Affective symptomatology in the prodromal and early stages of dementia

Affective symptomatology in the prodromal and early stages of dementia:

The role of the kynurenine pathway and systemic inflammation

Affective symptomatology in the prodromal and early stages of dementia: The role of the kynurenine pathway and systemic inflammation

Lay-Out: Kyonghwan (Chris) Choe
Cover Design: Perla Douven, Hyun Young (Hailey) Park
Published By: Maastricht University
ISBN: 978-94-6423-951-5
Printed By: Ipskamp Maastricht

Affective symptomatology in the prodromal and early stages of dementia:

The role of the kynurenine pathway and systemic inflammation

DISSERTATION

To obtain the degree of
Doctor at Maastricht University,
on the authority of the Rector Magnificus,
Prof. dr. Pamela Habibović
in accordance with the decision of the Board of Deans,

To be defended in public on
Tuesday 6 September 2022, at 13:00 hours

by

Kyonghwan (Chris) Choe

Supervisors:
Prof. Dr. Bart P.F. Rutten
Prof. Dr. Daniël L.A. van den Hove

Co-supervisors:
Dr. Gunter Kenis
Dr. Sebastian Köhler

Assessment Committee
Prof. Dr. Jos H.H.J. Prickaerts (Chair)
Prof. Dr. Pilar Martinez-Martinez
Dr. Martin P.J. van Boxtel
Dr. Livia De Picker (University of Antwerp, Belgium)
Prof. Dr. Ellen Nollen (University Medical Center Groningen, the Netherlands)

Table of Contents

CHAPTER 1

General Introduction

Alzheimer's disease, mild cognitive impairment, and subjective cognitive decline

Dementia is an umbrella term that describes a syndrome characterized by memory loss, language problems, and disturbances in social and problem-solving abilities; all severe enough to interfere with daily life [1]. According to the World Health Organization (WHO) 2021 datasheet, around 55 million people suffer from dementia worldwide, with 10 million new cases every year. In 2030 and 2050, this number is expected to rise to 78 and 139 million, respectively. Additionally, dementia has a significant impact not only on the patient and their caretakers, but also at the socio-economic level. In 2019, the global societal cost for dementia was estimated a total of 1.3 trillion US dollars [2].

The most common type of dementia is Alzheimer's disease (AD), accounting for 60%-70% of dementia cases, followed by vascular dementia (VaD), Lewy body dementia (LBD), frontotemporal dementia (FTD) and mixed dementia [2]. Notably, individuals with mild cognitive impairment (MCI) or subjective cognitive decline (SCD) are at an increased risk for developing AD. MCI patients experience similar symptoms as dementia patients with one major difference, i.e., that the symptoms do not yet affect their day-to-day life [3]. SCD refers to individuals who notice a decline in memory or other cognitive abilities, while clinical diagnosis shows no proof of such decline [4]. A recent large meta-analysis has shown that the annual conversion rate of SCD to MCI and dementia was 2.33% and 6.67%, respectively. Additionally, longitudinal studies over 4 years reported SCD to dementia development to be 14.1%, while 26.6% of SCD individuals converted to MCI during that same time frame [5].

The main pathological phenomenon observed in dementia is damage to or loss of nerve cells and their ability to communicate with other cells. Additionally, dementia is a heterogeneous syndrome involving impaired memory and judgment, confusion, changes in personality and behavior, difficulty in speech, motor alterations, and affects patients differently depending on the affected brain region(s) [6, 7]. Furthermore, the neuropathology is different for every dementia type. For example, AD-specific learning and memory impairments are mainly associated with the accumulation of amyloid-beta (Aβ) plaques and hyperphosphorylated tau protein in the hippocampus [8]. While VaD is characterized by blood vessel and vascular related brain damage due to reduced blood flow after stroke(s) [9], LBD is caused by abnormal aggregation of α-

synuclein protein as well as Aβ and tau [10]. Lastly, the main risk factor for dementia is aging, while genetic factors are also known to play an important role [11]. For example, different *apolipoprotein E* (*APOE*) alleles have been associated with differential risk for the development of AD. There are three major *APOE* allelic variants, *ε2*, *ε3*, and *ε4*, of which *APOE*ε2* is associated with decrease risk while *APOE*ε4* is associated with increased risk of developing MCI and AD [12].

Affective symptomatology in dementia: depressive- and anxiety-like symptoms

Studies have shown that depressive- and anxiety-like symptoms are commonly observed in patients with dementia or in individuals at risk of developing dementia. Depression is characterized by chronic sadness and a lack of interest or pleasure in previously rewarding or amusing activities while anxiety is characterized by intense, over-reactive, and continuous worry and fear, and that interfere with day-to-day functioning [13]. The exact cause of these affective symptoms are unknown, but a variety of factors may be involved including biological factors such as gender, and genetic and epigenetic variation, but also environmental factors such as stress and drug exposure. For example, a meta-analysis reported that the overall prevalence of depression was 32% in MCI patients [14]. With respect to anxiety, SCD patients with anxiety symptoms showed a hazard ratio (HR) of 1.3 for developing MCI/dementia [15]. Furthermore, a recent meta-analysis by Leung et al. (2021) investigated the prevalence of depression, anxiety and apathy symptoms across dementia stages over 20 studies and reported that the prevalence rates of depressive and anxiety symptoms in mild, moderate, and severe dementia were 38%, 41%, and 37%, respectively [16].

The mechanism of the kynurenine pathway

Recent studies point towards metabolic dysregulation as a potential mechanism that could explain the intricate link between neuropsychiatric [17, 18] and neurodegenerative disorders [19-21]. Tryptophan (TRP) is one of nine essential amino acids, indicating that it can only be obtained through diet or supplements. Moreover, TRP is a precursor to the 1) kynurenine pathway (KP), e.g., as part of the biosynthesis of nicotinamide adenine dinucleotide (NAD), 2) the neurotransmitter serotonin pathway, e.g., as a part of the biosynthesis of melatonin, and 3) tryptamine pathway [22] (Figure 1).

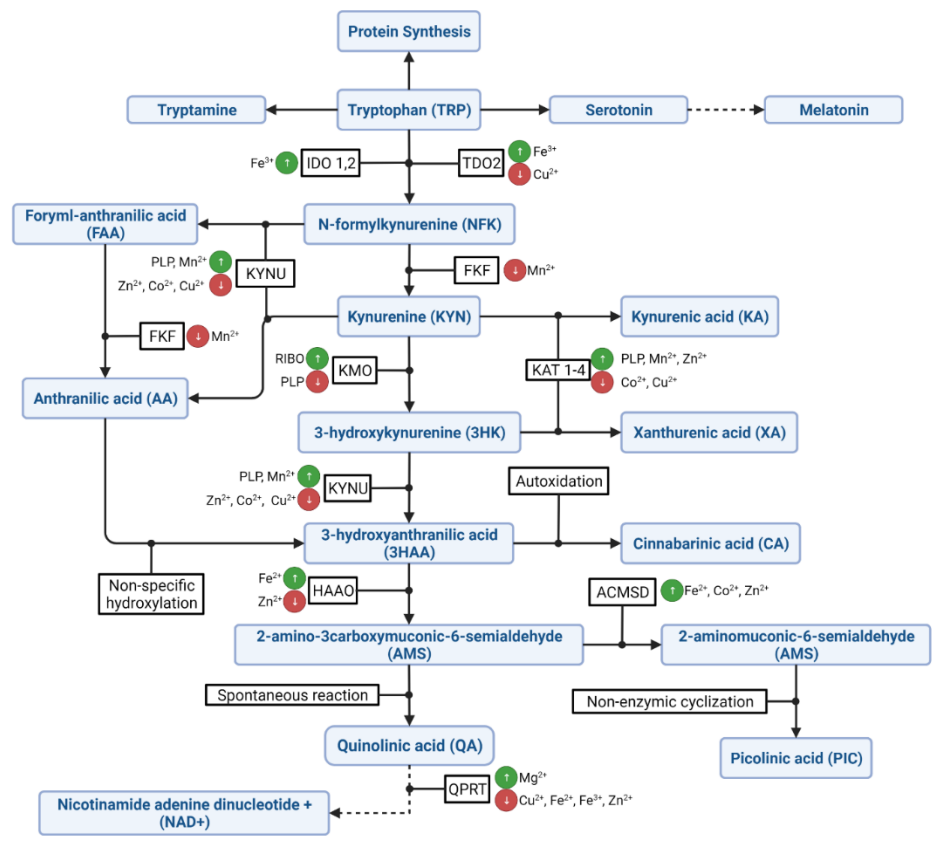


Figure 1. Overview of the kynurenine pathway. Blue boxes indicate metabolites, white boxes indicate the metabolic enzymes, green circles indicate coenzymes/cofactors that activate the enzyme, and red circles indicate coenzymes/cofactors that impair the associated enzyme. Abbreviations: IDO, indoleamine 2,3-dioxygenase; TDO2, tryptophan 2,3-dioxygenase; FKF, *N*-formylkynurenine formamides; KYNU, kynureninase; KMO, kynurenine 3-monoaxygenase; KAT, kynurenine aminotransferase; HAAO, 3-hydroxyanthranilate oxygenase; ACMSD, 2-amino-3-carboxymuconic-6-semialdehyde decarboxylase; PLP, pyridoxal phosphate; RIBO, riboflavin; Zn, zinc; Fe, iron; Co, cobalt; Cu, copper; Mn, manganese; Mg, magnesium.

The KP is the dominant pathway, accounting for about 90% of overall TRP metabolism. While it is mainly active in the liver, extrahepatic activities are also present, accounting for 5% - 10% of TRP degradation under normal physiological conditions [23]. The KP is initiated by two first and rate-limited enzymes, tryptophan 2,3-dioxygenase 2 (TDO2) and indoleamine 2,3-dixoygenase 1 and 2 (IDO1; IDO2). TDO2 is induced by cortisol and it is mainly expressed in the liver, but also present in the brain. Astrocytes, microglia, microvascular endothelial cells, and macrophages are the main cell types expressing IDO and induced by pro-inflammatory mediators [22, 24, 25]. Aside

from the aforementioned KP enzymes, there are coenzymes and cofactors that play a role in the KP [26] (Figure 1). These coenzymes are pyridoxal phosphate (PLP), active form of vitamin B6 [27-30], and riboflavin (RIBO), active form of vitamin B2 [31-33]. Furthermore, cofactors such as minerals can influence KP enzymes, which will not be discussed in-detail for this thesis [34-45], but still shown its influence in Figure 1.

The kynurenine pathway in the blood and the brain

The KP is particularly activated by inflammatory conditions. In such a scenario, unbound free TRP, which competes with other large neutral amino acids (LNAAs), is transported into the brain by the L-type amino acid transporter 1 (LAT1), located in the capillaries of the blood-brain-barrier (BBB). In the brain, TRP enters microglia or astrocyte and is converted to kynurenine (KYN), which can be further metabolized in two distinct metabolic branches, i.e., either through kynurenic acid (KA) or through quinolinic acid (QA). KYN is converted to KA through irreversible transamination by kynurenine aminotransferase (KAT) predominantly in astrocytes and neurons, while microglia and infiltrating macrophages are responsible for the production of QA [22]. Aside from the aforementioned KYN conversion inside the brain, peripheral unbound TRP can be converted to KYN and, subsequently, to 3-hydroxykynurenine (3-HK) by macrophages before passing the BBB (Figure 2).

Cytokine-induced kynurenine pathway activation

As mentioned before, inflammation has been associated with increased KP activity, especially in the brain. Multiple studies have investigated the role of pro-inflammatory cytokines on the activity of enzymes involved in the KP in healthy volunteers [46-54] and in a variety of disorders, including psychiatric [55], neurodegenerative [56], and inflammatory disorders [57, 58], cancer [59-61], respiratory disorders [62], arthritis [63], and obesity [64] (Figure 3). For example, numerous studies focus on IDO, that is the first and rate-limiting enzyme activated upon inflammatory stimulation. Studies have shown that under normal physiological or non-inflammatory conditions, IDO mRNA expression was either undetectable or very low, but upon interferon gamma (IFNγ) stimulation [46, 49, 57, 58], it is up-regulated substantially [64, 65]. Similarly, other pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin 6 (IL6), IL12, IL18, and IFNβ have been reported to up-regulate IDO expression [46, 56, 58-61], shifting the KP towards KYN. Aside from IDO

expression, pro-inflammatory cytokines can influence other downstream enzymes, reducing KAT expression and increasing KMO expression in dendritic cells and macrophages [49]. Additionally, KP metabolites, also known as kynurenines, can also affect the immune system. For example, studies have reported KYN, 3-HK, 3-HAA, and QA to induce apoptosis of T, B, neutrophils, and natural killer cells [66-68], while KA was shown to decrease TNFα and IL4 levels [69-71] (Figure 3).

