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Possible role of tryptophan metabolism along the microbiota-gut-brain axis on cognitive & behavioral aspects in Phenylketonuria

Sara Parolisi [a,1], Chiara Montanari [b,c,1], Elisa Borghi [d], Chiara Cazzorla [e], Juri Zuvadelli [f], Martina Tosi [b,d], Rita Barone [g], Giulia Bensi [h], Cristina Bonfanti [i], Carlo Dionisi Vici [j], Giacomo Biasucci [h], Alberto Burlina [e], Maria T. Carbone [a,2], Elvira Verduci [b,d,*,2], on behalf of SIMMESN Working Group for Gut Microbiota in Inborn Errors of Metabolism

[a] UOSD Metabolic Diseases, AORN Santobono-Pausilipon, Naples, Italy
[b] Department of Pediatrics, Vittore Buzzi Children's Hospital, University of Milan, Milan, Italy
[c] Department of Biomedical and Clinical Sciences, University of Milan, Milan, Italy
[d] Department of Health Science, University of Milan, Milan, Italy
[e] Division of Inherited Metabolic Diseases, DIDAS Department of Diagnostic Services, University Hospital, Padua, Italy
[f] Clinical Department of Pediatrics, San Paolo Hospital, ASST Santi Paolo e Carlo, Milan, Italy
[g] Child Neuropsychiatry Unit, Department of Clinical and Experimental Medicine, AOU Policlinico "G.Rodolico-San Marco", University of Catania, Catania, Italy
[h] Paediatrics & Neonatology Unit, Guglielmo da Saliceto Hospital, Piacenza, Italy
[i] Rare metabolic disease unit, Pediatric Department, San Gerardo Hospital, Monza, Italy
[j] Division of Metabolism, Bambino Gesù Children Hospital, Rome, Italy

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ABSTRACT

Cognitive and psychiatric disorders are well documented across the lifetime of patients with inborn errors of metabolism (IEMs). Gut microbiota impacts behavior and cognitive functions through the gut-brain axis (GBA). According to recent research, a broad spectrum of GBA disorders may be influenced by a perturbed Tryptophan (Trp) metabolism and are associated with alterations in composition or function of the gut microbiota. Furthermore, early-life diets may influence children's neurodevelopment and cognitive deficits in adulthood. In Phenylketonuria (PKU), since the main therapeutic intervention is based on a life-long restrictive diet, important alterations of gut microbiota have been observed. Studies on PKU highlight the impact of alterations of gut microbiota on the central nervous system (CNS), also investigating the involvement of metabolic pathways, such as Trp and kynurenine (KYN) metabolisms, involved in numerous neurodegenerative disorders. An alteration of Trp metabolism with an imbalance of the KYN pathway towards the production of neurotoxic metabolites implicated in numerous neurodegenerative and inflammatory diseases has been observed in PKU patients supplemented with Phe-free amino acid medical foods (AA-MF). The present review investigates the possible link

Abbreviations: IEMs, Inborn Errors of Metabolism; GBA, Gut-Brain Axis; Trp, Tryptophan; PKU, Phenylketonuria; KYN, Kynurenine; ENS, Enteric Nervous System; CNS, Central Nervous System; 5-HT, 5-hydroxytryptamine; 5-MTHF, 5-methyltetrahydrofolate; PUFAs, Polyunsaturated Fatty Acids; ADHD, Attention-Deficit Hyperactivity Disorder; GI, Glycaemic Index; BMI, Body Mass Index; IQ, Intellectual Quotient; MGBA, Microbiota Gut-Brain-Axis; MMSE, Mini Mental State Examination; BDNF, Brain Derived Neurotrophic Factor; ACTH, Adrenocorticotropic hormone; ASD, Autism Spectrum Disorders; SCFAs, Short Chain Fatty Acids; PAH, Phenylalanine Hydroxylase;; Phe, Phenylalanine; Tyr, Tyrosine; MHP, Mild Hyperphenylalaninemia; AA-MF, Amino Acid Medical Foods; LNAA, Large Neutral Amino Acids; GMP, Glycomacropeptide; LAT1, Large Neutral Amino Acid Transporter; PD, Parkinson's Disease; TPH, Tryptophan Hydroxylase; TPH1, Tryptophan hydroxylase 1; TPH2, Tryptophan hydroxylase 2; BBB, Blood-Brain Barrier; ECs, Enterochromaffin Cells; IDO, Indoleamine 2,3-dioxygenase; TDO, Tryptophan 2,3-dioxygenase; IBD, Inflammatory Bowel Disease; KYNA, Kynurenic Acid; QUIN, Quinolonic Acid; 3-HK, 3-hydroxykynurenine; PIC, picolinic acid; NMDA, N-Methyl D-Aspartate; GPR35, G protein-coupled receptor 35; TrpD, Tryptophan Decarboxylase; TnA, Tryptophanase; AhR, Aryl hydrocarbon receptor; IL4I1, Interleukin 4-induced 1; ILA, Indole-3-Lactic Acid; IPA, Indole-3-Propionic Acid; GF, Germ-Free; miRNAs, MicroRNAs; LPS, Lipopolysaccharide; TLRs, Toll-like Receptors; TS, Tryptophan Synthetase; 5-HT4, 5-hydroxytryptamine-4; FOS, Fructooligosaccharides; GOS, Galactooligosaccharides; GMP-MF, Glycomacropeptide Medical Foods; ROS, Reactive Oxygen Species; NO, Nitric Oxide; BH4, Tetrahydrobiopterin; FSMPs, Food for Medical Special Purposes.

* Corresponding author at: Department of Pediatrics, Vittore Buzzi Children's Hospital, University of Milan, Milan, Italy.
E-mail address: elvira.verduci@unimi.it (E. Verduci).

[1] These authors have contributed equally to this work and share first authorship.
[2] These authors have contributed equally to this work and share last authorship.

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between gut microbiota and the brain in IEMs, focusing on Trp metabolism in PKU. Considering the evidence collected, cognitive and behavioral well-being should always be monitored in routine IEMs clinical management. Further studies are required to evaluate the possible impact of Trp metabolism, through gut microbiota, on cognitive and behavioral functions in IEMs, to identify innovative dietetic strategies and improve quality of life and mental health of these patients.

1. Introduction

1.1. The gut-brain axis: the role of gut microbiota in cognitive and behavioral dysfunctions

Brain and intestine closely interact and modulate each other through a bi-directional communication [1]. The gut microbiota influences both healthy status and pathological conditions by contributing to the signaling along the gut-brain axis (GBA) [1]. The Central Nervous System (CNS), Enteric Nervous System (ENS), neuroendocrine and neuroimmune pathways, sympathetic and parasympathetic nerve systems, and the gut microbial community are all involved in the communication between the Gastrointestinal (GI) tract and the CNS [2].

Various disorders may interfere with the normal gut-brain communication that maintains homeostasis. First, intestinal microorganisms can alter brain function through the direct activation of the vagus nerve which, thanks to its afferent and efferent fibers, represents a direct and biunivocal connection between the ENS and the CNS [3]. Moreover, the influence on ENS and CNS may be mediated by the production of different metabolites by gut microbiota, including neurotransmitters [4].

Much emphasis has recently been paid on how the gut affects the brain function and behavior [2] and different pathways and neurobiological mechanisms are being investigated. An increasing variety of disease states and disorders have been found to correlate with the host microbiota, including cognitive and behavior alterations [5]. The gut microbiota has indeed been suggested as a further player in the GBA, delineating a microbiota-gut-brain axis (MGBA). The influence of gut microbiota composition on neuropsychiatric conditions like anxiety, depression, intellectual disability, and autism spectrum disorders (ASD), is largely accepted [6].

