis. PAGln levels are inversely correlated with the cortical volume and directly correlated with neurofilament light chain levels.[47] A study in the U.S. reported that PAGln levels were higher in women with short sleep duration (<7 h) compared to medium sleep duration (7−8 h), hence, strongly associating PAGln levels and sleep-related disorders which can further lead to neurological

https://doi.org/10.1021/acsomega.3c08184
ACS Omega 2024, 9, 3164−3172

complications.[79,80] Altered concentrations of phenylacetylglutamine are seen among autism spectrum disorder (ASD) patients when compared to non-ASD individuals.[81,82] Another evidence elucidating the role of altered phenylacetylglutamine levels was association with memory and executive functioning impairment among patients with renal disorders receiving dialysis.[83,84] Also a comparative metabolomic analysis identified dysregulated phenylacetyl-L-glutamine as a potential biomarker for cognitive impairment induced by lead.[85] The altered levels of PAGln are correlated with the initiation and progression of CVD, stroke, neurological disorders, and other disorders depicted in Table 1.

Table 1. PAGln Level Alterations in CVD, Stroke, and Neurological Disorders[a]

S. no.	control subjects	disease subjects	ref
CVD	0.08−152.10 μM	MACE: 0.108−267.1 μM	5
	1.249 ± 1.168 μM	HF: 3.322 ± 8.220 μM	86
	12.90−44.30 ng/mL	CHF: 142−1340.8 ng/mL	6
		ISR: 801.12 ± 291.1 ng/mL	87
		ISH: 526.75 ± 256.38 ng/mL	
		ISP: 367.18 ± 271.02 ng/mL	
		CVD with CKD: 2.99−13.18 μM	88
		HF: 0.76−3.12 μM	89
stroke		none−mild WMH: 1.8 (1.0−2.8) μmol/L	56
		moderate−severe WMH: 2.3 (1.5−3.8) μmol/L	
	1.0 (0.5−1.9) μmol/L	mild: 1.9 (1.0−3.2) μmol/L	55
		moderate−severe: 2.3 (1.3−3.5) μmol/L	
	556 ng/mL	first-onset stroke group: 933 ng/mL	7
		recurrent stroke group: 1014 ng/mL	
neurological disorders		traumatic brain injury and antemortem CNS damage: 81.9 (1.6−990) μg/mL	90
		AD and mild cognitive impairment: 264.1072 Da	77

[a]MACE, major adverse cardiovascular events; HF, heart failure; CHF, chronic heart failure; ISR, in-stent restenosis; ISH, in-stent hyperplasia; ISP, in-stent patency; CVD, cardiovascular diseases; WMH, white matter hyperintensity; CNS, central nervous system; AD, Alzheimer's disease.

OTHER DISORDERS

Metabolomic analysis in patients with sepsis and healthy volunteers shows elevated *n*-phenylacetylglutamine levels in sepsis patients indicating its biomarker potential in diagnosing sepsis.[91] PAGln appears to have a protective effect in rheumatoid arthritis.[92] Altered phenylacetic acid expression is seen in HIV patients with metabolic syndrome.[93] Plasma concentrations of phenylacetylglutamine are observed to inversely correlate with central adiposity among Chinese adults.[94] Metabolic profiling of plasma in patients with acute respiratory distress syndrome shows significant increase in levels of PAGln.[95] When compared with normal individuals, children with cystic fibrosis are reported to show increased urinary phenylacetylglutamine levels.[96] Increased body mass index (BMI) is also reported to be correlated with lower concentrations of phenylacetylglutamine.[97]

CONCLUSION

This Review summarizes the pathogenic role of gut bacteria-derived metabolite phenylacetylglutamine in cerebrovascular and cardiovascular diseases. Clinical and preclinical evidence indicate that increased level of PAGln potentiates the risk of the above-mentioned diseases. The present literature also explains the atherosclerotic formation pathway of PAGln in cardiovascular diseases and stroke. However, a thorough correlation between PGIn and particularly in stroke is need to be studied yet. Indeed, its role in inflammatory reactions and association with the opportunistic bacterial community need to be understood. Thus, more research is needed along this line to understand whether PGIn can be a biomarker for cerebrovascular and cardiovascular diseases. Such studies will help to develop potential therapeutic target(s) that can alleviate the inflammatory milieu in the above-mentioned diseases.