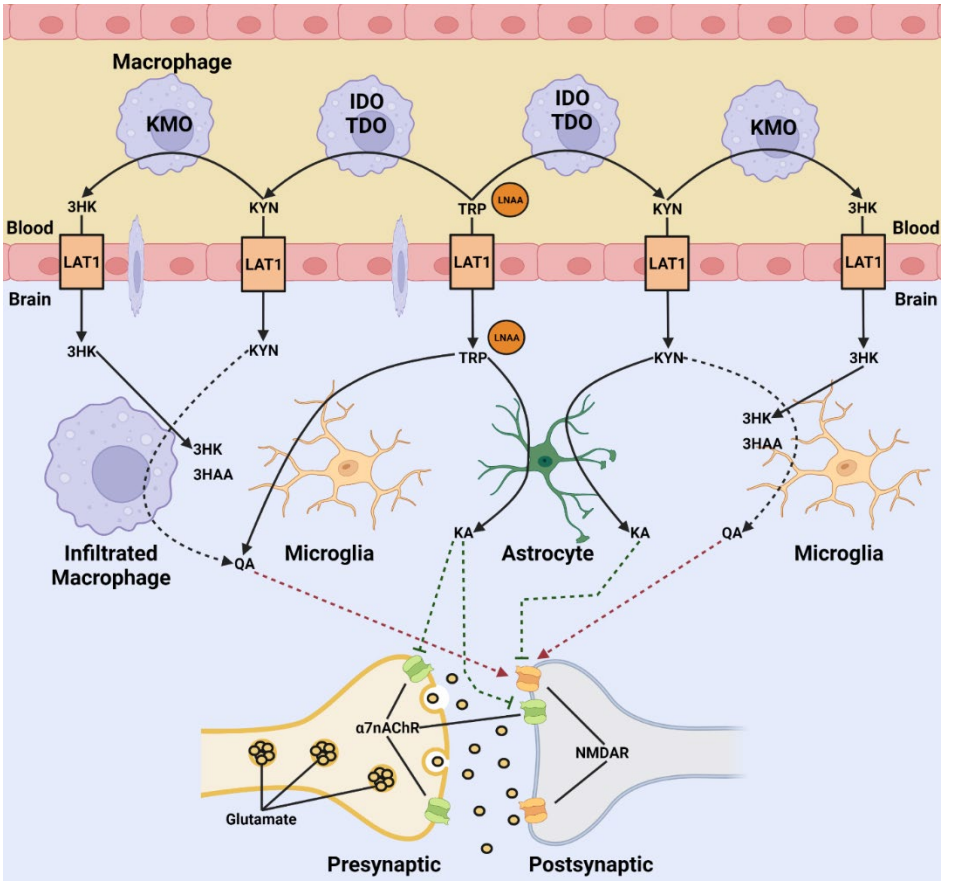


Figure 2. Overview of the kynurenine pathway in the blood and the brain. Unbound free TRP is transported to the brain by the LAT1 or, alternatively, converted to KYN and 3-HK by macrophages, before passing the BBB and entering the brain. Astrocytes and neurons are responsible for converting TRP and KYN to KA, while infiltrated macrophages and microglia produce QA. Abbreviations: BBB, blood-brain barrier; IDO, indoleamine 2, 3-dioxygenase; TDO2, tryptophan 2,3-dioxygenase; KMO, kynurenine 3-monooxygenase; TRP, tryptophan; KYN, kynurenine; 3-HK, 3-hydroxykynurenine; 3-HAA, 3-hydroxyanthranilic acid; KA, kynurenic acid; QA, quinolinic acid; α7nAChR, alpha 7 nicotinic acetylcholine receptor; NMDAR, N-methyl-D-aspartate receptor; LNAA, large neutral amino acid; LAT1, L-type amino acid transporter 1. Adapted from Schwarcz et al. (2012) [22].

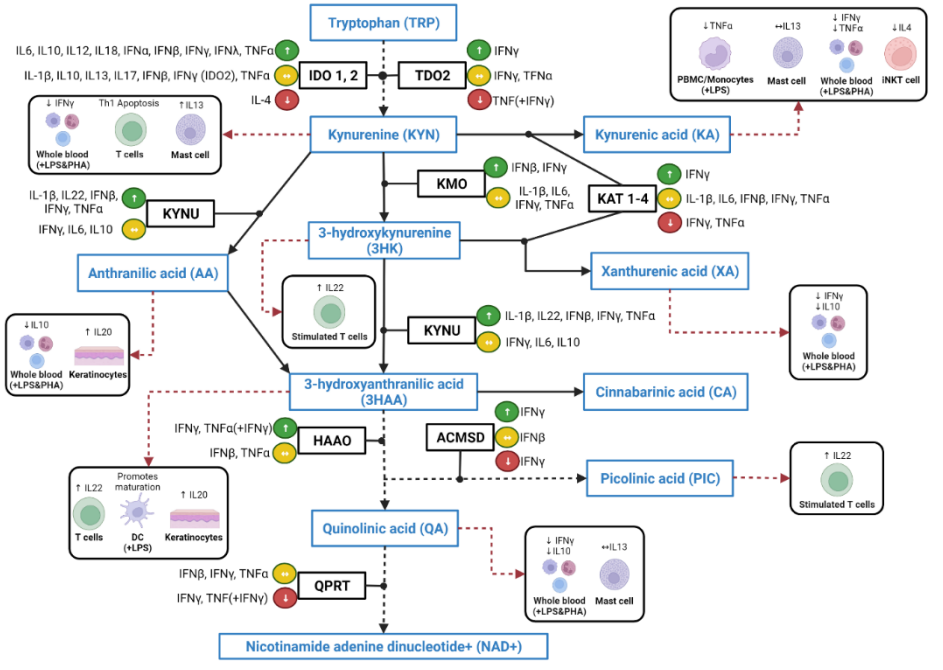


Figure 3. Overview of cytokine-induced KP enzymes and the effect of the KP on immune cells. Blue boxes indicate metabolites, black boxes indicate KP-associated enzymes, and red-dotted arrows indicate the effect of metabolites on immune cells. Cytokine-induced KP enzyme activities designated by green circles indicate increased activity, yellow arrows indicate no influence in activity, and red circles indicate reduced activity. Abbreviations: KP, kynurenine pathway; IDO, indoleamine 2,3-dioxygenase; TDO2, tryptophan 2,3-dioxygenase; KYNU, kynureninase; KMO, kynurenine 3-monoaxygenase; KAT, kynurenine aminotransferase; HAAO, 3-hydroxyanthranilate oxygenase; ACMSD, 2-amino-3-carboxymuconic-6-semialdehyde decarboxylase; QPRT, quinolinate phosphoribosyltransferase; IFN, interferon; IL, interleukin; TNFα, tumor necrosis factor alpha; LPS, lipopolysaccharide; PHA, phytohaemagglutinin; DC, dendritic cell; iNKT, invariant natural killer T; PBMC, peripheral blood mononuclear cell. Adapted from Baumgartner et al. (2019) [72].

Neuroactive properties of the kynurenine pathway metabolites

Certain kynurenines have been studied extensively in view of their neuroactive properties. KA is a well-studied metabolite owing to its neuroprotective properties. It is an antagonist of glutamatergic N-methyl-D-aspartate receptors (NMDARs), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPARs), and kainate receptors [73]. Furthermore, recently KA has been shown to inhibit α7 nicotinic acetylcholine receptors (α7nAChRs) [74]. In the periphery, KA acts as an agonist for the orphan G protein-coupled receptor 35 (GPR35) and aryl hydrocarbon receptor (AhR) [75]. Aside from its receptor properties, KA also has antioxidant properties by scavenging hydroxyl, superoxide anions and other free radicals [23]. Another well-studied metabolite

is QA, which is particularly known for its neurotoxic properties. In contrast to KA, it acts as an agonist of NMDARs, with the potential to induce excitotoxicity [23] (Figure 2).

The association between the KP and cognitive and affective disorders

Since kynurenines possess neuroactive properties and are highly active under inflammatory conditions, recent KP studies investigated the association between kynurenines and cognitive and affective disorders. For example, studies examining the serotonin hypothesis of major depression (MD) found that serotonergic dysregulation in MD patients is related to alterations in TRP metabolism through activation of the KP. It is suggested that this switch from the serotonin pathway to the KP is strongly influenced by pro-inflammatory cytokine-induced activation of IDO [76, 77]. In addition, AD pathology such as Aβ and tau also triggers an innate immune response, causing neuroinflammation in the brain [78]. Lastly, clinical studies have shown similar trends in TRP and KP metabolite concentrations for both disorders. For example, two independent systematic review and meta-analysis studies have both reported lower KA levels and no difference in 3-HK levels [17, 18], while one study reported lower TRP level in patients with depression compared to controls [17]. AD dementia studies, also reported lower TRP [21, 79-89], and KA [86, 90] levels, and no difference in 3-HK [19, 21, 83, 84, 86, 91] levels in AD dementia patients compared to controls. This was also shown in MCI studies, in which MCI patients showed lower TRP [21, 79, 92], and KA [21] levels, and no difference in 3-HK [21, 93] levels compared to controls. As such, there is an interplay between affective symptomatology and dementia and the involvement of TRP metabolic pathway, particularly the KP.

Overview of the thesis

The objectives of this thesis were to investigate the role of kynurenine pathway in different patient populations including individuals suffering from SCD, MCI, and AD dementia by identifying differences in KP-associated metabolites between patients with dementia and healthy controls, identifying whether other factors such as differential transcriptome and epigenome in TRP catabolic pathway-associated gene(s) are present in patients with AD compared to controls, and lastly, identify KP-associated metabolites and systemic inflammation markers associated with affective symptomatology, i.e., depressive- and anxiety-related symptoms in patients with or at risk of developing dementia.

CHAPTER 1 familiarizes the reader with dementia and associated risk groups, i.e., SCD and MCI, and the link between AD, depression and anxiety disorders as common comorbidities. In addition, it introduces the essential amino acid TRP, the KP as its dominant downstream metabolic pathway, as well as the potential role of the immune system in the development and course of AD and associated neurocognitive and neuropsychiatric symptomatology.

Next, in order to get a thorough understanding of KP-associated metabolite research in dementia performed to date, **CHAPTER 2** presents a systematic review and meta-analysis. It presents a summary of the available evidence for differences in TRP and KP metabolites between patients with evident cognitive impairment or dementia and neurologically healthy controls. In addition, the association between TRP and KP metabolites (in plasma, serum, cerebrospinal fluid [CSF], urine, saliva, fecal matter, and post-mortem brain tissue) and healthy aging and cognitive test scores was reviewed. Finally, a meta-analysis was performed in order to examine the effect sizes of KP metabolites in plasma, serum, and CSF when comparing AD dementia patients and controls.

In **CHAPTER 3**, we performed a transcriptomic, DNA (hydroxy)methylomic, associated gene regulatory network (GRN), and network perturbation analyses on the TRP metabolic and the NAD pathways, making use of data generated from middle temporal gyrus (MTG) tissue of AD patients and age-matched controls. Furthermore, the methylomic profiles were validated in an independent blood-based cohort, i.e., the German Study on Ageing, Cognition, and Dementia in Primary Care Patients (AgeCoDe) that compared baseline differential DNA

methylation profiles between AD converters and non-converters within 4.5 years follow-up. Based on the findings in the MTG and validation within the AgeCoDe cohort, we identified a potential candidate cytosine-phosphate-guanine (CpG) site in the *IDO2* gene, predicting the development of AD.

CHAPTERS 4 and **5** represents large cross-sectional molecular-epidemiology studies making use of various models to adjust for different factors. Previous studies have already investigated the association between KP metabolites, inflammatory and endothelial markers, and clinical symptoms in patients either suffering from a neurodegenerative or affective disorders. **CHAPTER 4** describes the first study investigating the association between plasma KP metabolites and affective symptoms associated with depression and anxiety in patients with or at risk of developing dementia (making us of the Biobank Alzheimer Centrum Limburg [BBACL] cohort). In **CHAPTER 5**, using the same cohort, we investigated the association between plasma inflammatory and endothelial markers in a similar context.

Finally, **CHAPTER 6** discussed the results of this thesis, **CHAPTER 7** summarized the main findings of each chapter, and **CHAPTER 8** discussed the scientific and societal impact in which the findings of this thesis brought.

References

1. Wallin K, Bostrom G, Kivipelto M, Gustafson Y. Risk factors for incident dementia in the very old. Int Psychogeriatr. 2013;25(7):1135-43.
2. Dementia Fact sheet: World Health Organization; 2021 [updated September 2. Available from: https://www.who.int/en/news-room/fact-sheets/detail/dementia.
3. Petersen RC. Mild cognitive impairment as a diagnostic entity. J Intern Med. 2004;256(3):183-94.
4. Jessen F, Amariglio RE, Van Boxtel M, Breteler M, Ceccaldi M, Chételat G, et al. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. Alzheimer's & dementia. 2014;10(6):844-52.
5. Mitchell A, Beaumont H, Ferguson D, Yadegarfar M, Stubbs B. Risk of dementia and mild cognitive impairment in older people with subjective memory complaints: meta-analysis. Acta Psychiatrica Scandinavica. 2014;130(6):439-51.
6. Raz L, Knoefel J, Bhaskar K. The neuropathology and cerebrovascular mechanisms of dementia. J Cereb Blood Flow Metab. 2016;36(1):172-86.
7. Ryan J, Fransquet P, Wrigglesworth J, Lacaze P. Phenotypic Heterogeneity in Dementia: A Challenge for Epidemiology and Biomarker Studies. Front Public Health. 2018;6:181.
8. Huang HC, Jiang ZF. Accumulated amyloid-beta peptide and hyperphosphorylated tau protein: relationship and links in Alzheimer's disease. J Alzheimers Dis. 2009;16(1):15-27.
9. T O'Brien J, Thomas A. Vascular dementia. The Lancet. 2015;386(10004):1698-706.
10. Walker Z, Possin KL, Boeve BF, Aarsland D. Lewy body dementias. The Lancet. 2015;386(10004):1683-97.
11. R AA. Risk factors for Alzheimer's disease. Folia Neuropathol. 2019;57(2):87-105.
12. Yamazaki Y, Zhao N, Caulfield TR, Liu C-C, Bu G. Apolipoprotein E and Alzheimer disease: pathobiology and targeting strategies. Nature Reviews Neurology. 2019;15(9):501-18.
13. Podell K, Torres K. Affective symptoms in early-onset dementia. Neurol Clin. 2011;29(1):99-114, viii.
14. Ismail Z, Elbayoumi H, Fischer CE, Hogan DB, Millikin CP, Schweizer T, et al. Prevalence of depression in patients with mild cognitive impairment: a systematic review and meta-analysis. JAMA psychiatry. 2017;74(1):58-67.
15. Liew TM. Subjective cognitive decline, anxiety symptoms, and the risk of mild cognitive impairment and dementia. Alzheimer's Research & Therapy. 2020;12(1):107.
16. Leung DKY, Chan WC, Spector A, Wong GHY. Prevalence of depression, anxiety, and apathy symptoms across dementia stages: A systematic review and meta-analysis. Int J Geriatr Psychiatry. 2021;36(9):1330-44.
17. Marx W, McGuinness AJ, Rocks T, Ruusunen A, Cleminson J, Walker AJ, et al. The kynurenine pathway in major depressive disorder, bipolar disorder, and schizophrenia: a meta-analysis of 101 studies. Mol Psychiatry. 2021;26(8):4158-78.
18. Ogyu K, Kubo K, Noda Y, Iwata Y, Tsugawa S, Omura Y, et al. Kynurenine pathway in depression: A systematic review and meta-analysis. Neurosci Biobehav Rev. 2018;90:16-25.
19. Sorgdrager FJH, Vermeiren Y, Van Faassen M, van der Ley C, Nollen EAA, Kema IP, et al. Age- and disease-specific changes of the kynurenine pathway in Parkinson's and Alzheimer's disease. Journal of Neurochemistry. 2019.
20. Oxenkrug G, van der Hart M, Roeser J, Summergrad P. Peripheral Tryptophan - Kynurenine Metabolism Associated with Metabolic Syndrome is Different in Parkinson's and Alzheimer's Diseases. Endocrinol Diabetes Metab J. 2017;1(4).
21. Whiley L, Chappell KE, D'Hondt E, Lewis MR, Jiménez B, Snowden SG, et al. Metabolic phenotyping reveals a reduction in the bioavailability of serotonin and kynurenine pathway metabolites in both the urine and serum of individuals living with Alzheimer's disease. Alzheimers Res Ther. 2021;13(1):20.

22. Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain: when physiology meets pathology. Nat Rev Neurosci. 2012;13(7):465-77.
23. Badawy AA. Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects. Int J Tryptophan Res. 2017;10:1178646917691938.
24. Salter M, Pogson CI. The role of tryptophan 2,3-dioxygenase in the hormonal control of tryptophan metabolism in isolated rat liver cells. Effects of glucocorticoids and experimental diabetes. Biochem J. 1985;229(2):499-504.
25. Guillemin GJ, Cullen KM, Lim CK, Smythe GA, Garner B, Kapoor V, et al. Characterization of the kynurenine pathway in human neurons. J Neurosci. 2007;27(47):12884-92.
26. Majewski M, Kozlowska A, Thoene M, Lepiarczyk E, Grzegorzewski WJ. Overview of the role of vitamins and minerals on the kynurenine pathway in health and disease. J Physiol Pharmacol. 2016;67(1):3-19.
27. Phillips RS, Scott I, Paulose R, Patel A, Barron TC. The phosphate of pyridoxal-5'-phosphate is an acid/base catalyst in the mechanism of Pseudomonas fluorescens kynureninase. Febs j. 2014;281(4):1100-9.
28. Okada K, Angkawidjaja C, Koga Y, Kanaya S. Structural and mechanistic insights into the kynurenine aminotransferase-mediated excretion of kynurenic acid. J Struct Biol. 2014;185(3):257-66.
29. Okuno E, Tsujimoto M, Nakamura M, Kido R. 2-Aminoadipate-2-oxoglutarate aminotransferase isoenzymes in human liver: a plausible physiological role in lysine and tryptophan metabolism. Enzyme Protein. 1993;47(3):136-48.
30. Goh DL, Patel A, Thomas GH, Salomons GS, Schor DS, Jakobs C, et al. Characterization of the human gene encoding alpha-aminoadipate aminotransferase (AADAT). Mol Genet Metab. 2002;76(3):172-80.
31. Amaral M, Levy C, Heyes DJ, Lafite P, Outeiro TF, Giorgini F, et al. Structural basis of kynurenine 3-monooxygenase inhibition. Nature. 2013;496(7445):382-5.
32. Stevens CO, Henderson LM. Riboflavin and hepatic kynurenine hydroxylase. J Biol Chem. 1959;234(5):1191-4.
33. Reyes Ocampo J, Lugo Huitrón R, González-Esquivel D, Ugalde-Muñiz P, Jiménez-Anguiano A, Pineda B, et al. Kynurenines with neuroactive and redox properties: relevance to aging and brain diseases. Oxid Med Cell Longev. 2014;2014:646909.
34. SCHARTAU W, LINZEN B. The Tryptophan 2,3-Dioxygenase of the Blowfly, Protophormia terrae-novae:Partial Purification and Characterization. 1976;357(1):41-50.
35. Serrano AE, Jr., Nagayama F. Inhibition studies on liver arylformamidases of rainbow trout and cattle. Comp Biochem Physiol B. 1991;99(2):281-5.
36. el-Sewedy SM, Abdel-Tawab GA, el-Zoghby SM, Zeitoun R, Mostafa MH, Shalaby SM. Studies with tryptophan metabolites in vitro. Effect of zinc, manganese, copper and cobalt ions on kynurenine hydrolase and kynurenine aminotransferase in normal mouse liver. Biochem Pharmacol. 1974;23(18):2557-65.
37. Zhang Y, Colabroy KL, Begley TP, Ealick SE. Structural studies on 3-hydroxyanthranilate-3,4-dioxygenase: the catalytic mechanism of a complex oxidation involved in NAD biosynthesis. Biochemistry. 2005;44(21):7632-43.
38. Calderone V, Trabucco M, Menin V, Negro A, Zanotti G. Cloning of human 3-hydroxyanthranilic acid dioxygenase in Escherichia coli: characterisation of the purified enzyme and its in vitro inhibition by Zn2+. Biochim Biophys Acta. 2002;1596(2):283-92.
39. Pucci L, Perozzi S, Cimadamore F, Orsomando G, Raffaelli N. Tissue expression and biochemical characterization of human 2-amino 3-carboxymuconate 6-semialdehyde decarboxylase, a key enzyme in tryptophan catabolism. Febs j. 2007;274(3):827-40.
40. Garavaglia S, Perozzi S, Galeazzi L, Raffaelli N, Rizzi M. The crystal structure of human alpha-amino-beta-carboxymuconate-epsilon-semialdehyde decarboxylase in complex with 1,3-dihydroxyacetonephosphate suggests a regulatory link between NAD synthesis and glycolysis. Febs j. 2009;276(22):6615-23.
41. Huo L, Liu F, Iwaki H, Li T, Hasegawa Y, Liu A. Human α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD): a structural and mechanistic unveiling. Proteins. 2015;83(1):178-87.

42. Liu H, Woznica K, Catton G, Crawford A, Botting N, Naismith JH. Structural and kinetic characterization of quinolinate phosphoribosyltransferase (hQPRTase) from homo sapiens. J Mol Biol. 2007;373(3):755-63.
43. Shibata K, Iwai K. Isolation and properties of crystalline quinolinate phosphoribosyltransferase from hog kidney. Biochim Biophys Acta. 1980;611(2):280-8.
44. Iwai K, Taguchi H. Crystallization and properties of quinolinate phosphoribosyltransferase from hog liver. Methods Enzymol. 1980;66:96-101.
45. Capece L, Lewis-Ballester A, Yeh SR, Estrin DA, Marti MA. Complete reaction mechanism of indoleamine 2,3-dioxygenase as revealed by QM/MM simulations. J Phys Chem B. 2012;116(4):1401-13.
46. Croitoru-Lamoury J, Lamoury FM, Caristo M, Suzuki K, Walker D, Takikawa O, et al. Interferon-γ regulates the proliferation and differentiation of mesenchymal stem cells via activation of indoleamine 2, 3 dioxygenase (IDO). PloS one. 2011;6(2):e14698.
47. Musso T, Gusella GL, Brooks A, Longo DL, Varesio L. Interleukin-4 inhibits indoleamine 2, 3-dioxygenase expression in human monocytes. 1994.
48. Sarkar SA, Wong R, Hackl SI, Moua O, Gill RG, Wiseman A, et al. Induction of indoleamine 2, 3-dioxygenase by interferon-γ in human islets. Diabetes. 2007;56(1):72-9.
49. Braidy N, Rossez H, Lim CK, Jugder B-E, Brew BJ, Guillemin GJ. Characterization of the kynurenine pathway in CD8+ human primary monocyte-derived dendritic cells. Neurotoxicity research. 2016;30(4):620-32.
50. Sheipouri D, Grant R, Bustamante S, Lovejoy D, Guillemin GJ, Braidy N. Characterisation of the kynurenine pathway in skin-derived fibroblasts and keratinocytes. Journal of cellular biochemistry. 2015;116(6):903-22.
51. Asp L, Johansson A-S, Mann A, Owe-Larsson B, Urbanska EM, Kocki T, et al. Effects of pro-inflammatory cytokines on expression of kynurenine pathway enzymes in human dermal fibroblasts. Journal of Inflammation. 2011;8(1):1-7.
52. Sa SM, Valdez PA, Wu J, Jung K, Zhong F, Hall L, et al. The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic adaptive immunity in psoriasis. The Journal of Immunology. 2007;178(4):2229-40.
53. Parlato S, Romagnoli G, Spadaro F, Canini I, Sirabella P, Borghi P, et al. LOX-1 as a natural IFN-α–mediated signal for apoptotic cell uptake and antigen presentation in dendritic cells. Blood, The Journal of the American Society of Hematology. 2010;115(8):1554-63.
54. Eminel S, Jin N, Rostami M, Dibbert S, Mrowietz U, Suhrkamp I. Dimethyl-and monomethylfumarate regulate indoleamine 2, 3-dioxygenase (IDO) activity in human immune cells. Experimental dermatology. 2017;26(8):685-90.
55. Johansson A-S, Owe-Larsson B, Asp L, Kocki T, Adler M, Hetta J, et al. Activation of kynurenine pathway in ex vivo fibroblasts from patients with bipolar disorder or schizophrenia: cytokine challenge increases production of 3-hydroxykynurenine. Journal of psychiatric research. 2013;47(11):1815-23.
56. Guillemin GJ, Kerr SJ, Pemberton LA, Smith DG, Smythe GA, Armati PJ, et al. IFN-β 1b Induces Kynurenine Pathway Metabolism in Human Macrophages: Potential Implications for Multiple Sclerosis Treatment. Journal of interferon & cytokine research. 2001;21(12):1097-101.
57. Belladonna ML, Grohmann U, Guidetti P, Volpi C, Bianchi R, Fioretti MC, et al. Kynurenine pathway enzymes in dendritic cells initiate tolerogenesis in the absence of functional IDO. The Journal of Immunology. 2006;177(1):130-7.
58. Harden JL, Lewis SM, Lish SR, Suárez-Fariñas M, Gareau D, Lentini T, et al. The tryptophan metabolism enzyme L-kynureninase is a novel inflammatory factor in psoriasis and other inflammatory diseases. Journal of Allergy and Clinical Immunology. 2016;137(6):1830-40.
59. Litzenburger UM, Opitz CA, Sahm F, Rauschenbach KJ, Trump S, Winter M, et al. Constitutive IDO expression in human cancer is sustained by an autocrine signaling loop involving IL-6, STAT3 and the AHR. Oncotarget. 2014;5(4):1038.

60. Liebau C, Baltzer A, Schmidt S, Roesel C, Karreman C, Prisack JB, et al. Interleukin-12 and interleukin-18 induce indoleamine 2, 3-dioxygenase (IDO) activity in human osteosarcoma cell lines independently from interferon-gamma. Anticancer research. 2002;22(2A):931-6.
61. Yu J, Wang Y, Yan F, Zhang P, Li H, Zhao H, et al. Noncanonical NF-κB activation mediates STAT3-stimulated IDO upregulation in myeloid-derived suppressor cells in breast cancer. The Journal of Immunology. 2014;193(5):2574-86.
62. Fox JM, Crabtree JM, Sage LK, Tompkins SM, Tripp RA. Interferon lambda upregulates IDO1 expression in respiratory epithelial cells after influenza virus infection. Journal of Interferon & Cytokine Research. 2015;35(7):554-62.
63. Lim J-Y, Im K-I, Lee E-S, Kim N, Nam Y-S, Jeon Y-W, et al. Enhanced immunoregulation of mesenchymal stem cells by IL-10-producing type 1 regulatory T cells in collagen-induced arthritis. Scientific reports. 2016;6(1):1-13.
64. Favennec M, Hennart B, Caiazzo R, Leloire A, Yengo L, Verbanck M, et al. The kynurenine pathway is activated in human obesity and shifted toward kynurenine monooxygenase activation. Obesity. 2015;23(10):2066-74.
65. Yeung AW, Terentis AC, King NJ, Thomas SR. Role of indoleamine 2,3-dioxygenase in health and disease. Clin Sci (Lond). 2015;129(7):601-72.
66. Hayashi T, Mo JH, Gong X, Rossetto C, Jang A, Beck L, et al. 3-Hydroxyanthranilic acid inhibits PDK1 activation and suppresses experimental asthma by inducing T cell apoptosis. Proc Natl Acad Sci U S A. 2007;104(47):18619-24.
67. Fallarino F, Grohmann U, Vacca C, Bianchi R, Orabona C, Spreca A, et al. T cell apoptosis by tryptophan catabolism. Cell Death Differ. 2002;9(10):1069-77.
68. Molano A, Illarionov PA, Besra GS, Putterman C, Porcelli SA. Modulation of invariant natural killer T cell cytokine responses by indoleamine 2,3-dioxygenase. Immunol Lett. 2008;117(1):81-90.
69. Wang J, Simonavicius N, Wu X, Swaminath G, Reagan J, Tian H, et al. Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. J Biol Chem. 2006;281(31):22021-8.
70. Maes M, Mihaylova I, Ruyter MD, Kubera M, Bosmans E. The immune effects of TRYCATs (tryptophan catabolites along the IDO pathway): relevance for depression - and other conditions characterized by tryptophan depletion induced by inflammation. Neuro Endocrinol Lett. 2007;28(6):826-31.
71. Fallarini S, Magliulo L, Paoletti T, de Lalla C, Lombardi G. Expression of functional GPR35 in human iNKT cells. Biochem Biophys Res Commun. 2010;398(3):420-5.
72. Baumgartner R, Forteza MJ, Ketelhuth DFJ. The interplay between cytokines and the Kynurenine pathway in inflammation and atherosclerosis. Cytokine. 2019;122:154148.
73. Perkins MN, Stone TW. An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. Brain Res. 1982;247(1):184-7.
74. Vecsei L, Szalardy L, Fulop F, Toldi J. Kynurenines in the CNS: recent advances and new questions. Nat Rev Drug Discov. 2013;12(1):64-82.
75. Kimura A, Naka T, Nohara K, Fujii-Kuriyama Y, Kishimoto T. Aryl hydrocarbon receptor regulates Stat1 activation and participates in the development of Th17 cells. Proc Natl Acad Sci U S A. 2008;105(28):9721-6.
76. Oxenkrug G. Serotonin-kynurenine hypothesis of depression: historical overview and recent developments. Curr Drug Targets. 2013;14(5):514-21.
77. Miura H, Ozaki N, Sawada M, Isobe K, Ohta T, Nagatsu T. A link between stress and depression: shifts in the balance between the kynurenine and serotonin pathways of tryptophan metabolism and the etiology and pathophysiology of depression. Stress. 2008;11(3):198-209.
78. Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's disease. Lancet Neurol. 2015;14(4):388-405.
79. Willette AA, Pappas C, Hoth N, Wang Q, Klinedinst B, Willette SA, et al. Inflammation, Negative Affect, and Amyloid Burden in Alzheimer's Disease: Insights from the Kynurenine Pathway. Brain Behav Immun. 2021.