Understanding the consequences of prolonged exposure of the brain to inflammatory conditions may help in clarifying how immunological status impacts behavior, stress, mood, thought, development of psychiatric illness and cognition [6]. Scientific research [7] has clearly shown that a condition of dysbiosis, through the production of pro-inflammatory cytokines (such as IL-6 and IFN-γ) and the activation of the immune signaling pathways, influences the brain and, consequently, the behavior, with anxiety and depressive manifestations named < <disease behavior> > by Bilbo and Schwarz [8]. In relation to functional connectivity of brain areas in the somatosensory network and GI sensorimotor function, differences in microbiota composition have been discovered in patients with inflammatory bowel disease (IBD) compared to healthy controls, suggesting changes in interactions of MGBA [9]. Komanduri et al. [10] reported that the increased level of pro-inflammatory cytokines is negatively associated with the score in the Mini Mental State Examination (MMSE) used to measure the presence of cognitive impairment. Studies on aged mice revealed memory deficiencies following acute inflammation [11]. Bercik et al. [12] showed that, in mice treated with antimicrobials, the transient perturbation of microbiota increased hippocampal expression of brain-derived neurotrophic factor (BDNF), but also exploratory behavior. In the hippocampus, BDNF is associated with memory and learning as well as with anxiolytic and antidepressant behavior [13]. Bercik et al. [12] believed that the changes in bacterial composition of the colon were responsible for changes of BDNF levels in the brain, behavior, and cognition; furthermore, they reported this condition as reversible upon normalization of the microbiota. Nevertheless, in this study, the perturbation of the microbiota by drug administration did not alter neither the intestinal cytokine profile nor the 5-hydroxytryptamine (5-HT), dopamine, or noradrenaline content [12]. It is important to consider that microbiota composition and its function are influenced by several factors such as age, diet, presence of non-communicable diseases (NCDs) [14], as well as culture and inter-individual variability. The assessment of cognitive and neuropsychological functioning itself could be influenced by methodological problems related to the sensitivity of neuropsychological tests and inter-individual characteristics. An intra-European consortium of researchers with expertise in the field of nutrition, pediatrics, psychology, psychiatry, and genetics, has developed a cross-cultural neuropsychological battery for cognitive assessment (NUTRIMENTHE) in order to highlight the long-term effects of cultural aspects on cognitive development. The results showed that the observed differences among countries disappeared when scores were standardized according to each country and sex. This evidence highlights the need for the development of specific procedures to compare neuropsychological performance among children from different cultural backgrounds [15].

Fernandez-Real et al. [16] observed an association among obesity, gut microbiota composition and cognitive alteration in motor speed, and attention and cognitive flexibility. The same association was found in thirty-five adult subjects with obesity and insulin resistance, characterized by lower fecal glutamate and glutamate/glutamine ratio together with a higher relative abundance of Streptococcaceae and a depletion in Corynebacteriaceae family. These subjects presented alteration in processing speed, mental flexibility, and executive functioning [17].

Moreover, a higher relative abundance in Verrucomicrobia and Lentisphaerae phila, which are usually reduced in subjects with low-grade inflammation and obesity, was related to an amelioration of cognitive dysfunction and sleep quality [18]. Veronese et al. [19] also suggested that a decrease in the inflammation status due to weight loss could improve the cognitive function, whose decline has been associated with peripheral and central inflammation. Several studies reported a significant relationship between gut microbiota in chronic-inflammatory condition and cognitive dysfunction, immediate and delayed visual memories and visual-spatial constructional, (measured by Rey-Osterrieth Complex Figure Test), phonemic and semantic verbal fluency (measured by Verbal Fluency Test) [20] executive functions, attention, and mental flexibility (measured by Stroop Color-Word Test and Trail Making Test) [17,18]. The exact role of gut microbiota on these neurological effects is not known yet, but the positive influence of a balanced diet on microbiota health has already been established. Lastly, considering the quantity of variables that may influence the host microbiota, it is important to consider physiological stressors such as sleep loss and acute circadian misalignment, which could be potentially linked to gut microbiota alterations [14]. The relationships among these factors are related to increased cortisol levels, considered as markers of stress, potential alterations of gut microbiota and metabolic health [21]. Diaz Heijtz et al. [5] demonstrated that the gut microbiota can modulate the levels of adrenocorticotropic hormone (ACTH) in young mice [5] and the exposure to microbial pathogens resulted in behavioral abnormalities including anxiety-like behavior and impaired cognitive function. In addition, psychological stress has the capacity to deregulate the gut microbiota, contributing to increased gut permeability and poor general health [14].

A central role has been attributed to the circulating Trp availability, with focus on regulation by gut microbiota on 5-HT synthesis and

kynurenine pathway (KYN) metabolism. The role of products derived from Tryptophan (Trp) metabolism by gut bacteria are gaining increasing importance in understanding signaling mechanisms in the GBA. Intestinal homeostasis may impact on Trp metabolism in the GI tract by host cells and bacteria, interfering with the formation of neurotransmitters, neurotoxins, and antimicrobial metabolites [2]. An array of neuropsychiatric disturbances, such as depression and anxiety, as well as cognitive performance, social behaviors and visceral pain perception are influenced by the modulation of neuroactives derived from Trp by microbiota [22].

5-HT, a key monoamine neurotransmitter synthesized from Trp, takes part in the modulation of central neurotransmission and in the enteric physiological function [23]. A dysfunctional 5-HT signaling is reported in GI disorders with psychiatric comorbidity, such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) and in other CNS disorders with GI dysfunction like autism spectrum disorders (ASD). This could indeed explain pathological symptoms related to both gastrointestinal and psychiatric pathologies [9,24].

Gut microbiota participates in the KYN pathway of Trp, modulating its metabolism in the host [2] and its metabolites production. An altered metabolism of Trp along the KYN pathway can occur during life; fluctuations of metabolites during specific "critical windows" of neurodevelopmental seem to have an impact on cognitive impairments [25]. It has been demonstrated that a prenatal KYN pathway inhibition in rats leads to different morphology of hippocampal neurons as well as different protein expressions in neocortical and cerebellar, which persist into adulthood. Therefore, gut microbiota and KYN pathway appears to be involved in early neurodevelopmental programming, specifically during the first 1000 days of life, which is a vulnerable period of both CNS glutamatergic and serotonergic system development [22].

An increasing body of evidence suggests that MGBA perturbation in early life may influence neurogenesis, myelination [26], microglial cells maturation [27] as well as blood-brain barrier development and integrity [28]. An experimental perturbation of the maternal gut microbiota in mice, induced by exposure of dams to antibiotics during a critical perinatal period, showed that the onset of neurobehavioral changes in the male offspring is associated with modifications of several gut bacterial species and modified brain gene expression related to neurodevelopmental disorders [29]. Furthermore, possible relationships between MGBA and neurodevelopment may be inferred from the occurrence of microbiota perturbations in children with neurodevelopmental disorders such as ASD [30]. It is largely acknowledged that subjects with ASD have a higher abundance of bacteria such as Clostridium spp. and Bacteroides spp., which have been linked to the production of pro-inflammatory metabolites and improper synthesis of short chain fatty acids (SCFAs) that, in turn, affect brain development by modulating 5-HT and dopamine production [31]. Evidence suggests that a significant increase of urinary metabolites derived from gut bacterial degradations of Trp, such as indole3-acetic acid, indoxyl sulfate, and most prominently, indolyl lactate [32], characterized patients with ASD, confirming the association between Trp and ASD [33]. Moreover, GI symptoms are frequently connected to ASD and may be explained by the disruption of tryptophan metabolism in the gut [8,24,34]. The authors of a study on children with ASD showed increased levels of xanthurenic acid and quinolinic acid (QUIN) in urine, indicating a preferential transition from Trp, at the expense of kynurenic acid (KYNA) [9]. Thus, it has been recognized that a deeper knowledge of the interface between MGBA and neurodevelopmental disorders might shed light on the role of microbiota metabolites and proper brain development and functioning in health and disease.

1.2. Impact of nutrition on cognitive and behavioral development in children

Early-life diet and nutritional status may contribute to children's neurodevelopment and to adults' cognitive function. The associations between specific dietary patterns and cognitive outcomes in children from high-income countries have been little investigated [34]. The determinant role in the development of the brain of micronutrients, such as vitamin B12, folic acid, zinc, iron, and iodine, has been explored [35,36]. There are sensitive and critical stages of development, such as the last trimester of gestation and the first two years of post-natal life, which are characterized by rapid brain growth and during which diet and nutrition can have a long-lasting influence on later cognitive development and behavior [37,38]. Several studies demonstrated the presence of significant correlations among the quality of nutrients, such as essential fatty acids, iron, and zinc [39–41], and brain development, neuropsychological functioning [15,36,42], behavior [43] and mood [44]. Moreover, the positive effect of micronutrients on health, especially in pregnant women to maximize their child's cognitive and behavioral outcomes, is commonly acknowledged. For example, children born from mothers supplemented with 5-methyltetrahydrofolate (5-MTHF) during pregnancy, have shown better performance on working memory tasks [36]. Many authors underlined that low maternal folate status during pregnancy is associated with an increased risk of internalizing and externalizing problems in young children [45,46], which have not been found in children of folate-supplemented mothers. Folate supplementation ameliorates verbal and executive functions, reducing hyperactivity at 4 and 8 years [47,48]. Alterations in the folate status seem to play a crucial role for memory functioning [49] and depression [50]. Fattal et al. [51] underlined the correlation between thiamine deficiency during the first year of life and specific impairment in language domains, particularly in syntax and lexical retrieval.

Fatty acids, both omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) have shown to play an important role in the development of attention and problem-solving abilities, and to be implicated in a cluster of neurodevelopmental disorders, including attention-deficit hyperactivity disorder (ADHD), dyslexia, dyspraxia, and the autistic spectrum [52,53]. Different studies have reported the beneficial effects of breastfeeding, even with long-term impact, on the intelligence quotient (IQ), child cognition and infant temperament. These aspects appear to be strictly dependent on the fatty acid content of breast milk [54–57].