ASSOCIATED CONTENT

Data Availability Statement

The data that support the findings of this study are available in standard research databases such as PubMed, Science Direct, or Google Scholar and/or on public domains that can be searched with either keywords or DOI numbers.

AUTHOR INFORMATION

Corresponding Authors

Arehally M. Mahalakshmi − *Department of Pharmacology, JSS College of Pharmacy and Centre for Experimental Pharmacology and Toxicology, Central Animal Facility, JSS Academy of Higher Education and Research, Mysuru 570015, India;* orcid.org/0000-0003-0966-8333; Email: ammahalakshmi@jssuni.edu.in

Meena Kishore Sakharkar − *Drug Discovery and Development Research Group, College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5E5, Canada;* Email: meena.sakharkar@usask.ca

Authors

Naveen Kumar Krishnamoorthy − *Department of Pharmacology, JSS College of Pharmacy and Centre for Experimental Pharmacology and Toxicology, Central Animal Facility, JSS Academy of Higher Education and Research, Mysuru 570015, India*

Manjunath Kalyan − *Department of Pharmacology, JSS College of Pharmacy and Centre for Experimental Pharmacology and Toxicology, Central Animal Facility, JSS Academy of Higher Education and Research, Mysuru 570015, India*

Tousif Ahmed Hediyal − *Department of Pharmacology, JSS College of Pharmacy and Centre for Experimental Pharmacology and Toxicology, Central Animal Facility, JSS Academy of Higher Education and Research, Mysuru 570015, India*

Nikhilesh Anand − *Department of Pharmacology, College of Medicine, American University of Antigua, W-1451 Saint John's, Antigua and Barbuda*

Pavan Heggadadevanakote Kendaganna − *Centre for Experimental Pharmacology and Toxicology, Central Animal Facility, JSS Academy of Higher Education and Research, Mysuru 570015, India*

https://doi.org/10.1021/acsomega.3c08184
ACS Omega 2024, 9, 3164−3172

Gurudutt Pendyala − *Department of Anesthesiology, University of Nebraska Medical Center (UNMC), Omaha, Nebraska 68198, United States; Department of Genetics, Cell Biology, and Anatomy, Child Health Research Institute, and National Strategic Research Institute, UNMC, Omaha, Nebraska 68198, United States*

Sowmya V. Yelamanchili − *Department of Anesthesiology, University of Nebraska Medical Center (UNMC), Omaha, Nebraska 68198, United States; Department of Genetics, Cell Biology, and Anatomy and National Strategic Research Institute, UNMC, Omaha, Nebraska 68198, United States;* orcid.org/0000-0003-1530-0527

Jian Yang − *Drug Discovery and Development Research Group, College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5E5, Canada;* orcid.org/0000-0002-4935-7380

Saravana Babu Chidambaram − *Department of Pharmacology, JSS College of Pharmacy and Centre for Experimental Pharmacology and Toxicology, Central Animal Facility, JSS Academy of Higher Education and Research, Mysuru 570015, India;* orcid.org/0000-0003-2357-056X

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c08184

Author Contributions

N.K.K.: literature collection, compilation, manuscript drafting; M.K., T.A.H., N.A., P.H.K., and J.Y.; assistance in manuscript editing; A.M.M., S.B.C., M.K.S., G.P., and S.V.Y: conceptualization, editing and finalization of manuscript; image drawing.

Funding

This work was funded by Agricultural Development Fund (ADF) grant from the Provincial Government of Saskatchewan, Canada (422905).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank their respective institutions for providing the facilities during the manuscript preparation.

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https://doi.org/10.1021/acsomega.3c08184
ACS Omega 2024, 9, 3164−3172