80. Gonzalez-Dominguez R, Garcia-Barrera T, Gomez-Ariza JL. Metabolite profiling for the identification of altered metabolic pathways in Alzheimer's disease. Journal of Pharmaceutical and Biomedical Analysis. 2015;107:75-81.
81. Widner B, Leblhuber F, Walli J, Tilz GP, Demel U, Fuchs D. Degradation of tryptophan in neurodegenerative disorders. Adv Exp Med Biol. 1999;467:133-8.
82. Shao Y, Ouyang Y, Li T, Liu X, Xu X, Li S, et al. Alteration of Metabolic Profile and Potential Biomarkers in the Plasma of Alzheimer's Disease. Aging Dis. 2020;11(6):1459-70.
83. van der Velpen V, Teav T, Gallart-Ayala H, Mehl F, Konz I, Clark C, et al. Systemic and central nervous system metabolic alterations in Alzheimer's disease. Alzheimers Res Ther. 2019;11(1):93.
84. Giil LM, Midttun O, Refsum H, Ulvik A, Advani R, Smith AD, et al. Kynurenine Pathway Metabolites in Alzheimer's Disease. Journal of Alzheimer's Disease. 2017;60(2):495-504.
85. Trushina E, Dutta T, Persson XM, Mielke MM, Petersen RC. Identification of altered metabolic pathways in plasma and CSF in mild cognitive impairment and Alzheimer's disease using metabolomics. PLoS One. 2013;8(5):e63644.
86. Gulaj E, Pawlak K, Bien B, Pawlak D. Kynurenine and its metabolites in Alzheimer's disease patients. Advances in Medical Sciences. 2010;55(2):204-11.
87. Li NJ, Liu WT, Li W, Li SQ, Chen XH, Bi KS, et al. Plasma metabolic profiling of Alzheimer's disease by liquid chromatography/mass spectrometry. Clinical Biochemistry. 2010;43(12):992-7.
88. Fekkes D, van der Cammen TJ, van Loon CP, Verschoor C, van Harskamp F, de Koning I, et al. Abnormal amino acid metabolism in patients with early stage Alzheimer dementia. J Neural Transm (Vienna). 1998;105(2-3):287-94.
89. Watkins SE, Thomas DE, Clifford M, Tidmarsh SF. Plasma amino acids in patients with senile dementia and in subjects with Down's syndrome at an age vulnerable to Alzheimer changes. Journal of Mental Deficiency Research. 1989;33(2):159-66.
90. Hartai Z, Juhasz A, Rimanoczy A, Janaky T, Donko T, Dux L, et al. Decreased serum and red blood cell kynurenic acid levels in Alzheimer's disease. Neurochemistry International. 2007;50(2):308-13.
91. Jacobs KR, Lim CK, Blennow K, Zetterberg H, Chatterjee P, Martins RN, et al. Correlation between plasma and CSF concentrations of kynurenine pathway metabolites in Alzheimer's disease and relationship to amyloid-β and tau. Neurobiology of Aging. 2019;80:11-20.
92. Ramos-Chavez LA, Roldan-Roldan G, Garcia-Juarez B, Gonzalez-Esquivel D, Perez de la Cruz G, Pineda B, et al. Low serum tryptophan levels as an indicator of global cognitive performance in nondemented women over 50 years of age. Oxidative Medicine and Cellular Longevity. 2018;2018 (no pagination).
93. González-Sánchez M, Jiménez J, Narváez A, Antequera D, Llamas-Velasco S, Martín AH, et al. Kynurenic Acid Levels are Increased in the CSF of Alzheimer's Disease Patients. Biomolecules. 2020;10(4).

CHAPTER 2

Relation of the kynurenine pathway with age and cognitive functioning, and differences in patients with cognitive impairment or dementia: a systematic review and meta-analysis

Kyonghwan Choe[1†], Lieke Bakker[1†], Daniël L.A. van den Hove[1, 2], Simone, J.P.M. Eussen[3, 4], Gunter Kenis[1], Inez H.G.B. Ramakers[1], Bart P.F. Rutten[1], Frans R.J. Verhey[1], Sebastian Köhler[1]

†Authors contributed equally to this work.

1 *Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience (MHeNs) and European Graduate School of Neuroscience (EURON), Faculty of Health, Medicine and Life Sciences (FHML) Maastricht University, Maastricht, the Netherlands.*

2 *Department of Psychiatry, Psychosomatics and Psychotherapy, University of Würzburg, Würzburg, Germany.*

3 *Department of Epidemiology, Maastricht University, Maastricht, the Netherlands.*

4 *School for Cardiovascular Diseases (CARIM) and Care and Public Health Research Institute (CAPHRI), Maastricht University, Maastricht, the Netherlands.*

Abstract

Studies suggest that the kynurenine pathway (KP) is involved in the pathophysiological processes associated with dementia, while alterations in KP metabolites have also been associated with age, the most important risk factor for dementia. However, as clinical studies reported contradictory results and often do not correct for important confounders, the role of the KP in dementia remains unclear. Therefore, the aims of this systematic review and meta-analysis were to summarize the available evidence for i) differences in KP metabolites in patients with evident cognitive impairment, ii) associations of KP metabolites with healthy aging and cognitive functioning. English, full length articles published in PubMed, Embase, PsycINFO, or the Cochrane Database of Systematic Reviews with a prospective, cross-sectional, case-control or randomized controlled study design, up to April 21 2021, were included. A random effect meta-analysis of standardized mean differences (SMD) was performed. Heterogeneity, meta-regression, small study bias, and study quality assessments were carried out. Out of 7233 retrieved studies, 103 were eligible for the systematic review. Twenty six studies were included in meta-analysis comparing patients with Alzheimer's disease (AD) dementia with neurologically healthy controls and showed that AD dementia patients had lower blood levels of tryptophan (SMD = -0.42 [95% CI -0.55, -0.29]), kynurenic acid (-0.37 [-0.53, -0.21]), xanthurenic acid (-0.40 [-0.59, -0.21]), and anthranilic acid (-0.33 [-0.63, -0.03]). Quinolinic acid displayed a tendency towards lower blood levels in AD dementia, but this did not reach statistical significance. Studies included in the systematic review suggest that age is associated with lower blood levels of tryptophan, whereas blood levels of kynurenine and the kynurenine-tryptophan ratio and cerebrospinal fluid levels of kynurenic acid and quinolinic acid were generally higher with increasing age. Associations with cognitive test scores were inconclusive and generally non-significant. These results suggest alterations in the KP metabolism in AD, but challenge current assumptions of higher quinolinic acid blood levels.

Keywords: kynurenine pathway, dementia, Alzheimer's disease, aging, cognitive impairment

Introduction

Dementia is a syndrome characterized by cognitive decline in multiple domains resulting in dysfunction in activities of daily living, and has a considerable impact on patients, caregivers and society overall [1, 2]. According to the World Health Organization (WHO), around 55 million people lived with dementia worldwide in 2021, with 10 million new cases every year [3]. Alzheimer's disease (AD) dementia is the most common type of dementia, but there are many others [3]. Additionally, aging is the major risk factor for developing dementia, but other factors play a role as well, including lifestyle, inflammation, oxidative stress and metabolic dysregulation [4, 5]. In normal aging, patients experience mild decrease in short term memory and mild accumulation of amyloid plaque while in pathological aging such as AD, patients experience severe short and long term memory loss and severe accumulation of plaques [6].

Amongst others, recent studies point towards a role for the kynurenine pathway (KP) as a common pathway involved in the pathophysiology of several neurological and psychiatric disorders including AD, Parkinson's disease (PD), Huntington's disease (HD), schizophrenia, and major depressive disorder (MDD) [7-10]. The KP (Figure 1) is the dominant pathway of tryptophan (TRP) degradation, accounting for more than 90% of TRP metabolism both in the periphery and in the central nervous system [11]. Kynurenine (KYN) is the central KP metabolite which can be further degraded into downstream metabolites, collectively called kynurenines, i.e., kynurenic acid (KA), 3-hydroxykynurenine (3-HK) and anthranilic acid (AA). 3-HK can be further metabolized to xanthurenic acid (XA) via kynurenine aminotransferase (KAT), or to 3-hydroxyanthranilic acid (3-HAA) via kynureninase (KYNU). 3-HAA, in turn, is synthesized to quinolinic acid (QA) and picolinic acid (PIC). Finally, QA is metabolized into nicotinamide adenine dinucleotide (NAD^+). Kynurenines have been implicated to have diverse effects, with some having neurotoxic (e.g., QA) and others having neuroprotective (e.g., KA) properties [12]. Associations between kynurenines and age are recently gaining more attention [13-16].

Clinical studies suggest that kynurenines are altered in blood [17-20] and cerebrospinal fluid (CSF) [8, 18, 21, 22] of patients with dementia, with a tendency towards higher neurotoxic load. Age is the main confounder in clinical studies, but studies do not always control for it, which might explain

contradictory findings. In this systematic review and meta-analysis, we investigated 1) differences in levels of TRP and kynurenines in patients with evident cognitive impairment compared to neurologically healthy controls, and 2) associations between metabolite levels and cognitive functioning and normal aging.

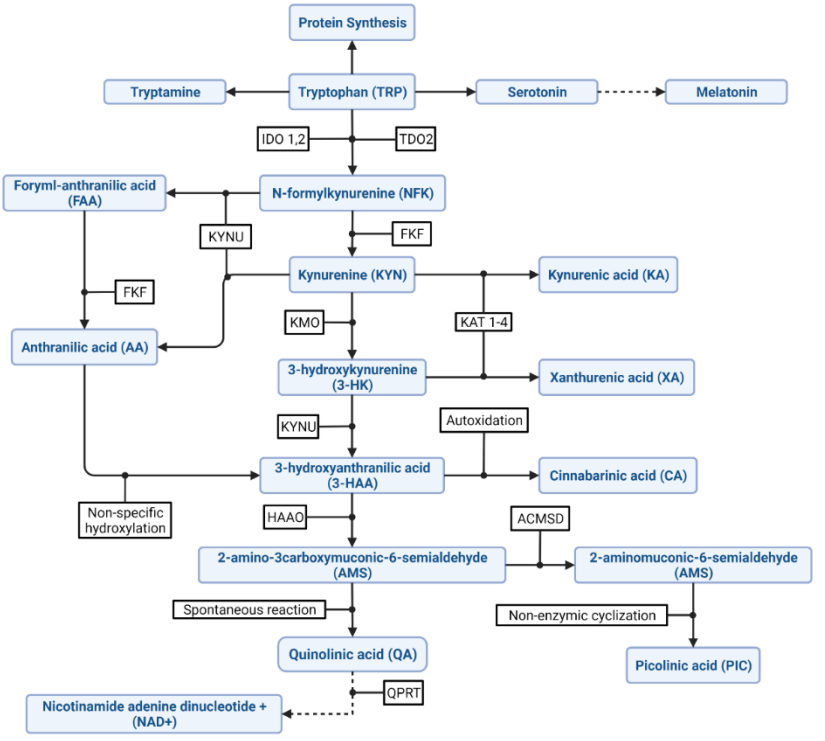


Figure 1. Overview of the kynurenine pathway. Blue boxes indicate metabolites and white boxes indicate the metabolic enzymes. Abbreviations: IDO, indoleamine 2,3-dioxygenase; TDO2, tryptophan 2,3-dioxygenase; FKF, *N*-formylkynurenine formamides; KYNU, kynureninase; KMO, kynurenine 3-monoaxygenase; KAT, kynurenine aminotransferase; HAAO, 3-hydroxyanthranilate oxygenase; ACMSD, 2-amino-3-carboxymuconic-6-semialdehyde decarboxylase.

Methods

Literature Search

This systematic review and meta-analysis was performed according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 guidelines [23, 24]. A comprehensive systematic literature search was done using PubMed, Embase, PsycINFO, and the Cochrane Database of Systematic Reviews. An initial search was done to include studies published

through November 6 2019, and an updated search was performed to include studies published until April 21 2021. The final search term was as follows*: (tryptophan OR kynuren* OR anthranil* OR xanthurenic OR cinnabar* OR Picolinic OR Quinolinic) AND (Alzheim* OR dementia OR demented OR cogn* OR neurocogn* OR memory OR amnestic OR amnesia OR neuropsychol* OR aging)*. No filters were used during the search. Additionally, a snowballing systematic literature approach, by means of screening the references in the selected studies, was carried out. This study was registered in the International Prospective Register of Systematic Reviews (PROSPERO) database on March 11, 2020 (ID: CRD42020159274).

Study selection

Two main reviewers (K.C. and L.B.) independently read and assessed the eligibility of the articles based on title and abstract using Endnote x9 software, followed by assessment of the full text, where applicable. Differences in opinion were resolved through structured discussion or by consulting a third reviewer (S.K.). Data was extracted using a pre-specified data extraction form (Supplementary Appendix S1). Missing data were handled by contacting the corresponding author, and if the corresponding author did not reply after two reminders or could not provide the information, the paper was excluded from the systematic review and/or meta-analysis. Out of 38 data requests, data for seven articles (18%) were received.