Considering macronutrients and whole food, Gispert-Llaurado et al. [58] reported a reduction in social, attention, and behavioral problems in 7–9-year-old children eating only two fish meals per week [58]. Sugar intake doesn't appear to affect behavior or cognition development in children [59], while other recent studies found that sugar-sweetened beverages intakes can predict worse verbal development in 3-year-old American children [60]. Low-glycaemic index (GI) breakfasts seem to be beneficial for attention and memory [61].

Nutritional status was found to mediate the relationship among socio-demographic factors and language, motor function and executive functioning [15,36]. In animal models, an association among prenatal protein-energy malnutrition and cellular, electrophysiological, behavioral and neurotransmitter synthesis alterations, resembling those seen in patients with schizophrenia, has been observed [62,63]. Reduced brain weight, altered formation of the hippocampus, altered neurotransmitter systems and changes in protein phosphorylation are the direct effects of protein deprivation in the brain [15,36]. In addition, developmental delay, altered behavior, and lower academic performance have been generally found in undernourished children under 3 years of age [64]. The limitation of this field of research is that most studies have been conducted in school children, where the influence of education may represent a confounding factor (bias). To date, we still know very little about preschool children. Indeed, only a few studies have investigated the impact of dietary patterns in cognitive development [65]. Granziera et al. [34] found a protective effect of very high adherence to Mediterranean Diet on cognition in preschool children and a negative impact of a high body mass index (BMI) regardless of intellectual quotient (IQ) estimates, parents' socioeconomic status, exclusive/non-exclusive breastfeeding, actual age at cognitive assessment and gender [58].

1.3. Cognitive and behavioral aspects in inborn error of phenylalanine metabolism

In agreement with the previous considerations, it is useful to consider the connection among the gut microbiota, the inflammatory processes, and the brain, especially in IEMs, where different dietary restrictions and supplementation are commonly employed.

1.3.1. Physiopathology and dietary treatment in PKU

Phenylketonuria (PKU; OMIM 261600) is an autosomal recessive inborn error of phenylalanine metabolism, caused by mutation of phenylalanine hydroxylase (PAH), resulting in inadequate conversion of phenylalanine (Phe) into tyrosine (Tyr), due to the impaired activity of the enzyme PAH. Phe accumulates in the blood and in the brain, where it becomes toxic. Different types of PAH mutation result in different biochemical phenotype, ranging from mild, moderate, and classical (or severe) PKU (when blood Phe levels are >360 μmol/L) to mild hyperphenylalaninemia (MHP, blood Phe levels ranging 120–360 μmol/L) [66]. According to European guidelines for PKU management [66], no intervention is required for MHP patients, while PKU patients need a special "low Phe intake" diet. The dietary treatment should be started as soon as possible, and it must be continued throughout life, in order to maintain Phe concentration within safe ranges to prevent neurodevelopmental damage [66]. Dietary treatment consists in three components: (i) natural protein restriction (ii) Phe-free amino acid medical foods (AA-MF) as protein substitutes, and (iii) low protein foods (LP foods). The limited consumption of natural proteins to maintain the plasmatic level of Phe within the safety range must, at the same time, ensure the minimal Phe intake to promote an optimal protein synthesis and support an adequate growth, since Phe is an essential amino acid [67]. Since the natural protein intake could be very restrictive, it is essential to ensure an adequate intake of Phe-free AA-MF to prevent protein deficiency, maintain normal physiological function and optimize metabolic control. Compliance to diet therapy with AA-MF could be difficult, especially in adolescence and adulthood, due to the low palatability of these products [68]. Nowadays, different alternative strategies are available including the use of large neutral amino acids (LNAA) and glycomacropeptide (GMP). Phenylalanine crosses the blood-brain barrier via the Large Neutral Amino Acid Transporter (LAT1), competing with other LNAA for its transport. Thus, supplementation with LNAA can have several important advantages, reducing brain Phe concentrations and increasing brain concentrations of essential amino acids and of brain neurotransmitters [69]. GMP is a protein derived from cheese whey, enriched in some specific essential amino acids, principally threonine and isoleucine. It provides an alternative source of low-Phe protein for the management of PKU, since it is naturally almost free of Phe, Tyr, and Trp, and it is more palatable than other protein substitutes [70]. GMP formulations are generally supplemented with Tyr and Trp to avoid eventual deficiencies.

1.3.2. Neurological involvement in PKU

In untreated PKU, the neurological and behavioral impairment can be highly variable [71] but the susceptibility of the CNS due to the high metabolic rate can have possible effects on mood [44], behavior [43], and cognition [72]. Although the most serious consequences of PKU have been averted by early diagnosis with the timely start of the diet therapy treatment, psychiatric disorders, behavioral problems, and cognitive deficits have been well documented throughout the life course of people with PKU even on early treatment [73]. Affected patients often do not feel the effects of poor metabolic control. Early treated patients can display the so-called "hidden disabilities''. Indeed, subtle deficits in executive function, mild reductions in mental processing speed, social difficulties, and emotional problems may remain unnoticed for years [74,75]. Reduced levels of monoaminergic neurotransmitters, such as dopamine and 5-HT, have been described in PKU. Altered phenylalanine metabolism would expose the CNS to a lower availability of Tyr and Trp, precursors of the synthesis of dopamine and 5-HT respectively [76,77]. These compounds play a key role in the onset of neurocognitive and neuropsychiatric sequelae observed in PKU subjects. Although the complex interactions among neurotransmitter systems are not fully understood, dopamine, norepinephrine, and 5-HT are all involved in the regulation of mood, emotions, and cognitive processes [78]. Burlina et al. [79] revealed a severe deficiency of the metabolism of dopamine neurotransmitters and 5-HT in the cerebrospinal fluid of early-treated PKU despite the dietary compliance and the good metabolic control, with the magnetic resonance showing white matter and myelin anomalies. Other authors [80] observed an attenuation of levodopa cerebral influx in affected adult patients comparable to that seen in patients with Parkinson's disease (PD) [81], regardless of the absence of typical clinical manifestations of this syndrome. This could be due, for a compensatory mechanism, to a reduced activity of the dihydroxyphenylalanine-decarboxylase, involved in the catabolism of the dopamine, as shown by the authors themselves [80]. Because Tyr and Trp also enter the brain via the LAT1 carrier, it has been hypothesized that elevated concentrations of phenylalanine in the blood can influence the metabolism of monoaminergic neurotransmitters by competitively blocking the Tyr and Trp transport to the brain, in addition to the possible inhibitory effect of high concentrations of Phe on the enzyme tryptophan hydroxylase (TPH) [82] increasing the potential for neurotransmitter dysfunction and their availability for protein synthesis [83,84].

2. Materials and methods

In this narrative review, a comprehensive literature search was conducted using the PubMed/Medline database. The authors independently identified the most relevant studies on the topic, published in English language, including original papers, pre-clinical studies, observational studies, clinical trials, systematic and narrative reviews.

The following research strategies were used:

1. (("Metabolism, Inborn Errors"[Mesh]) OR ("Phenylketonurias"[Mesh])) AND (tryptophan[Title/Abstract]). Number of publications collected: 468
2. (("Tryptophan"[Mesh]) OR (Kynurenine[MeSH])) AND ((’microbiota-gut-brain-axis’[Title/Abstract]) OR (‘gut microbiota’[Title/Abstract])). Number of publications collected: 282
3. (("Phenylketonurias"[Mesh]) OR ("Metabolism, Inborn Errors"[Mesh])) AND (’microbiota-gut-brain-axis’ OR ’gut microbiota’). Number of publications collected: 141
4. (("Metabolism, Inborn Errors"[Mesh]) OR ("Phenylketonurias"[Mesh])) AND (("Diet" [Mesh]) OR ("Nutrition")) AND ((’microbiota-gut-brain-axis’) OR (’gut microbiota’)). Number of publications collected: 48
5. (("Metabolism, Inborn Errors"[Mesh]) OR ("Phenylketonurias"[Mesh])) AND ((‘cognitive’) OR (‘behavioral’)) AND ((’microbiota-gut-brain-axis’) OR (’gut microbiota’)). Number of publications collected: 22

The search was narrowed to articles published between 2013 and 2023. The total number of documents collected in PubMed/Medline database was n = 961. The flow selection data is illustrated in Fig. 1. Documents were excluded through three steps: listed twice (n = 467), for titles and abstract not relevant (n = 236) and for full text not available and not focusing on Trp metabolism (n = 228). Although numerous studies highlighted a connection between gut microbiota and Inherited Metabolic Disorders, and in particular PKU, the authors chose to focus the review only on papers regarding the Trp metabolism. Moreover, the review included documents identified through other sources (n = 3) and published before 2013, because of their significance (n = 8), for a final number of 41 of selected articles (Fig. 1).