Study inclusion and exclusion

English full-length articles were included if they:

1. Measured TRP or one of the KP metabolite(s): N-formylkynurenine (N-f-KYN), KYN, 3-HK, KA, XA, AA, formyl-anthranilic acid, 3-HAA, cinnabarinic acid (CA), PIC, or QA;
2. Represented either a prospective cohort study, a cross-sectional study, a case-control study, or a randomized controlled trial;
3. Involved human participants and their biomaterials (CSF, plasma, serum, red blood cells, saliva, urine, feces, post-mortem tissues);
4. Included patients with subjective cognitive decline (SCD), mild cognitive impairment (MCI), or dementia (AD, vascular dementia [VaD], Creutzfeldt-Jakob disease, Lewy body dementia [LBD], frontotemporal dementia [FTD], HD, PD, Korsakoff syndrome, mixed dementia), with or without neuropsychiatric symptoms (e.g., depression);

5. Compared patients to neurologically healthy control participants or compared patients with different levels of cognitive decline or different types of dementia;
6. Investigated associations between metabolites and cognitive test scores in patients or controls, or between metabolites and age in neurologically healthy individuals.

Studies were excluded in cases where a clinical diagnosis was absent.

Quality assessment

Study quality was assessed using the Newcastle-Ottawa Scale (NOS) for case-control studies or cohort studies (Supplementary Table S14) [25]. An adapted version was used for cross-sectional data and data comparing different patient groups (Supplementary Appendix S2 and S3) [26]. The scales consist of three categories (Selection, Exposure, and Comparability), of which a maximum of one star for each numbered item within the category (Selection n = 4, Exposure n = 2, Comparability n = 3) can be awarded, nine in total.

Meta-analysis

Meta-analyses were done in STATA 17.0 with the *metan* package. To investigate differences in metabolite levels between cases and controls, a random effect model was used that produced standardized mean difference (SMD) and its 95% confidence interval (95% CI). For this, the mean, standard deviation (SD), and sample size (n) were used. If a study reported standard error of the mean (SEM) instead, the SD was calculated using the following equation: $SD = SEM * \sqrt{n}$. Additionally, if a study reported the metabolites in weight/volume (e.g., µg/mL), the unit was converted to the correct molarity (µM or nM) using the molecular weight. Meta-analyses were only performed on metabolites reported in at least three independent articles. Effect size was interpreted using the guidelines from Cohen: small, SMD = 0.2; moderate, SMD = 0.5; and large, SMD = 0.8, with positive values indicating elevated metabolite levels in cases [27]. A p-value < 0.05 was considered statistically significant in two-sided tests.

Heterogeneity and publication bias

Heterogeneity of the included studies was assessed by I^2 and Cochrane's Q-tests [28-30]. Since there is no golden standard in interpreting the I^2 value, the

Cochrane Handbook for Systematic Reviews of Interventions version 6.2, 2021 (section 10.10.2) was used as a rough guideline [31]:

- 0% to 40%: might not be important;
- 30% to 60%: may represent moderate heterogeneity;
- 50% to 90%: may represent substantial heterogeneity;
- 75% to 100%: considerable heterogeneity

Small study bias was examined by funnel plots and Egger's regression tests. A conservative p-value < 0.1 was considered statistically significant, as previously described [32]. Bi- and multivariable meta-regressions were performed with metabolites reported in at least three independent studies with pooled estimates showing an $I^2 > 50\%$.

Meta-regression

A Knapping-Hartung modification analysis was done to investigate potential sources of heterogeneity, including year of publication, overall sample's mean age (if not provided, this was calculated by formula $=\frac{n1x1+n2x2}{n1+n2}$, where n_i is subsample (e.g., AD dementia, controls) size and x_i is the subsample's mean age), sex (percentage female), biomaterial (CSF/ blood, CSF/ plasma/serum, or plasma/serum), analytical technique (high-performance liquid chromatography [HPLC]/enzyme-linked immunosorbent assay [ELISA]/liquid chromatography with tandem mass spectrometry [LC-MS/MS]/others), and, in plasma samples, type of anticoagulant tube (heparin/ ethylenediaminetetraacetic acid [EDTA]/others). As insufficient information was available about education level, kidney function, or dementia severity (e.g., mini mental state examination [MMSE], Clinical Dementia Rating Scale [CDR]), assessment of these variables was not possible. Residual variation (I^2_{res}), adjusted (adj.) R^2, tau^2 (τ_2), 2-tailed p-value, F test p-value (Prob. > F), and parameter estimates were reported.

Certainty assessment

This review did not perform a certainty assessment using the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) guideline because it does not represent frameworks for developing and presenting summaries for making clinical practice recommendations. Nevertheless, this study provides class II (moderately low risk) evidence.

Results

Characteristics of the included studies

After de-duplication, 7233 abstracts were retrieved from the search, of which 140 full-text studies were assessed for eligibility, and 74 were finally included in the qualitative synthesis. An additional 29 studies were found through reference lists, review papers or were sent by authors contacted for other reasons as part of the systematic review. This yielded a total of 103 studies for the systematic review, of which 26 studies met the meta-analysis criteria (Figure 2). Of these 103 articles, 68 studies compared metabolite levels between patients with cognitive impairment or dementia and neurologically healthy controls. Forty-one studies reported associations with normal aging, and 19 studies reported associations between kynurenines and cognitive test scores. These studies were not mutually exclusive. Data included in this systematic review consisted of case-control data (n = 64), data comparing different patient groups (n = 3), cross-sectional data (n = 33) and prospective data (n = 3). In most studies, TRP or KYN were determined, with only a few studies investigating the downstream metabolites. A detailed overview of all the included studies in this systematic review is provided in Supplementary Table S1.

Systematic review of prospective studies on dementia risk

There were only two prospective studies investigating associations between kynurenines and dementia risk: Chouraki et al. (2017) [36] and Toledo et al. (2017) [37] both investigated associations between metabolite levels and the risk for AD dementia. In the former, higher AA plasma levels were associated with a greater risk of both incident all-type dementia and AD dementia after a mean follow-up of 15.8 ± 5.2 years, while correcting for age, gender, education, APOE ε4 status, and total homocysteine levels. Higher levels of plasma KYN/TRP ratio (KTR) were associated with a greater risk of dementia, but not AD dementia. 3-HAA showed a tendency for an association with a lower risk of dementia and AD dementia, but did not reach statistical significance. No associations were found for TRP, KYN, KA, XA, or QA [36]. Similarly, in the latter study, Toledo et al (2017) found no association between serum levels of TRP and KYN with risk of conversion from MCI to AD dementia after 3 years, while correcting for age, gender, APOE ε4 status, and education [37]. No other metabolites were measured.

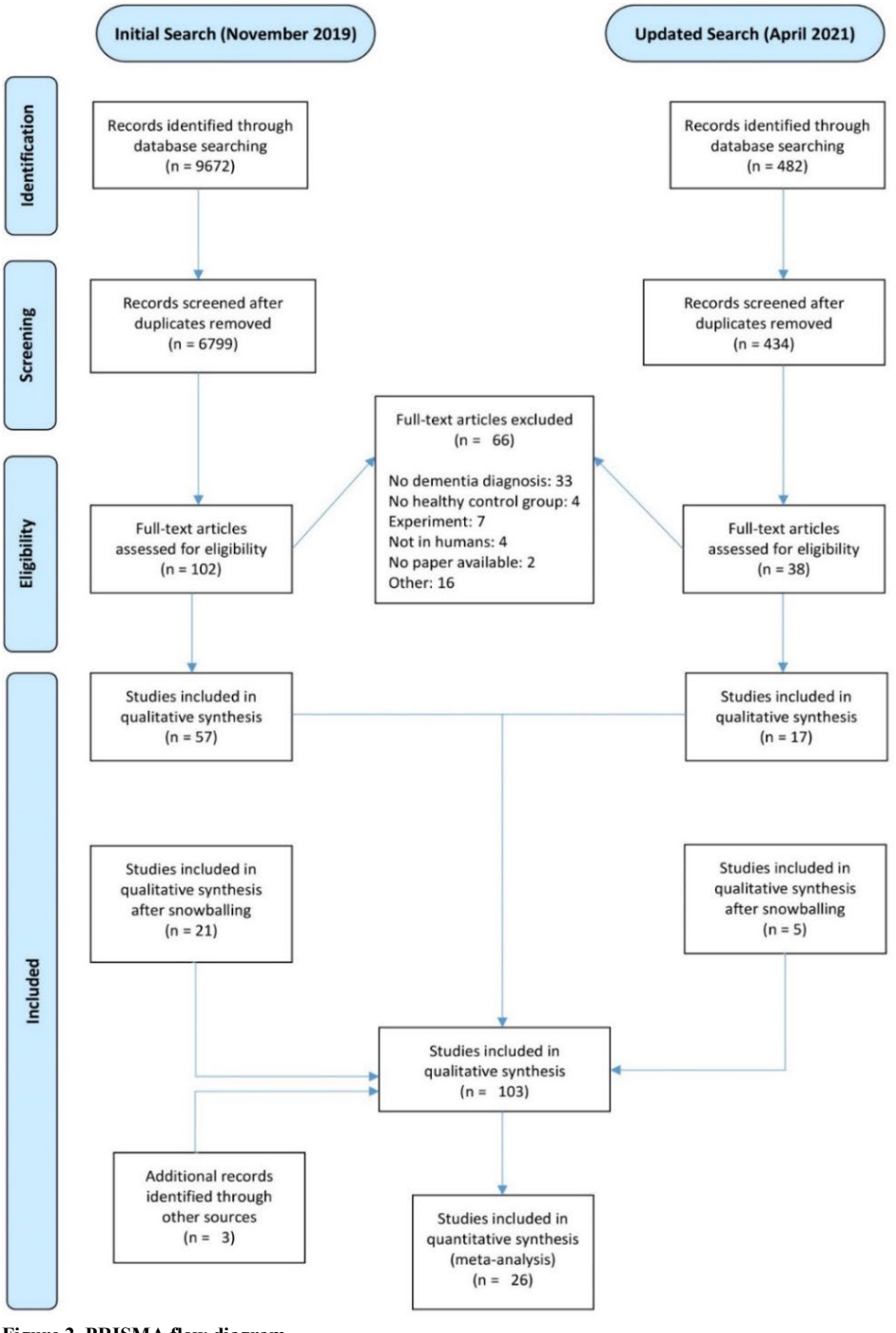


Figure 2. PRISMA flow diagram.

Systematic review of case-control studies in CSF and blood

Case-control studies measuring kynurenines in CSF

Case-control studies with kynurenines measured in CSF were found for AD dementia (n = 19 studies [8, 18, 21, 22, 38-52]), VaD (n = 1 [49]), LBD (n = 1 [38]), FTD (n = 2 [21, 53]), and MCI (n = 2 [21, 40]) (Table 1; Supplementary Table S2). In studies comparing AD dementia patients with neurologically healthy controls, TRP (n = 15 studies [8, 18, 21, 22, 39-44, 46-50]), KYN (n = 8 studies [8, 18, 40, 42, 43, 47, 48, 50]) and KA (n = 8 studies [8, 18, 21, 22, 38, 45, 48, 51]) were, respectively, the most and second most commonly measured metabolites. The majority of studies with TRP and KYN reported non-significant differences [8, 18, 21, 22, 39-44, 46-49], while only one study [50] reported significant lower levels of TRP and KYN in AD dementia patients. KA showed inconsistent associations when comparing AD dementia patients to controls, with studies showing higher (n = 4 [18, 21, 22, 45]), lower (n = 2 [8, 48]) or no differences (n = 2 [38, 51]) in concentrations. Other downstream kynurenines and ratios were less frequently studied. 3-HK levels were either found to be lower or not significantly different in AD dementia [8, 18, 47, 50], whereas QA levels were higher in one study [22] but not significantly different from controls in all others [8, 18, 48, 52]. All studies investigating other downstream kynurenines (XA, AA, 3-HAA, PIC) and KTR reported no significant differences between AD dementia patients and controls [8, 18, 22, 40, 43, 50].

Studies in patients with other dementia disorders (VaD, FTD and LBD) reported no significant differences in any of the measured metabolites compared to controls [21, 38, 49, 53]. Studies in MCI patients reported an increase in KYN, KA, and KTR levels, as well as a decrease or no significant difference in TRP levels compared to controls [21, 39, 40].

Table 1. Differences in CSF TRP and KP metabolites between cases and controls

Study	Cases			Controls			Covariates in analyses	Metabolites									
	N	Age	% female	N	Age	% female		TRP	KYN	3-HK	KA	XA	AA	3-HAA	QA	PIC	KTR
Alzheimer's disease dementia																	
González-Sánchezal (2020)[21]	Mod (20)	73.3 ± 7.2	65.0	23	64.7 ± 10.8	34.8	None	ns	-	-	↑	-	-	-	-	-	-
	Mild (41)	71.9 ± 8.1	53.7					ns	-	-	↑	-	-	-	-	-	-
van der Velpen (2019)[22]	40	74.9 ± 6.4	60.0	34	65.4 ± 6.2	67.7	None	ns	-	-	↑	-	ns	-	↑	-	-
Jacobs (2019)[18]	20	77.9 ± 7.5	55.0	18	73.1 ± 7.9	16.7	Sex	ns	ns	ns	↑	-	ns	ns	ns	ns	ns
Sorgdrager (2019)[8]	33	73.7 ± 6.0	54.5	39	71.3 ± 10.7	53.8	Age, sex	ns	ns	ns	↓	ns	-	-	ns	-	-
Wennström (2014)[38]	19	75.0	52.6	20	76.0	50.0	None	-	-	-	ns	-	-	-	-	-	-
Ibanez (2013)[39]	21	69 ± 9.6	71.4	21	58 ± 8.9	57.1	None	ns	-	-	-	-	-	-	-	-	-
Kaddurah-Daouk (2013)[40]	40	69.0	75.0	38	69.5	66.8	unk	ns	ns	-	-	-	-	-	-	-	ns
Trushina (2013)[41]	15	82.7 ± 4.2	20.0	15	78.6 ± 3.5	33.3	unk	ns	-	-	-	-	-	-	-	-	-
Fonteh (2007)[44]	8	77.9 ± 7.4	50.0	8	79.5 ± 5.5	50.0	None	ns	-	-	-	-	-	-	-	-	-