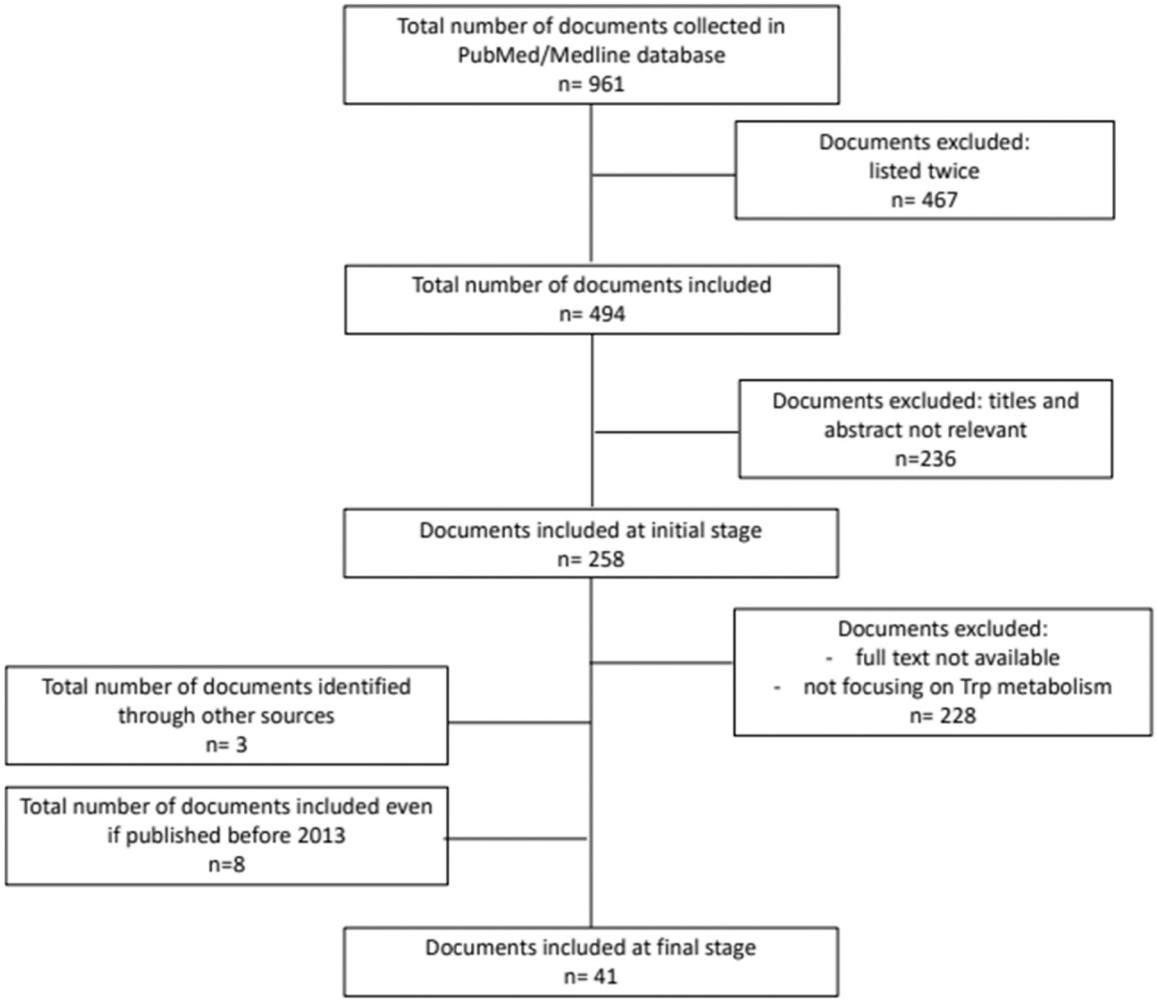


Fig. 1. Flow chart of study selection.

3. Results

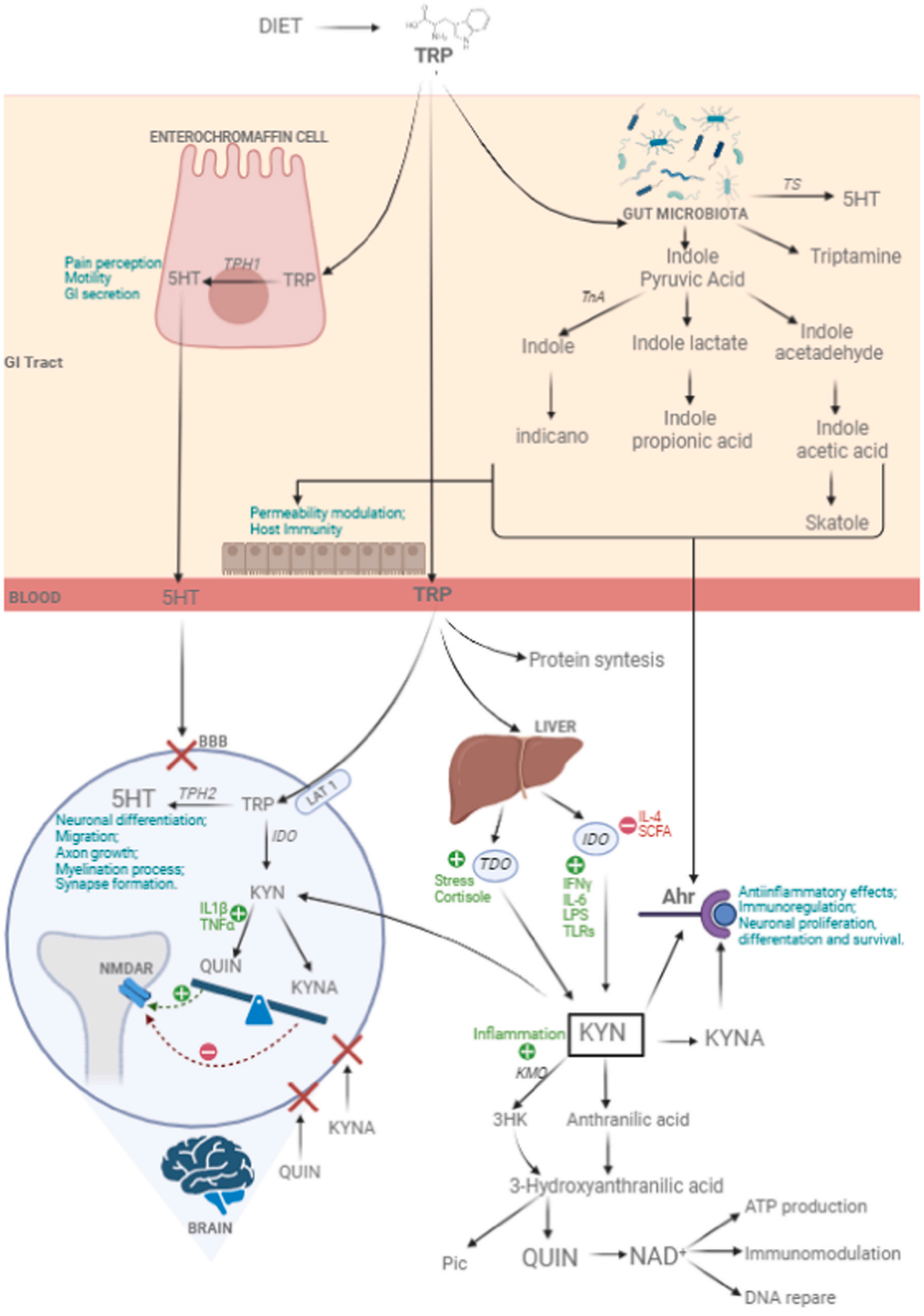
3.1. Tryptophan in the gut-brain axis

3.1.1. Tryptophan metabolism pathways

Trp is an essential amino acid and, consequently, an important nutrient for protein synthesis involved in the growth and the health of the organism [2,23]. Most part of dietary Trp enters the peripheral circulation by absorption from the small intestine and reaches the CNS crossing the blood-brain barrier (BBB) through LAT1 [85] (Fig. 2). The body contains Trp in two different states: free or bound to albumin, both in a balanced state. Only the free form passes through BBB [2]. Important metabolites are produced from Trp metabolism, through two main pathways in balance with each other: of synthesis, with 5-HT production, and of degradation, or KYN pathway, with *de novo* synthesis of nicotinamide adenosine dinucleotide (NAD) through the conversion of neuroactive intermediates [86] (Fig. 2).

3.1.1.1. Biosynthesis pathway. Trp is the only precursor for the biosynthesis of 5-HT that takes place in gut mucosal enterochromaffin cells (ECs) and in ENS neuron, as well as in CNS [87]. TPH is the key enzyme in the synthesis pathway of 5-HT from Trp: TPH1 is the isoform expressed in the enterochromaffin cells (ECs) of the gastrointestinal tract and is responsible for the 90% of the synthesis, while TPH2 is the isoform expressed in the CNS and in the ENS [23] (Fig. 2). Serotonergic neurons are among the first to be present in the enteric nervous system, and during CNS development [23]; 5-HT can peripherally mediate different gastrointestinal functions as well as pain perception, motility and GI secretion [88,89], while in the brain is important for 5-HT signaling pathways that are implicated in regulating mood and cognition [90]; 5-HT plays an important role in modulating neuronal differentiation and migration, axon growth, myelination process, and synapse formation [23]. Since under physiological conditions peripheral 5-HT cannot cross the BBB, different pools of peripheral and central 5-HT coexist [91]. The equilibrium between 5-HT's excitatory and inhibitory activity is modulated by tryptamine, a neuromodulator produced by Trp [2].

3.1.1.2. Degradation pathway. About 90% of Trp is oxidized into the KYN pathway [92]. Two main enzymes are involved in this pathway, indoleamine 2,3-dioxygenase (IDO), ubiquitously expressed, including the brain and the gastrointestinal tract, and tryptophan 2,3-dioxygenase (TDO), expressed mainly in the liver [85] (Fig. 2). There are two isoforms of IDO enzyme, IDO1 and IDO2, both with the same action, with different activity rates. Liver is the main organ involved in the Trp oxidation [93], influencing its availability [23]. However, an excessive activation of KYN pathway with an elevated KYN/Trp ratio and a consequence of reduced availability of Trp, is considered a prognostic factor for chronic disease [94], besides a reduced availability of 5-HT, influencing the development of cognitive and behavioral symptoms. Therefore, a dysregulation between the synthesis of 5-HT and the KYN pathway, with an excessive activation of KYN metabolism, seems to have some specific neurotoxic effects [9], influencing neuropsychiatric disorders, such as depression [23].



(caption on next page)

Fig. 2. Dietary Trp is the only source for the synthesis of 5-HT: TPH1 is the isoform expressed in the enterochromaffin cells of the gastrointestinal tract, while TPH2 is the isoform expressed in the CNS and in ENS. Dietary Trp reaches the brain crossing the blood brain barrier through large neutral amino acids transporters (LAT1) and is converted into 5-HT, since it cannot cross the BBB. Moreover, commensal bacteria directly convert the Trp of the intestinal lumen in 5-HT. Dietary Trp is oxidized by gut commensal bacteria in tryptamine and indole pyruvic acid derivatives. These metabolites act locally, influencing intestinal permeability and host immunity and, at CNS level, activate Ah receptor impact on neuronal proliferation, differentiation, and survival, and on CNS inflammation. The KYN pathway is the main process of Trp degradation, involving two main enzymes: indoleamine 2,3-dioxygenase (IDO), here represented in liver, but ubiquitously expressed, and tryptophan 2,3-dioxygene (TDO), expressed mainly in the liver. Trp availability modulates TDO activity, while liver expression of the enzyme is primarily affected by stress. IDO, both peripheral and central, is enhanced by bacterial inflammation metabolites (lipopolysaccharide, LPS) with subsequent Toll-like receptors (TLRs) activation and by proinflammatory cytokines. On the other hand, SCFAs are able to down-regulate IDO intestinal expression, suppressing KYN production from Trp, together with anti-inflammatory cytokine, as IL-4. By degradation of KYN derive KYNA, a neuroprotective factor, and QUIN, a weak agonist of the NMDA receptor with neurotoxic properties. They cannot cross the BBB, but KYN can be either produced by Trp or pass the BBB. Production of QUIN is stimulated by inflammation. QUIN is converted into the essential cofactor NAD+ . KYN and KYNA are ligands of Ah receptor.

Immunological mediators, cytokines, and inflammatory molecules such as interferon-γ (IFN-γ), amyloid peptides, and lipopolysaccharides (LPS), can activate IDO, representing the most efficient inducer of the enzyme [2,23]. In contrast, interleukin-4, a suppressor of inflammation, inhibits IDO activity [95]. In vitro and in vivo studies showed that the activation of IDO by IFN-γ leads to a greater Trp conversion to KYN with depressive symptoms observed [2]. The availability of Trp stably modulates TDO activity, while liver expression of the free apoenzyme of TDO is inducible by glucocorticoids [96] and so primarily affected by stress, through the activation of hypothalamic-pituitary adrenal axis [97] (Fig. 2). KYN can be metabolized through two pathways producing two main neuromodulators called KYNA and QUIN [92]. In the first step KYN is converted into KYNA, 3-hydroxykynurenine (3-HK), and anthranilic acid; then 3-HK and anthranilic acid are converted into 3-hydroxyanthranilic acid. Finally, it can be transformed through enzymatic or non-enzymatic reactions either in picolinic acid (PIC) or in QUIN [2,94] (Fig. 2).

KYNA and QUIN are not able to reach the CNS through the BBB [94] but can be directly produced in the CNS by KYN [85]. In the brain, astrocytes degrade KYN primarily through the KYNA arm of the pathway, while microglia through the QUIN one [85] (Fig. 2). KYNA is considered a neuroprotective factor at physiologic concentrations [85], acting as an antagonist of the N-methyl Daspartate (NMDA) receptor, while QUIN is a weak agonist of the NMDA receptor with a potential neurotoxic properties [2,6,25,85] (Fig. 2). An overexcitation of NMDA receptors could lead to "excitotoxic" loss of neurons in the CNS, suggesting the NMDA role in the etiology of neurodegenerative disorders such as Alzheimer's, epilepsy, or stroke [95]. The KYN 3-monooxygenase (KMO) is the enzyme for the production of QUIN, through the intermediate neurotoxic product of 3-HK, which can cross the BBB, inducing free radicals' production and vasodilatation [94]. KMO is an enzyme sensitive to proinflammatory cytokines [94]. TNF-α promotes QUIN production, while interleukin-1β potentiates quinolinate excitotoxicity [95]. Thus, in inflammatory conditions, the pathway of KYN is pushed toward an imbalance between the neurotoxic and neuroprotective factors [94] (Fig. 2). Since a dynamic equilibrium between KYNA and QUIN is necessary for an adequate glutamate neurotransmission [94], the KYNA/QUIN ratio is raising a great interest in relation to health and disease status [98,99]. QUIN excess was associated with neuronal damage and the degeneration associated disorders [95], while KYNA has neuroprotective and anticonvulsant effects [95]. Moreover, KYNA and QUIN are important compounds in neuro-gastroenterology: they bind NMDA and α7 nicotinic acetylcholine receptors also in the enteric nervous system with immunoregulatory role; KYNA has anti-inflammatory and inhibitory action on colon cancer [2]. PIC is another metabolite with similar function to KYNA, being an antagonist of NMDA [94]; it can decrease the neurotoxic effects of quinolinic acid by a different mechanism, possibly by chelating zinc and/or reducing the calcium-dependent glutamate release, much like KYNA, but to a lesser extent [2]. Finally, QUIN has a role in the cellular energy homeostasis through the synthesis *de novo* of NAD+ , which is involved in ATP production, immunomodulation, and DNA repair (Fig. 2). The increased production of NAD+ via the KYN pathway during inflammatory conditions could be explained as response to the increased energy demand in chronic diseases [94].

Besides the neuroactive properties, KYN has an immunomodulatory role. Aryl hydrocarbon receptors (AhR) and G protein-coupled receptor 35 (GPR35) [25], expressed in various tissues, are associated with anti-inflammatory effects, stimulating regulatory T cells generation with suppression of effector T cells and natural killer cells, and through regulation on adipose tissue (e.g., thermogenesis). These receptors can be activated by KYN (AhR) and KYNA (AhR and GPR35) [94]. AhR is a receptor that promotes the metabolism of environmental toxins, functions in immune-regulation, but it's also present on tumor cells [95] (Fig. 2).

Another pathway of Trp metabolism, besides KYN pathway, leads to the production of indole-3-pyruvic acid, which is actually an alternative pathway towards the production of KYNA, and that has been recently discovered as an intermediate product of interleukin 4-induced 1 (IL4I1) enzyme, with a higher affinity for Ah receptors than KYNA [94].

3.1.2. The role of gut microbiota in Trp metabolism and KYN pathway

The gut microbiota influence on Trp metabolism is an emerging driving force in the modulation of the amino acid metabolism pathways [23]. About 5% of dietary Trp reaches the large intestine, escaping the absorption in the small intestine [23], where intestinal bacteria can participate in Trp metabolism. Gut microbiota is able to consume Trp, competing with the host for Trp metabolism [92], and it can participate in the KYN pathway modulating its metabolism in the host [2] and its metabolites production, playing a critical role in the interaction between the gut and the brain [23]. In addition, Trp seems to have a role in gut health, protecting the intestinal epithelium and maintaining local immunity [92]. Gut microbiota can influence Trp metabolism via KYN pathway in the host in association with the immune system [2] which has a role in activating or not kynurenine pathway, making a link between immune system dysfunction, microbiota, and psychopathologies, such as stress-related disorders [25].