Czech (2012)[42]	Light to mild (MMSE > 22) (53)	69.7 ± 9.5	56.6	51	63.1 ± 7.7	52.9	Age, sex	ns	ns	-	-	-	-	-	-	-	-
	Mod to strong (MMSE 14-22) (26)	69.6 ± 10.1	53.8					ns	ns	-	-	-	-	-	-	-	-
Kaddurah-Daouk (2011)[43]	15	80.0 ± 1.1	73.0	15	82.0 ± 8.8	73.0	Clinical/ demographic measures, compound ratios	ns	ns	-	-	-	-	ns	-	-	-
Baran (1999)[45]	2	73.2	unk	5	72.3 ± 7.8	unk	None	-	-	-	↑	-	-	-	-	-	-
Molina (1998)[46]	37	70.9 ± 8.5	54.1	32	67.9 ± 9.2	53.1	None	ns	-	-	-	-	-	-	-	-	-
Tohgi (1995)[47]	15	68.0 ± 6.0	unk	10	68.5 ± 6.1	unk	None	ns	ns	↓	-	-	-	-	-	-	-
Heyes (1992)[48]	39	63.8 ± 1.2	unk	30	59.1 ± 14.2	unk	None	ns	ns	-	↓	-	-	-	ns	-	-
Martinez (1993)[49]	13	68.0 ± 6.0	69.2	15	66.0 ± 8.0	46.7	None	ns	-	-	-	-	-	-	-	-	-
Tohgi (1992)[50]	14	68.4 ± 10.1	unk	10	68.5 ± 6.1	unk	None	↓	↓	↓	-	-	-	-	-	-	ns
Beal (1990)[51]	9	76.7 ± 7.2	unk	50	43.8 ± 3.2	unk	None	-	-	-	ns	-	-	-	-	-	-
Mourdian (1989)[52]	35	64.0 ± 5.9	unk	23	65.0 ± 9.6	unk	None	-	-	-	-	-	-	-	ns	-	-

Table 1. (Continue)

Study	Cases			Controls			Covariates in analyses	Metabolites									
	N	Age	% female	N	Age	% female		TRP	KYN	3-HK	KA	XA	AA	3-HAA	QA	PIC	KTR
Vascular dementia																	
Martinez (1993)[49]	13	71.0 ± 6.0	46.2	15	66.0 ± 8.0	46.7	None	ns	-	-	-	-	-	-	-	-	-
Lewy body dementia																	
Wennström (2014)[38]	18	77.0	55.6	20	76.0	50.0	None	-	-	-	ns	-	-	-	-	-	-
Frontotemporal dementia																	
Janssens (2020)[53]	39	67.4 ± 11.6	48.7	25	67.3 ± 8.1	44.0	None	ns	ns	ns	ns	ns	ns	-	ns	ns	ns
González-Sánchez (2020)[21]	8	66.4 ± 5.2	37.4	23	64.7 ± 10.8	34.8	None	ns	-	-	ns	-	-	-	-	-	-
Mild cognitive impairment																	
González-Sánchez (2020)[21]	24	72.0 ± 7.1	58.4	23	64.7 ± 10.8	34.8	None	ns	-	-	↑	-	-	-	-	-	-
Kaddurah-Daouk (2013)[40]	36	69.9	52.8	38	69.5	66.8	unk	↓	↑	-	-	-	-	-	-	-	↑

↑ Sig. higher in cases, ↓ Sig. lower in cases, *ns* non-significant, *unk* unknown, - metabolite not measured. Abbreviations: CSF, cerebrospinal fluid; Sig., significant; SCD, subjective cognitive decline; Mod, moderate; TRP, tryptophan; KYN, kynurenine; KA, kynurenic acid; AA, anthranilic acid; 3-HK, 3-hydroxykynurenine; 3-HAA, 3-hydroxyanthranilic acid; XA, xanthurenic acid; QA, quinolinic acid; PIC, picolinic acid; KTR, kynurenine-tryptophan ratio.

Case-control studies measuring kynurenines in blood

Case-control studies investigating metabolite levels in blood (plasma/serum) consisted of patients with AD dementia (n = 30 studies [8, 17-22, 37, 41, 44-46, 49, 54-70]), VaD (n = 1 [49]), LBD (n = 1 [70]), FTD (n = 3 [53, 70, 71]), all type dementia (n = 3 [72-74]), MCI and AD dementia combined (n = 2 [75, 76]), MCI (n = 10 [19-21, 37, 41, 54, 69, 77, 78]), and post-stroke cognitive impairment (PSCI) (n = 2 [79, 80]). Two studies compared AD dementia patients with SCD (Table 2; Supplementary Table S3) [81, 82].

TRP (n = 24 studies [8, 17-22, 37, 41, 44, 46, 49, 56, 57, 60, 62-70]) and KYN (n = 14 studies [8, 17-20, 22, 37, 55, 56, 59-63]) were again the most and second most commonly measured metabolites in studies comparing patients with AD dementia to healthy controls, respectively. Studies on TRP either reported no difference (n = 12 [8, 18, 21, 37, 44, 46, 49, 56, 62, 64, 66, 70]) or lower levels (n = 12 [17, 19, 20, 22, 41, 57, 60, 63, 65, 67-69]) in AD dementia. Results for KYN were inconsistent, with most studies reporting no significant difference (n = 10 [8, 18, 20, 22, 37, 56, 60-63]), whereas some reported higher (n = 2 [55, 59]) or lower (n = 2 [17, 19]) levels in AD dementia. Studies investigating differences in KA (n = 11 [8, 17-19, 21, 22, 45, 56, 60, 61, 82]) and QA (n = 6 [8, 17-19, 22, 60]), the more commonly studied downstream metabolites, were inconclusive, although most studies reported no differences [8, 18, 19, 21, 22, 56, 82]. Interestingly, many other downstream kynurenines exhibited more consistent findings (Table 2 and Supplementary Table S3). 3-HK, PIC, and KTR showed either higher levels in AD dementia or no significant difference [8, 17-20, 22, 54, 56, 58, 60, 62, 63], whereas for XA and AA the opposite trend was reported with either lower levels in AD dementia or also no significant difference [8, 17-19, 56, 60]. Only 3-HAA levels were consistently lower in AD dementia [17-19]. Studies in other dementia disorders were limited.

Table 2. Differences in plasma and serum TRP and KP metabolites between

Study	Cases			Controls			Blood type	Covariates in analyses
	N	Age	% female	N	Age	% female		
Alzheimer's disease dementia								
Whiley (2021)[19]	103	76.5 ± 6.0	51.5	86	75.9 ± 5.2	48.8	S	None
Willette (2021)[20]	112	74.8 ± 8.1	42.0	58	75.1 ± 5.8	48.3	S	Age, sex
Sorgdrager (2019)[8]	33	73.7 ± 6.0	54.5	39	71.3 ± 10.7	53.8	S	Age, sex
Atukeren (2017)[55]	14	78.9 ± 8.0	42.9	32	77.3 ± 6.7	56.3	S	None
Oxenkrug (2017)[56]	20	unk	60.0	24	unk	50.0	S	None
Toledo (2017)[37]	175	75.6	51.4	199	75.3	50.3	S	Age, gender, education, APOE ε4 status
González-Dominguez (2015a)[57]	23	79.2 ± 5.9	65.2	21	72.1 ± 5.4	57.1	S	None
González-Dominguez (2015b)[58]	30	80.3 ± 5.0	60.0	30	73.5 ± 5.9	66.7	S	None
González-Dominguez (2014)[59]	22	78.5 ± 5.0	54.6	18	70.7 ± 4.1	61.1	S	None
Tsuruoka (2013)[70]	3	64.3 ± 16.9	0.0	9	68.1 ± 13.7	100	S	None
Widner (2000)[62]	21	74.4 ± 5.4	71.4	20	73.4 ± 7.4	50.0	S	None
Baran (1999)[45]	1	73.2	unk	4	72.3 ± 7.8	unk	S	None
Widner (1999)[63]	24	unk	unk	unk	unk	unk	S	None
Martinez (1993)[49]	13	68.0 ± 6.0	69.2	15	66.0 ± 8.0	46.7	S	None
González-Sánchez (2020)[21]	Mod (20) Mild (41)	73.3 ± 7.2 71.9 ± 8.1	65.0 53.7	23	64.7 ± 10.8	34.8	P	None

cases and controls

Metabolites										
TRP	**N-f-KYN**	**KYN**	**3-HK**	**KA**	**XA**	**AA**	**3-HAA**	**QA**	**PIC**	**KTR**
↓	-	↓	ns	ns	↓	-	ns	ns	ns	ns
↓	-	ns	-	-	-	-	-	-	-	ns
ns	-	ns	ns	ns	↓	-	-	ns	-	-
-	↑	↑	-	-	-	-	-	-	-	-
ns	-	ns	↑	ns	ns	↓	-	-	-	ns
ns	-	ns	-	-	-	-	-	-	-	-
↓	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	↑	-
-	-	↑	-	-	-	-	-	-	-	-
ns	-	-	-	-	-	-	-	-	-	-
Ns	-	ns	-	-	-	-	-	-	-	↑
-	-	-	-	↑	-	-	-	-	-	-
↓	-	ns	-	-	-	-	-	-	-	ns
ns	-	-	-	-	-	-	-	-	-	-
ns	-	-	-	ns	-	-	-	-	-	-
ns	-	-	-	ns	-	-	-	-	-	-

Table 2. (Continue)

Study	Cases			Controls			Blood type	Covariates in analyses
	N	Age	% female	N	Age	% female		
Alzheimer's disease dementia								
Shao (2020)[69]	30	71.6 ± 8.8	66.7	43	65.5 ± 7.9	41.9	P	None
Jacobs (2019)[18]	20	77.9 ± 7.5	55.0	18	73.1 ± 7.9	16.7	P	Sex
Lin (2019)[54]	15	76.9 ± 8.0	unk	15	66.8 ± 6.5	unk	P	None
van der Velpen (2019)[22]	40	74.9 ± 6.4	60.0	34	65.4 ± 6.2	67.7	P	None
Giil (2017)[17]	42	78.5 ± 6.3	unk	42	78.6 ± 6.8	unk	P	Age, sex, creatinine
Trushina (2013)[41]	15	82.7 ± 4.2	20.0	15	78.6 ± 3.5	33.3	P	unk
Gulaj (2010)[60]	34	78.8 ± 5.7	70.6	18	76.2 ± 7.3	72.2	P	None
Li (2010)[68]	20	68.0 ± 10	50.0	20	70.0 ± 9	50.0	P	None
Fonteh (2007)[44]	8	77.9 ± 7.4	50.0	8	79.5 ± 5.5	50.0	P	None
Hartai (2007)[61]	28	77.0 ± 6.3	78.6	31	73 ± 8.3	67.7	P	None
Bonaccorso (1998)[64]	15	78.4 ± 10.3	80.0	15	75.6 ± 9.1	46.7	P	Age, sex
Fekkes (1998)[65]	14	73.6 ± 6.3	71.4	17	70.1 ± 1.3	0	P	None
Molina (1998)[46]	37	70.9 ± 8.5	54.1	32	67.9 ± 9.2	53.1	P	None
Basun (1990)[66]	22	74.0 ± 9.0	59.1	11	79.0 ± 2.0	54.5	P	None
Watkins (1989)[67]	22	77.3	68.2	22	76.0	68.2	P	None
Schwarz (2013)[82]	20	74 ± 7.6	80.0	SCD (19)	59.5 ± 10.2	42.1	S	Age, sex
de Leeuw (2017)[81]	127	65.1 (9.1)[a]	50	SCD (121)	62.7 (8.0)[a]	46	P	Age, sex, clinical characteristics

Metabolites										
TRP	**N-f-KYN**	**KYN**	**3-HK**	**KA**	**XA**	**AA**	**3-HAA**	**QA**	**PIC**	**KTR**
↓	-	-	-	-	-	-	-	-	-	-
ns	-	ns	ns	ns	-	ns	↓	ns	ns	ns
-	-	-	-	-	-	-	-	-	-	ns
↓	-	ns	ns	ns	-	-	-	ns	-	-
↓	-	↓	ns	ns	↓	ns	↓	↓	-	ns
↓	-	-	-	-	-	-	-	-	-	-
↓	-	ns	ns	↓	-	ns	-	↑	-	↑
↓	-	-	-	-	-	-	-	-	-	-
ns	-	-	-	-	-	-	-	-	-	-
-	-	ns	-	↓	-	-	-	-	-	-
ns	-	-	-	-	-	-	-	-	-	-
↓	-	-	-	-	-	-	-	-	-	-
ns	-	-	-	-	-	-	-	-	-	-
ns	-	-	-	-	-	-	-	-	-	-
↓	-	-	-	-	-	-	-	-	-	-
ns	-	ns	↑	ns	-	-	-	ns	ns	-
ns	-	ns	-	-	-	-	-	-	-	-

Table 2. (Continue)

Study	Cases			Controls			Blood type	Covariates in analyses
	N	Age	% female	N	Age	% female		
Vascular dementia								
Martinez (1993)[49]	13	71.0 ± 6.0	46.2	15	66.0 ± 8.0	46.7	S	None
Lewy body dementia								
Tsuruoka (2013)[70]	3	75.3 ± 4.9	33.3	9	68.1 ± 13.7	100	S	None
Frontotemporal dementia								
Janssens (2020)[53]	39	67.4 ± 11.6	48.7	26	67.0 ± 8.0	46.2	S	None
Santos (2020)[71]	9	65.5 ± 9.5	33.3	15	67.7 ± 8.4	66.7	P	None
Tsuruoka (2013)[70]	4	72.0 ± 2.9	0.0	9	68.1 ± 13.7	100	S	None
All type dementia								
Rudman (1989)[72]	17	73.0	0.0	21	75.0	0.0	P	None
Thomas (1986)[73]	23	77.2	60.9	23	76.1	60.9	P	None
Shaw (1981)[74]	32	77.1	unk	70	70.1	unk	P	Sex
Mild cognitive impairment + Alzheimer's disease dementia								
Rommer (2016)[75]	16	63.3 ± 13.7	56.3	15	62.8 ± 3.6	73.3	P	None
Greilberger (2010)[76]	16	63.3 ± 13.7	56.3	15	62.8 ± 3.6	73.3	P	None
Mild cognitive impairment								
Whiley (2021)[19]	165	76.3 ± 6.0	57.0	86	75.9 ± 5.2	48.8	S	None
Willette (2021)[20]	396	74.7 ± 7.4	35.4	58	75.1 ± 5.8	48.3	S	Age, sex
Ramos-Chavez (2018)[77]	23	unk	unk	54	unk	unk	S	Age, TRP
Toledo (2017)[37]	356	75.1	64.6	199	75.3	50.3	S	Age, gender, education, APOE ε4