1) Gut Trp metabolism

   Many bacteria within the large intestine encode enzymes responsible for conversion of Trp into neuroactive metabolites [23]: indole and indican (i) indole acid derivatives (ii) (e.g., indole lactate and indole propionic acid) skatole (iii) and tryptamine (iv) [2] (Fig. 2). These are key molecules in the communication pathways between the immune system and GI tract [2,100]. The genera *Clostridium, Ruminococcus, Blautia,* and *Lactobacillus,* for instance, express the tryptophan decarboxylase (TrpD), which converts Trp to Tryptamine [101]. Other species, both Gram-negative and Gram positive, including *Escherichia coli, Clostridium spp.,* and *Bacteroides spp.* express tryptophanase (TnA), which converts Trp to indole [102]. Other microorganisms, i.e., *Lactobacillus* and *Bifidobacterium*, encode enzymes involved in the production of indole acid derivatives as indole-lactic acid (ILA), which is further converted in indole-propionic acid (IPA) from bacteria of the Clostridium and Peptostreptococcus genera [23] (Table 1). These Trp metabolites are signal molecules capable of acting locally at the intestinal mucosa,

Table 1
Modulation of the Trp pathway by gut microbiota and characteristics of gut microbiota profiles in PKU.

Reference	Bacteria and metabolites	Action in Trp pathway	Reference	Microbiota profile in PKU (PKU vs Controls)
Lee et al., 2010	*Escherichia coli, Clostridium and Bacteroides* spp.	Express TnA[a]: Trp → indole	Bassanini et al., 2019	↓α-diversity[b] β- diversity[c] ($p \leq 0.05$) ↑*Lachnospiraceae (other), Blautia* and *Clostridium* spp. ↓*Ruminococcaceae (other), Faecalibacterium* and *Dialister* spp. ↓Total SCFAs[d] and butyrate (in feces)
Williams et al., 2014	*Clostridium, Ruminococcus, Blautia, and Lactobacillus*	Express TrpD[e] Trp → Tryptamine	Mancilla et al., 2021	↑ *Clostridium* spp. ↓ *Faecalibacterium* and *Blautia* spp.
O'Mahony et al., 2015	*Lactococcus, Lactobacillus, Streptococcus, Escherichia coli, and Klebsiella*	Trp → 5-HT		
Kennedy et al., 2017 Martin-Gallausiaux et al., 2018	SCFAs[d] (butyrate)	↓IDO[f]		
Kan et al.,2020	*Lactobacillus and Bifidobacterium* *Clostridum and Peptostreptococcus* spp.	Indole → ILA[g] ILA → IPA[h]	Pinheiro de Oliveira et al., 2016	↓α-diversity[b] β- diversity[c] ($p \leq 0.003$) ↑ Bacteroidetes, Verrucomicrobia ↓ Firmicutes ↑ Peptostreptococcaceae, *Akkermansia,Prevotella* spp. ↓*Coprococcus, Dorea, Lachnospira, Odoribacter, Ruminococcus* and *Veillonella*
Kan et al., 2020	Lipopolysaccharide and lipoteichoic acid	↑IDO[f]		
Hyland et al., 2022	Proteobacteria (Bacteroidetes, Actinobacteria)	↑Kinurenine pathway		

[a] tryptophanase;
[b] α-diversity, the diversity in the bacterial composition within each sample;
[c] β- diversity, the diversity between sample groups;
[d] SCFAs, short-chain fatty acids;
[e] tryptophan decarboxylase;
[f] indoleamine 2,3- dioxygenase iversity;
[g] indole 3-lactic acid;
[h] indole-3-propionic acid.

influencing intestinal permeability and host immunity, and on distant organs, including the brain [23] (Fig. 2). Indolyl metabolites may be significant signaling molecules binding Ah receptors and activate locally and systemically [100]. Indeed, they cross the BBB and activate the Ah receptor expressed in neurons, astrocytes, and microglia, impacting on neuronal proliferation, differentiation, and survival. Ah receptors modulate CNS inflammation [103], playing a role in neuropsychiatric disorders (Fig. 2).

2) Gut microbiota and KYN pathway

Gut microbiota has a role in the KYN pathway by influencing Trp metabolites production and Trp availability [2].

a) KYN/Trp ratio

Several bacteria encode for the enzyme responsible for KYN production and downstream metabolites [92]. In germ-free (GF) animals there is an increase in plasma Trp with a decreased KYN/Trp ratio due to the reduced actions of IDO and TDO. Administering microbiota could reinstate the regular action of these enzymes [2] with important consequences on Trp availability [92]. When compared to conventional mice, germ-free mice show an increased anxiety-like behavior, and higher levels of 5-HT in the hippocampus, in line with a higher concentration of the precursor Trp in the bloodstream. These observations suggest a humoral pathway through which the gut microbiota influences the CNS serotonergic neurotransmission. Of note is the pivotal role of gut microbiota influence during early life, since hippocampal 5-HT levels remain unchanged despite a gut microbiota normalization during adult age [82].

b) KYN pathway

If gut microbiota restores the KYN/Trp ratio, it also degrades Trp to various metabolites that limit the availability of Trp for the KYN pathway and 5-HT production. Therefore, in physiological conditions IDO and gut microbiota have feedback control on each other: IDO has an immunosuppressive and immune regulation response in the GI tract, gut microbiota can influence IDO activity by affecting Trp availability [100]. Moreover, the interaction of Trp-derived metabolites of gut microbiota (kynurenines) with Ah receptor could stimulate the expression of IL-6 in macrophages, and IFN-γ production in natural killer cells, with a consequent activation of IDO [100]. The activation of IDO can be explained because of its anti-inflammatory and immunosuppressive response in intestinal mucosa, by controlling the host's immunomodulatory actions through KYN synthesis and its influence on T cells, balancing the pro- and anti-inflammatory status [2]. Moreover, kynurenines have antimicrobial activity and Toll-like receptors (TLRs), important for identification of the microbial components, are decreased in germ-free animals. Ah receptors are considered the main mediator of GI microbiota, KYN pathway and the host immune system, regulating inflammation, intestinal homeostasis, and carcinogenesis; they can be linked by KYN metabolites, and they can modulate the expression of IDO and TDO [2]. Molecular analyses of bacterial genomes have found homologs of KYN pathway enzymes, conferring to microbiota the potential to produce neuroactive metabolites [100]. Bacteria expressing KYN pathways belong to Bacteroidetes, Actinobacteria, and Proteobacteria phyla, with the last one having a predominant role [92]. Proteobacteria are generally associated with an increased release of inflammatory mediators pushing the pathway preferentially towards the production of KYN, rather than 5-HT [9] (Table 1), stimulating the activation of IDO [22]. Since the gut-microbial colonization plays a role in the host immunity response, by bidirectional cross-talk [104], the release of inflammatory molecules, such as LPS and lipoteichoic acids, with subsequent TLRs activation, are identified as key factors in the starting up of the KYN pathway through the IDO activation [23]

(Fig. 2). LPS enhances the KYN pathway and reduces the 5-HT levels of the prefrontal cortex of mice [23]. On the other hand, SCFAs, produced by fermentation metabolism of carbohydrates, can modulate IDO intestinal expression (Fig. 2), suppressing the production of KYN from Trp [22,105]. A high KYN/Trp ratio has been reported associated with inflammatory diseases and cancers [2]. Moreover, an overexpression of IDO is observed in the colonic mucosa and in damaged tissues of patients with inflammatory bowel disease (IBD) [2106]. Thus, we can assert that bacteria behave as a two-faced Janus, as gut microbiota protect the host from Trp excess, but an excessive activation of the Trp-KYN pathway can mean pro-inflammatory status [85,92].

c) 5-HT synthesis:

Moreover, bacteria can directly synthesize Trp by means of the bacterial enzyme complex of tryptophan synthetase (TS) [107]. Other commensal bacteria (i.e., *Lactococcus, Lactobacillus, Streptococcus, Escherichia coli,* and *Klebsiella*) directly convert the Trp of the intestinal lumen in 5-HT [107], while other bacteria, including Bacillus (aerobic) and Clostridium (anaerobic), are involved in the modulation of 5-HT synthesis, promoting the enterochromaffin cells action [87,108] (Table 1). Intestinal microbiota modulating the central synthesis of 5-HT, influencing the brain availability of Trp [23,85]. The SCFA butyrate exerts neuroprotective effects in stressed mice by increasing the cerebral concentration of 5-HT and restoring damaged BBB [23]. Colonization of GF mice with the microbiota of conventional mice modifies the neuroanatomy of the ENS and enhances intestinal transit, with increased production of neuronal and mucosal 5-HT and proliferation of enteric neuronal progenitors in the adult intestine. The activation of the 5-hydroxytryptamine-4 (5-HT4) receptors in the ENS is linked to neurogenesis and neuroprotection in adults. The pharmacological modulation of the receptor has been identified as a bridge mechanism through the 5HT-dependent activation of the 5-HT4 receptor, between the intestinal microbiota and the maturation of the adult ENS [23].