Metabolites										
TRP	**N-f-KYN**	**KYN**	**3-HK**	**KA**	**XA**	**AA**	**3-HAA**	**QA**	**PIC**	**KTR**
↑	-	-	-	-	-	-	-	-	-	-
ns	-	-	-	-	-	-	-	-	-	-
ns	-	ns	ns	ns	ns	ns	-	ns	ns	ns
↓	-	-	-	-	-	-	-	-	-	-
ns	-	-	-	-	-	-	-	-	-	-
ns	-	-	-	-	-	-	-	-	-	-
↓	-	-	-	-	-	-	-	-	-	-
↓	-	-	-	-	-	-	-	-	-	-
↓	-	ns	-	-	-	-	-	-	-	↑
↓	-	ns	-	-	-	-	-	-	-	↑
↓	-	↓	ns	ns	↓	-	ns	ns	ns	ns
↓	-	ns	-	-	-	-	-	-	-	ns
↓	-	-	-	-	-	-	-	-	-	ns
ns	-	ns	-	-	-	-	-	-	-	-

Table 2. (Continue)

Study	Cases			Controls			Blood type	Covariates in analyses
	N	Age	% female	N	Age	% female		
Mild cognitive impairment								
González-Sánchez (2020)[21]	24	72.0 ± 7.1	58.4	23	64.7 ± 10.8	34.8	P	None
Peña-Bautista (2020)[78]	25	70 (67-73)[a]	60.0	25	66 (62-70)[a]	36.0	P	Age, gender
Shao (2020)[69]	13	67.9 ± 7.2	38.5	43	65.5 ± 7.9	41.9	P	None
Lin (2019)[54]	10	74.6 ± 8.5	unk	15	66.8 ± 6.5	unk	P	None
Graham (2015)[83]	16	72.4 ± 7.3	50.0	37	73.1 ± 8.9	51.4	P	None
Post-stroke cognitive impairment								
Cogo (2021)[79]	13	69.4 ± 17.8	38.5	PSNCI (10)	64.7 ± 13.3	40.0	S	None
Liu (2015)[80]	30	unk	unk	PSNCI (30)	unk	unk	S	None

↑ Sig. higher in cases, ↓ Sig. lower in cases, *ns* non-significant, *unk* unknown, - metabolite not measured, [a]Age decline; Mod, moderate; PSNCI, post-stroke no cognitive impairment; S, serum; P, plasma TRP, tryptophan; 3-HK, 3-hydroxykynurenine; 3-HAA, 3-hydroxyanthranilic acid; XA, xanthurenic acid; QA, quinolinic acid;

Metabolites										
TRP	**N-f-KYN**	**KYN**	**3-HK**	**KA**	**XA**	**AA**	**3-HAA**	**QA**	**PIC**	**KTR**
ns	-	-	-	ns	-	-	-	-	-	-
ns	-	-	-	-	-	-	-	-	-	-
ns	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	ns
unk	unk	-	unk	-	-	-	-	-	-	-
ns	↑	-	-	ns	-	-	-	↑	-	↑
↓	-	↑	-	-	-	-	-	-	-	-

in median (IQR). Abbreviations: Sig., significant; IQR, interquartile range; SCD, subjective cognitive
N-f-KYN, N-formyl-kynurenine; KYN, kynurenine; KA, kynurenic acid; AA, anthranilic acid;
PIC, picolinic acid; KTR, kynurenine-tryptophan ratio.

Systematic review of case-control studies measuring kynurenines in other tissues

Although KP metabolites have been studied the most in CSF and in blood, other tissues have been investigated as well, including post-mortem brain tissue [52, 84-90], red blood cells [61], saliva [70, 91], urine [19, 44, 92, 93], and fecal samples [94] (Supplementary Table S4). A meta-analysis with these studies was not possible due to the limited number of studies.

Kynurenines in post-mortem brain tissue

Studies in post-mortem brain tissue mostly investigated differences in TRP levels between AD dementia patients and controls [84-90]. Xu et al. (2016), reported that AD dementia patients had higher TRP levels in the hippocampus, entorhinal cortex, middle temporal gyrus, motor cortex, and cingulate gyrus

compared to controls [90], whereas all other studies reported no differences in metabolite levels in any of the examined brain areas [52, 84-89]. Only one other study investigated differences in QA levels, but also did not find any differences [52].

Kynurenines in saliva

The two studies investigating differences in TRP levels in saliva were inconclusive [70, 91]. Liang et al. (2016) reported that TRP levels were higher in AD dementia patients compared to MCI [91], while Tsuruoka et al. (2013), reported no difference in TRP levels when comparing patients with AD dementia, FTD or LBD with controls [70].

Kynurenines in urine and feces

Studies investigating urine levels of TRP in AD dementia and MCI patients either reported lower levels or no differences compared to controls [19, 44, 92, 93]. One study also investigated more downstream kynurenines and reported lower concentrations of KA and XA, and higher KTR in both AD dementia and MCI patients compared to controls, while KYN, 3-HK, and 3-HAA were not significantly different [19]. Lastly, one study investigated KP metabolites in fecal samples of AD dementia and MCI patients and reported no differences in KYN or KA compared to controls [94].

Systematic review on associations of the kynurenine pathway with age

Twenty six studies looked at associations with age as a continuous variable [8, 14-17, 38, 48, 49, 51, 52, 61, 64, 66, 69, 95-106] and 23 studies investigated different age groups [16, 48, 62, 64, 74, 95, 97, 100, 101, 107-120] (Supplementary Tables S5-S8). These were not mutually exclusive. In studies that included age as a continuous variable, TRP, 3-HK, and XA were either negatively or not significantly associated with age, with only one study reporting a positive association with TRP levels in CSF (Supplementary Table S5). In contrast, KYN, KA, and QA showed a positive or no association with age, while KTR and single study with AA and PIC only showed positive associations. One study investigated an association with 3-HAA which was non-significant.

A similar trend appeared in studies comparing metabolites between younger and older age groups (Supplementary Table S6). TRP and XA levels were either lower or not significantly different in the older group. In contrast, KYN and most

of its downstream metabolites, 3-HK, KA, AA, QA, and KTR, were all higher or not significantly different in the older group. 3-HAA was again investigated in only one study and was higher in the older group as well.

Systematic review on associations of the kynurenine pathway with cognitive test scores

Cross-sectional associations between kynurenines and cognitive test scores were investigated as well (Supplementary Tables S9-S11). Most studies (n = 15; [8, 13, 19, 20, 38, 50, 60, 62, 63, 66, 69, 121-124]) investigated associations between kynurenines and MMSE scores, whereas eight studies investigated associations with other cognitive tests [13, 14, 20, 37, 52, 60, 66, 102] (Supplementary Tables S9 and S10). A higher KTR was associated with lower scores on several cognitive tests [13, 20], whereas most associations of other metabolites with cognitive test scores were non-significant.

Meta-analysis of case-control studies

Since the number of cross-sectional and prospective studies were too small, meta-analyses were only performed on case-control studies with kynurenines measured in CSF and/or blood. The studies by Greilberger et al. (2010) [76] and Rommer et al. (2016) [75] and the studies by Watkins et al. (1989) [67] and Thomas et al. (1986) [73] used data from the same participants; therefore, only the initial study by Greilberger et al. (2010) and the study by Watkins et al (1989), which reported overall estimates for patients and controls, were included in the meta-analysis.

Meta-analysis on differences in metabolite levels between AD dementia patients and controls

Twenty six studies were included in the meta-analysis investigating differences in kynurenines between AD dementia patients and controls (Table 3; Figure 3; Supplementary Figures S1-S4). In the overall analyses (CSF and blood combined), TRP, KA, XA, AA, and QA levels were all lower in AD dementia patients compared to controls. No evidence of small study effects was shown in the funnel plots or Egger's regression tests. Levels of other metabolites (KYN, 3-HK) and KTR were not different between AD dementia cases and controls.

Subsequently, subgroup analyses were done while stratifying for biofluid type (CSF and blood). For some metabolites (TRP, KYN, KA), CSF levels were not

different, while for other metabolites (3-HK, XA, AA, QA, and KTR) not enough independent articles were available to conduct a meta-analysis. Blood levels of TRP, KA, XA, and AA were all reported to be lower in AD dementia patients, although TRP showed a considerable to large heterogeneity and small study bias. Blood levels of other metabolites (KYN, 3-HK, QA) and KTR were not different. QA displayed a tendency towards lower levels in AD dementia patients, which did not reach statistical significance ($p = 0.053$; Table 3). Separate analyses for plasma and serum consistently showed that levels of TRP and KA were lower in AD dementia. Additionally, for XA, serum levels were lower in AD dementia as well. Not enough studies were available to do a separate meta-analysis for XA in plasma, or for AA in plasma and serum.

Table 3. Effect sizes and Egger's bias coefficients of AD dementia-control studies

		Effect size		Heterogeneity		Publication bias	
	N	**SMD (95% CI)[a]**	**p-value**	**I^2 (%)**	**p-value**	**Egger's bias coefficient**	**p-value**
Tryptophan							
Overall	20	-0.33 (-0.44, -0.22)	**<0.001**	65.8	**<0.001**	-1.85	0.104
CSF	6	-0.03 (-0.27, 0.20)	0.772	0.0	0.488	-2.23	0.210
Blood	14	-0.42 (-0.55, -0.29)	**<0.001**	69.8	**<0.001**	-2.51	0.059
Plasma	8	-0.73 (-0.94, -0.52)	**<0.001**	70.0	**0.001**	-4.53	0.154
Serum	6	-0.23 (-0.40, -0.07)	**0.006**	19.2	0.288	-0.08	0.955
Kynurenine							
Overall	11	-0.11 (-0.24, 0.03)	0.114	58.0	**0.008**	2.15	0.125
CSF	3	-0.15 (-0.50, 0.20)	0.398	0.0	0.893	-1.10	0.139
Blood	8	-0.10 (-0.25, 0.04)	0.174	70.2	**0.001**	3.79	0.055
Plasma	2	-	-	-	-	-	-
Serum	6	-0.09 (-0.25, 0.07)	0.253	64.3	**0.015**	3.74	0.052
3-hydroxykynurenine							
Overall	7	-0.09 (-0.26, 0.07)	0.266	60.4	**0.019**	-1.21	0.521
CSF	2	-	-	-	-	-	-
Blood	5	-0.05 (-0.23, 0.13)	0.565	43.0	0.135	0.87	0.692
Plasma	2	-	-	-	-	-	-
Serum	3	0.05 (-0.16, 0.25)	0.671	47.2	0.150	2.78	0.308
Kynurenic acid							
Overall	10	-0.27 (-0.42, -0.13)	**<0.001**	80.6	**<0.001**	-1.07	0.671
CSF	3	0.08 (-0.23, 0.39)	0.603	93.0	**<0.001**	-8.87	0.644
Blood	7	-0.37 (-0.53, -0.21)	**<0.001**	48.1	0.072	-1.34	0.457
Plasma	3	-0.37 (-0.67, -0.07)	**0.017**	68.3	**0.043**	-2.80	0.803
Serum	4	-0.37 (-0.57, -0.18)	**<0.001**	43.0	0.154	-1.61	0.507
Xanthurenic acid							
Overall	5	-0.44 (-0.62, -0.27)	**<0.001**	0.0	0.638	0.31	0.832
CSF	1	-	-	-	-	-	-
Blood	4	-0.40 (-0.59, -0.21)	**<0.001**	0.0	0.840	1.11	0.229
Plasma	1	-	-	-	-	-	-
Serum	3	-0.40 (-0.61, -0.19)	**<0.001**	0.0	0.658	1.26	0.372

Table 3. (Continue)

		Effect size		Heterogeneity		Publication bias	
	N	**SMD (95% CI)[a]**	**p-value**	**I^2 (%)**	**p-value**	**Egger's bias coefficient**	**p-value**
Anthranilic acid							
Overall	3	-0.33 (-0.63, -0.03)	**0.033**	28.8	0.246	-4.95	0.380
CSF	0	-	-	-	-	-	-
Blood	3	-0.33 (-0.63, -0.03)	**0.033**	28.8	0.246	-4.95	0.380
Plasma	2	-	-	-	-	-	-
Serum	1	-	-	-	-	-	-
Quinolinic acid							
Overall	5	-0.17 (-0.34, -0.00)	**0.049**	0.0	0.583	-1.25	0.422
CSF	2	-	-	-	-	-	-
Blood	3	-0.20 (-0.39, 0.00)	0.053	24.1	0.268	-2.96	0.306
Plasma	1	-	-	-	-	-	-
Serum	2	-	-	-	-	-	-
KTR							
Overall	10	0.01 (-0.13, 0.15)	0.868	71.5	**<0.001**	2.03	0.240
CSF	2	-	-	-	-	-	-
Blood	8	0.08 (-0.07, 0.23)	0.309	72.0	**0.001**	3.72	**0.034**
Plasma	2	-	-	-	-	-	-
Serum	6	-0.00 (-0.17, 0.16)	0.960	42.1	0.125	2.55	0.060

[a]SMD > 0 trend towards higher in AD dementia, SMD < 0 trend towards lower in AD dementia compared to controls. Statistically significant *p*-values are in bold. Abbreviations: SMD, standardized mean difference; 95% CI, 95% confidence interval; AD, Alzheimer's disease; CSF, cerebrospinal fluid; KTR, kynurenine-tryptophan ratio.

Meta-analyses on differences in metabolite levels between AD dementia and SCD, and between MCI and controls

Due to a limited number of studies investigating differences in metabolite levels between AD dementia patients and individuals with SCD, only overall analyses could be done combining measurements in blood and CSF (Figure 3; Supplementary Table S12; Supplementary Figures S5 and S6). These showed that, compared to SCD subjects, levels of TRP were lower in AD dementia patients, while levels of 3-HK, KA, and PIC were all higher. No overall differences were found for KYN or QA. In analyses investigating differences between MCI patients and controls (Figure 3; Supplementary Table S13; and Supplementary Figures S7-S9), blood levels of TRP were lower in MCI patients, whereas no overall differences were found for TRP, KYN, KA, and KTR. Other metabolites had insufficient or no articles to conduct a meta-analysis.