3.1.3. Potential influencing factors on tryptophan pathway: nutrients and probiotics

Diet plays a pivotal role in modulating Trp metabolism [23] with different effects according to various macro- and micronutrients. The availability of indigestible carbohydrates in the colon, such as fructooligosaccharides (FOS) and resistant starches, can reduce the breakdown of Trp. Dietary fibers can lead to increased SCFAs production, reducing Trp degradation, and indole derivatives production in the large intestine, as reported in animal studies [109,110]. Clearly, the intake of food high in Trp (e.g., nuts, seed of sesame, pumpkin, sunflower and soybeans, grains) increase Trp availability and Trp metabolites deriving from degradation pathways, as 5-HT and KYN and its catabolites, respectively. However, high dietary Trp intake does not necessarily correspond to a greater concentration of 5-HT in CNS, since 5-HT is not able to pass the BBB and its availability depends on the transport of Trp into the brain. Because of the competition between Trp and LNAA for the transport system, the Trp/LNAA ratio influences the Trp availability into the brain and thus 5-HT synthesis. Hence, the effects of high Trp intake can be lost in case of competition with LNAA for the brain transporters [111]. The carbohydrates available in the large intestine seem to be able to suppress the microbial degradation of Trp, with an increase in intestinal and plasma levels of the amino acid and the synthesis of intestinal 5-HT. The increased microbial production of SCFAs would be involved in this process, stimulating the release of 5-HT in the enterochromaffin cells of the colon [23]. Furthermore, there is evidence that eating food source of carbohydrates along with food rich in protein can increase Trp availability in the brain. Dietary fats could also exert modulatory effects. It appears that a high-fat diet vs a low-fat diet may attenuate bacterial breakdown of Trp in mice [23]. The type of fats, saturated or unsaturated, could display different effects. A diet rich in proteins was found to be associated with saturated fats, promoting a shift in the metabolism of Trp, and resulting in an increase of QUIN with possible neurotoxic effects [112]. Karlsson et al. [113] reported an association between fish intake and plasma metabolites of the KYN in patients with diabetes. The authors observed a negative correlation between PUFAs intake and KYN/Trp ratio, suggesting a possible lower activation of the IFN-γ mediated immune response with a higher fish intake. Besides lipids and carbohydrates that seem to modify the metabolism of Trp, the availability of this amino acid in the intestine is another important factor to consider. For example, feeding mice with a diet low in Trp led to a reduction in the biodiversity of intestinal bacteria, with an increase of circulating pro-inflammatory cytokines [114]. On the contrary, diets rich in Trp can have a protective role on the intestinal epithelium, preventing chemically induced damage in a murine colitis model [92,115]. In addition to the availability of different nutrients, some functional foods, such as breast milk, can restore the metabolism of Trp. In an animal model it has been demonstrated that formula milk induced alterations in gut microbiota shifting the Trp metabolism from 5-HT to tryptamine in the neonatal porcine colon [116]. Trp metabolism is highly influenced and modulated by dietary constituents like phytochemicals, colorants, and probiotics [6]. In particular, the most sensitive pathway to environmental influences is the IDO-mediated Trp-catabolism. Foods enriched in antioxidant compounds (e.g., vitamin C and E) could improve both Trp and 5-HT status in the brain. These compounds counteract pro-inflammatory response and Trp breakdown by IDO [6]. The prediction of the effects of phytochemicals (e.g., resveratrol) on Trp metabolism in humans is somewhat limited due to the paucity of in vivo studies. Though polyphenol-rich food/beverages and phytochemicals as resveratrol are generally considered to act as antioxidant, data suggest that the net effect depends on an individual's immune status, being immunostimulatory in healthy individuals while dampening of responses may occur on an inflammatory milieu [6]. It would be interesting to discuss the role of supplementation with probiotics, but up-to-date human data focusing on the influence of probiotics on Trp metabolism are still scarce. Preliminary evidence seems to highlight individual effects dependent on the host immune status and epithelial barrier function. The type of probiotic and the treatment duration also play a role [6]. Probiotics, such as bacteria belonging to the genera *Lacto-bacillus* and *Bifidobacterium*, are reported to exert beneficial effects [91] by directly enhance 5-HT production [107], or indirectly, by modulating gene expression of TPH1, as reported for *Lactobacillus casei* 327 [117]. In mice, *Lactobacillus johnsonii* cell-free supernatant has been shown to reduce IDO activity and circulating KYN, while in humans the administration of *Lactobacillus johnsonii* was shown to lightly decrease serum levels of KYN, along with increased amounts of Trp [118]. Another study also revealed that administration of *Bifidobacterium infantis* in rats induces an increase in Trp levels, with a consequent decrease in the KYN/Trp ratio in the blood circulation [119].

3.2. Trp metabolism in PKU patients

As reported above, a disturbed Trp metabolism could be triggered by an alteration of gut microbiota [100]. We can translate this consideration in PKU, where a condition of gut dysbiosis is well-known [120–122]. To date, few studies on gut microbiota in PKU addressed the repercussions of an altered microbial environment on the CNS and on metabolic pathways that can participate in the pathophysiological processes of neuroinflammatory diseases. Alterations of the gut microbiota observed in PKU [120,121,123] (Table 1) have been identified by Sawin et al. as a possible factor able to influence the metabolism of dopamine and 5-HT through an increase of the intestinal degradation of Tyr and an alteration of Trp-metabolism, respectively [124]. Despite the low protein diet and the high consumption of fruit and vegetables ensuring good fiber amounts, LP foods have a high GI and only a small amount of undigested carbohydrates reaches the colon [122]. Furthermore, the content of Tyr and Trp in medical foods for the management of

PKU may affect dopamine and 5-HT synthesis [125]. An increase in concentrations of cerebrospinal metabolites of dopamine and 5-HT has been observed in PKU patients supplemented with LNAA, including Tyr and Trp [69,77,79]. Ney et al. [125] reported a reduced availability of Tyr and an altered Trp metabolism with ingestion of medical foods in PKU patients. Subjects with PKU taking AA-MF show a significantly higher Tyr than those consuming glycomacropeptide medical foods (GMP-MF), without significant differences in fasting plasma amino acid concentrations. This data suggests an increased degradation of Tyr by intestinal microbiota, impacting on its availability for neurotransmitter synthesis [125]. Concerning Trp metabolism, adult PKU subjects display plasma Trp concentration similar to the general population, but a different ratio of its metabolites [82]. Interestingly, in PKU subjects consuming AA-MF compared with GMP-MF, Trp is preferentially metabolized via the KYN pathway, instead of the 5-HT pathway [125]. The increased synthesis of KYN could be associated with a more pronounced inflammatory response, coherently with the observations of Sawin et al. [124]. Indeed, in a mouse model of PKU, the authors reported an increase in inflammatory cytokines in animals fed with AA compared to a GMP-based diet [124]. The neuroprotective metabolite KYNA shows similar levels in AA-MF and GMP-MF animals, while the neurotoxic QUIN shows higher levels in AA-MF in both plasma and urine [125]. Of note, an unbalanced metabolism of KYN towards QUIN production can induce oxidative stress, apoptosis, and mitochondrial dysfunction, resulting in neuronal damage. Indeed, various neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, are characterized by elevated concentrations of QUIN in the cerebrospinal fluids. Extensive studies are needed to understand the implications of metabolism of Trp, through the KYN route, in the PKU [125,126].

4. Discussion

During the years, life expectancy of patients with IEMs has significantly improved, thanks to the advent of newborn screening, the development of medical foods and improvements in diet therapy. Currently, the first early-treated PKU patients have reached middle age, representing a new challenge in terms of overall life expectancy and therapeutic possibilities. Adulthood may represent a moment of greater vulnerability for PKU patients, since the compensation mechanisms for the accumulation of Phe may be reduced by the normal process of cerebral aging, with evidence of signs of intellectual disability and mild parkinsonism in some adult patients with PKU [127]. PKU patients are often unaware of their symptoms [68] and are therefore unable to appreciate their influence on the ability to carry out daily activities. They often do not seek psychiatric or psychosocial support on their own initiative [73]. In the light of this, we would like to underline the importance of the patient's mental health management. The emotional and behavioral well-being of affected individuals should be monitored as a routine part of the clinical management of the patient, to intervene appropriately before psychiatric symptoms and dysfunctional behavior patterns occur and a vicious cycle is established with the worsening of symptoms. As above depicted, Trp metabolism is complex as there is a balance between the synthesis and degradation pathways, in order to guarantee an adequate level of plasmatic and CNS metabolites. Inflammation, immunomodulation, and the intestinal microbiota are all actors that can contribute to this delicate balance, which, in case of a perturbation of one or more of them, can determine a disturbance of Trp metabolism pathways. Kynurenines may influence redox reactions, with consequences for some biological functions and pathologies onset [25]. Immune response is a strong stimulator of reactive oxygen species (ROS) production. In case of prolonged immune activation, the antioxidant pools are depleted. In patients with low blood antioxidant concentrations, an increase of inflammation biomarkers and Trp breakdown is observed, with consequent lower Trp levels [111]. Dopamine, epinephrine, norepinephrine, 5-HT, and nitric oxide (NO) enzymatic biosynthesis require tetrahydrobiopterin (BH4) as a cofactor, which "suffers'' the oxidation state. An "antioxidant environment" can promote BH4 activity, increasing its lifespan and neurotransmitter synthesis (5-HT, dopamine, epinephrine, and norepinephrine) explaining the reason why an antioxidant diet may be considered as a mood and cognitive abilities improver [111]. A pro-inflammatory condition seems to be associated with increased KYN production and KYN/Trp ratio, correlating with immune biomarkers enhancement, indicating higher IDO activity. Such an association has been described for neuropsychiatric disorders, for example depressive mood, led to considering the KYN pathway as a target of treatment [111]. The literature reports two interpretations about the different concentration and ratios of serum and cerebrospinal fluid (CSF) that Trp products found in patients with chronic diseases compared with healthy controls: is the KYN pathway a driving force responsible for the disease progression or a compensatory mechanism in response to the pathology [94]? It has been postulated that the timing and the duration of KYN pathway activation could be the key factor: in patients with autoimmune encephalomyelitis, a short-term IDO activation could be a compensatory action against inflammation, while a prolonged IDO stimulation has a negative effect on neuroinflammation [94].

In patients with PKU, a state of intestinal dysbiosis and a consequent pro-inflammatory condition has been reported, with potential consequence on systemic inflammation, increased oxidative stress and risk of NCDs [128]. We assume that through the MGBA, the same pro-inflammatory state may influence the complex sphere of neurocognitive and behavioral problems in PKU subjects. In this review, we explored the Trp metabolism that will depict the complexity of the relationship among Trp metabolism, inflammation, gut microbiota, and brain.

Since an alteration of Trp metabolism has been described in PKU patients supplemented with AA-MF, with accumulation of potentially neurotoxic compounds implicated in numerous neurodegenerative and inflammatory pathologies [117,124], it remains to be seen whether these observations can be rationally integrated into a framework that enables oriented alternative therapeutic targeting of the KYN route, along the MGBA, for PKU.

4.1. Novelty nutritional challenges in PKU: potential therapeutic approaches targeting the TRP metabolism

The diet plays a central role in the Trp availability and, through the modulation of the intestinal microbiota composition, impacts on Trp metabolism. The ability of the brain to synthesize 5-HT is highly influenced by the availability of Trp. Since the only source of Trp comes from diet, 5-HT synthesis in the CNS depends on the Trp amount in the GI tract and consequently in the brain. A low Trp diet leads to lower 5-HT levels in the CNS of both human and animal models [100]. PKU requires complex and life-long management. The strong recommendation to follow a lifelong diet is necessary to avoid damage from Phe long-term accumulation [66].

The contribution of gut microbiota in brain/neurological impairment observed in PKU is still unclear, but the modulation by gut microbiota in anti-inflammatory and anti-oxidative direction, thanks to the use of biotics and of improved Food for Medical Special Purposes (FSMPs) in PKU, could represent both a preventive and therapeutic novelty dietetic strategy. In particular, the supplementation of amino acids mixtures with prebiotics (non-digestible dietary components that promote a selective growth and activity of certain bacterial species) probiotics (live microorganisms) or postbiotics (functional bioactive compounds resulting from microbial fermentation), confers a health benefit for the host, restoring the gut microbiota eubiosis and so modulating the Trp metabolism [129]. FOS and galactooligosaccharides (GOS) supplementation could also have a double effect: fibers represent an alternative substrate for bacteria, leading to a reduction in Trp degradation and indolic compound amounts and an increase of SCFAs

production [109,110] with a consequent activation of 5-HT synthesis. On the other hand, as reported by MacDonald et al. [130], the supplementation of amino acid mixture with oligosaccharides (GOS/FOS), commonly present in breast milk and in healthy newborns gut, can promote the selective growth of Bifidobacteria and Lactobacilli-Enterococci. Bacteria of these genera can influence the Trp metabolism both by the endogenous 5-HT production and modulation of the TPH1 gene expression [117]. Moreover, those genera are reported to reduce KYN levels and KYN/Trp ratio in the blood circulation [119]. Further, the supplementation with probiotics encoding for enzymes able to drive Trp metabolism towards 5-HT synthesis, could be a therapeutic strategy in PKU patients. On the other hand, the dietetic approach can include the possible use of alternative protein substitutes, such as GMP and prolonged-release AA-MF.

As mentioned above, GMP is considered a useful product for the dietary management of PKU patients exerting health-promoting properties [131]. In particular, it has shown a possible prebiotic activity with beneficial effects on the gut microbiota [124,131]. GMP modifies the microbial signature in an anti-inflammatory sense promoting bacterial diversity and increasing the production of SCFAs, if compared to a diet only supplemented with Phe-free AA-MF [124,131]. SCFAs stimulate the release of 5-HT from intestinal cells [23] and modulate IDO intestinal expression suppressing the production of KYN from Trp [22,105]. In this way GMP impacts on Trp metabolism, lowering its breakdown in the KYN pathway and neurotoxic metabolites production, with a contemporary increase of 5-HT biosynthesis [125]. Furthermore, GMP expresses other biological properties with a positive impact on systemic health. It also ameliorates calcium phosphate homeostasis and bone health, with increasing levels of vitamin D [132] and it is able to mitigate oxidative stress, inflammation, lipoprotein biogenesis as well as improve insulin sensitivity on human intestinal Caco-2 cells [131], representing an appealing product for PKU patients.

Prolonged-release AA-MF simulates the physiological absorption of intact dietary proteins. The same author of the study on GMP [131] in an ongoing preclinical study showed that slow-release L-AAs are a valid and health-promoting product for the dietary management patients with PKU. The slow absorption of amino acids can reduce their plasmatic fluctuations and oxidation, likely improving the protein metabolism, the intestinal oxidative and inflammatory status and ameliorating the Trp availability for the brain [133].

Overall, protein substitutes such as GMP or prolonged-release AA-MF, with their anti-inflammatory and anti-oxidative properties [131], could therapeutically target the gut microbiota and have an indirect positive potential effect on Trp metabolism; they may represent a valid nutritional strategy for an optimal management of PKU, with a broader vision in terms of long-term health promoting in these patients.

5. Conclusion

Despite many IEMs being characterized by a brain/neurological impairment, the role of the MGBA has not been yet well investigated. The gut microbiota is a potential actor in the phenotypic expression of pathology, with an impacting role on quality of life and general health of IEMs patients. Neuropsychological and behavioral assessments are considered major aspects in the management of IEMs patients, since the CNS is one of the most important systems that can be compromised in these patients. In light of this consideration, PKU provides a model of disease where the exact interaction existing among dietary treatment, biochemical control, and neuropsychological clinical manifestations will need to be further investigated. This paper wants to explain the importance of the MGBA in this interaction, starting from the example of Trp metabolism. Further studies should consider the potential correlation between an inflammatory profile and cognitive and behavioral impairment, which has been described in early treated adults with PKU, in order to ameliorate the complex management, such as that of mental health, in patients with PKU. Intestinal inflammatory processes, mediated by selective alteration of gut microbiota towards pro-inflammatory signature would persistently activate the CNS, causing neuroinflammation with mechanisms similar to those seen in some neurodegenerative and inflammatory diseases, even in the absence of overt symptoms, potentially involving Trp-metabolism with an over-expression of the KYN pathway on the one hand and loss of protective effects of SCFAs on the other. Finally, modeling the intestinal microbiota profile through non-pharmacological nutritional interventions, based on the use of more physiological alternative protein substitutes, or specific biotics, may represent an innovative approach to improve health outcomes of PKU patients, focused on restoring gut microbiota balance, having an anti-inflammatory and anti-oxidative status as a new target of dietetic therapy in PKU.

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E.V. and M.T.C and conceptualized and reviewed the article; S.P. and C.M. collected all the data; S.P., C.M., C.C., J.Z., M.T., R.B., G.B., and C. B. drafted the manuscript; E.B. critically revised the manuscript; C.D.V., G.B. and A.B. gave the final approval. All authors read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

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