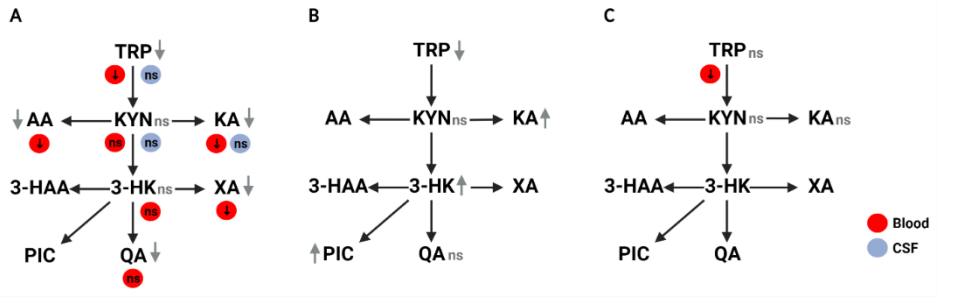


Figure 3. Results of meta-analyses comparing kynurenines. A) AD dementia and neurologically healthy controls, B) AD dementia and SCD, C) MCI and neurologically healthy controls. Overall differences are shown in grey, blood differences are shown in red, and CSF differences are shown in blue. ↑ Sig. higher in cases, ↓ Sig. lower in cases. Abbreviations: CSF, cerebrospinal fluid; Sig., significant; ns, non-significant; AD, Alzheimer's disease; MCI, mild cognitive impairment; SCD, subjective cognitive decline; TRP, tryptophan; KYN, kynurenine; KA, kynurenic acid; 3-HK, 3-hydroxykynurenine; 3-HAA, 3-hydroxyanthrnailic acid; XA, xanthurenic acid; PIC, picolinic acid; QA, quinolinic acid.

Meta-regression

Studies comparing levels of TRP, KYN, 3-HK, KA, and KTR between AD dementia patients and controls were included as these showed considerable heterogeneity ($I^2 > 50\%$; Table 3). Sources of heterogeneity were found in studies investigating overall metabolite levels and in blood specifically (Table 4). The covariate that most frequently showed significance was 'analytical techniques'. Results from the meta-regression in KTR (in blood) showed that the analytical technique, both LC-MS/MS versus HPLC and other technique versus HPLC, had an influence on the estimates. For KA (overall), this was the case for the comparison of ELISA versus HPLC. The type of biomaterial (CSF versus

plasma) was also a source of heterogeneity in overall analyses in TRP. Additionally, in the meta-analysis with TRP levels in blood, the comparison in plasma was different compared to serum. Sex was a source of heterogeneity for KYN (overall and in blood) and KTR (overall and in blood), and year of publication for KA (overall) and KTR (in blood).

Table 4. Summary of Meta-regression

	I^2_{res} (%)	Adj. R^2 (%)	τ^2	P > |t|	Prob. > F
Tryptophan					
Overall (n = 20)					
Year of publication	63.99	11.47	0.140	0.130	-
Age *(n = 19)*	61.60	28.96	0.123	0.062	-
Sex (% female) *(n = 19)*	66.55	-2.18	0.166	0.592	-
Biomaterial					
CSF/ blood	62.07	21.22	0.125	0.064	-
CSF/ plasma/ serum	49.99	53.94	0.073	-	**0.012**
Plasma/ CSF	-	-	-	**0.005**	-
Serum/ CSF	-	-	-	0.464	-
Analytical technique *(n = 18)*	51.82	37.92	0.068	-	0.117
ELISA/ HPLC	-	-	-	0.060	-
LC-MS/MS/ HPLC	-	-	-	0.233	-
Other/ HPLC	-	-	-	0.032	-
Blood (n = 14)					
Year of publication	65.71	18.47	0.143	0.130	-
Age (n = 13)	68.87	21.96	0.152	0.110	-
Sex (% female)	67.27	2.69	0.170	0.467	-
Biomaterial					
Plasma/ serum	59.39	38.93	0.107	**0.035**	-
Analytical technique *(n = 12)*	57.75	39.31	0.080	-	0.206
ELISA/ HPLC	-	-	-	0.270	-
LC-MS/MS/ HPLC	-	-	-	0.359	-
Other/ HPLC	-	-	-	0.047	-
Plasma (n = 8)					
Year of publication	73.46	-13.59	0.253	0.502	-
Age	58.32	33.12	0.149	0.181	-
Sex (% female)	73.64	-20.99	0.269	0.919	-
Analytical technique *(n = 6)*	27.16	84.75	0.037	-	0.250
ELISA/ HPLC	-	-	-	0.177	-
LC-MS/MS/ HPLC	-	-	-	0.438	-
Other/ HPLC	-	-	-	0.107	-
Anticoagulant	70.96	-13.06	0.252	-	0.638
EDTA/ Heparin	-	-	-	0.414	-
Other/ Heparin	-	-	-	0.519	-
Kynurenine					
Overall (n = 11)					
Year of publication	48.37	34.02	0.050	0.166	-
Age *(n = 10)*	63.27	-9.00	0.096	0.687	-
Sex (% female) *(n = 10)*	40.27	60.95	0.033	**0.035**	-

Table 4. *(Continue)*

	I^2_{res} (%)	Adj. R^2 (%)	τ^2	P > |t|	Prob. > F
Kynurenine					
Biomaterial					
CSF/ blood	62.11	-16.28	0.089	0.547	-
CSF/ plasma/ serum	66.25	-37.68	0.105	-	0.811
Plasma/ CSF	-	-	-	0.744	-
Serum/ CSF	-	-	-	0.531	-
Analytical technique	27.65	67.23	0.025	-	0.059
ELISA/ HPLC	-	-	-	-	-
LC-MS/MS/ HPLC	-	-	-	0.038	-
Other/ HPLC	-	-	-	0.031	-
Blood (n = 8)					
Year of publication	49.87	59.11	0.047	0.055	-
Age *(n = 7)*	74.13	-9.17	0.153	0.526	-
Sex (% female)	38.82	70.51	0.034	**0.023**	-
Biomaterial					
Plasma/ serum	74.44	-22.48	0.142	0.860	-
Analytical technique	35.09	73.55	0.031	-	0.066
ELISA/ HPLC	-	-	-	-	-
LC-MS/MS / HPLC	-	-	-	0.040	-
Other/ HPLC	-	-	-	0.039	-
Serum (n = 6)					
Year of publication	30.84	67.52	0.024	0.091	-
Age *(n = 5)*	64.55	-0.96	0.096	0.457	-
Sex (% female)	48.99	44.78	0.041	0.128	-
Analytical technique	36.78	61.19	0.029	-	0.220
ELISA/ HPLC	-	-	-	-	-
LC-MS/MS/ HPLC	-	-	-	0.319	-
Other/ HPLC	-	-	-	0.106	-
3-hydroxykynurenine					
Overall (n = 7)					
Year of publication	38.37	74.32	0.025	0.067	-
Age *(n = 6)*	58.42	-936.74	0.079	0.552	-
Sex (% female) *(n = 6)*	42.95	0	0.019	0.973	-
Biomaterial					
CSF/ blood	64.09	-41.19	0.137	0.320	-
CSF/ plasma/ serum	62.88	-31.17	0.127	-	0.392
Plasma/ CSF	-	-	-	0.729	-
Serum/ CSF	-	-	-	0.229	-
Analytical technique	59.66	-0.52	0.097	-	0.285
ELISA/ HPLC	-	-	-	-	-
LC-MS/MS/ HPLC	-	-	-	0.264	-
Other/ HPLC	-	-	-	0.136	-
Kynurenic acid					
Overall (n = 10)					
Year of publication	74.55	40.01	0.188	**0.047**	-
Age *(n = 9)*	84.67	-11.61	0.398	0.589	-
Sex (% female) *(n = 9)*	76.65	28.25	0.203	0.101	-
Biomaterial					
CSF/ blood	80.02	-1.67	0.319	0.439	-

Table 4. *(Continue)*

	I^2_{res} (%)	Adj. R^2 (%)	τ^2	P > |t|	Prob. > F
Kynurenic acid					
CSF/ plasma/ serum	82.52	-21.46	0.381	-	0.762
Plasma/ CSF	-	-	-	0.576	-
Serum/ CSF	-	-	-	0.499	-
Analytical technique	38.98	86.44	0.043	-	**0.019**
ELISA / HPLC	-	-	-	**0.003**	-
LC-MS/MS/ HPLC	-	-	-	0.111	-
Other/ HPLC	-	-	-	0.051	-
CSF (n = 3)					
Year of publication	92.87	32.60	0.804	0.401	-
Age	94.52	9.38	1.080	0.470	-
Sex (% female)	-	-	-	-	-
Analytical technique	-	-	-	-	-
Plasma (n = 3)					
Year of publication	0.00	100.00	0.000	0.241	-
Age	74.93	-21.99	0.219	0.527	-
Sex (% female)	42.46	70.21	0.054	0.335	-
Analytical technique	-	-	-	-	-
Anticoagulant	-	-	-	-	-
KTR					
Overall (n = 10)					
Year of publication	70.61	-5.84	0.205	0.626	-
Age *(n = 9)*	74.80	11.92	0.200	0.170	-
Sex (% female) *(n = 8)*	55.66	71.58	0.061	**0.020**	-
Biomaterial					
CSF/ blood	68.21	28.14	0.139	0.093	-
CSF/ plasma/ serum	64.59	36.55	0.123	-	0.120
Plasma/ CSF	-	-	-	0.046	-
Serum/ CSF	-	-	-	0.147	-
Analytical technique	63.30	31.45	0.130	-	0.300
ELISA / HPLC	-	-	-	-	-
LC-MS/MS/ HPLC	-	-	-	0.155	-
Other/ HPLC	-	-	-	0.196	-
Blood (n = 8)					
Year of publication	28.08	100.00	0.000	**0.013**	-
Age *(n = 7)*	79.58	-23.61	0.250	0.625	-
Sex (% female) *(n = 7)*	22.24	88.61	0.022	**0.012**	-
Biomaterial					
Plasma/ serum	69.40	9.69	0.148	0.247	-
Analytical technique	0.00	100.00	0.000	-	**0.016**
ELISA / HPLC	-	-	-	-	-
LC-MS/MS / HPLC	-	-	-	**0.015**	-
Other/ HPLC	-	-	-	**0.006**	-

Statistically significant p-values are in bold. Abbreviations: I^2_{res}, residual variation; τ^2, tau^2; P > |t|, 2-tailed p-value; Prob. > F, F test p-value; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography with tandem mass spectrometry; EDTA, ethylenediaminetetraacetic acid; KTR, kynurenine-tryptophan ratio.

Discussion

This systematic review and meta-analysis identified and summarized 103 studies that investigated KP metabolites in patients with cognitive impairment or dementia or in normal aging. Twenty six articles were included in the meta-analyses, in which TRP and kynurenines were compared between patients with AD dementia and neurologically healthy controls. Results showed that patients with AD dementia had lower blood levels of TRP, KA, XA and AA, whereas blood levels of KYN, 3-HK, and QA, and CSF levels of TRP, KYN, and KA were non-significant. QA was lower overall and displayed a tendency towards lower blood levels in AD dementia patients. For other metabolites, not enough independent articles were available to conduct a meta-analysis. Studies investigating associations with age suggested that age was associated with lower blood levels of TRP and higher blood levels of KYN and KTR, next to higher CSF levels of KA and QA. Associations of other metabolites with age, and of kynurenines with cognitive test scores, were inconclusive and generally non-significant.

Lower overall levels of QA in AD dementia patients

The findings of the meta-analysis are different than reported and interpreted in previous studies. As such, these results strengthen previous observations of changes in various kynurenines in cognitive impairment and AD dementia, but also challenge current hypotheses regarding the role of several others. The meta-analysis did not find elevated QA levels in CSF or blood of patients with AD dementia. Even more, the meta-analysis limited to studies in blood showed that, if anything, QA displayed a non-significant tendency towards lower levels in AD dementia patients. Similarly, post-mortem studies investigating QA levels in several brain areas of patients with AD dementia pathology and controls found no differences [52, 125]. Moreover, with respect to cognitive test scores, studies generally report no significant associations as well [8, 14, 35, 52, 60]. Taken together, these findings strongly contradict the general notion that QA levels are higher in AD dementia and other dementias due to its presumed neurotoxic properties [126, 127].

Multiple lines of evidence have suggested QA's involvement in the pathophysiology of AD dementia. Levels of QA increase after inflammation or immune activation and can lead to neuronal death via different mechanisms,

including oxidative stress, glutamatergic excitotoxicity, neuroinflammation, and mitochondrial dysfunction [127-131]. In post-mortem studies, QA has been associated with AD neuropathological features, since the highest expression of QA was found in the perimeter of senile plagues in the hippocampus [132, 133], and QA was shown to increase tau phosphorylation in human primary neurons [134]. In turn, $A\beta_{1-42}$ was shown to induce the production of QA in human microglia and macrophages [131]. However, it is important to note that lesions in brains of AD dementia patients are micro-local and investigating entire brain areas (as was mostly done thus far) might lack the potential to detect subtle or localized differences in QA levels. Therefore, the findings in this systematic review and meta-analysis emphasize the need for further research in order to identify the exact role of QA in AD dementia and other neurodegenerative disorders.

Lower peripheral levels of KA in AD dementia patients

Results from our meta-analysis suggest that AD dementia is associated with lower blood levels of KA, both in plasma and in serum, but not in CSF. In line with these findings, previous studies investigating KA levels in red blood cells or urine of AD dementia patients reported a decrease in KA levels as well [19, 61]. In contrast to QA, KA is considered a neuroprotective metabolite through its role as an antagonist of the N-methyl-D-aspartate (NMDA) receptor and alpha 7 nicotinic acetylcholine (α7nACh) receptor, and has antioxidant and anti-inflammatory properties [126]. *In vivo* studies demonstrated that KA or its analogue provided neuroprotection in rodent models of AD-like pathology [135, 136] and cerebral ischemia [137, 138]. More specifically, experimentally increasing levels of KA or its analogue in the brain of these models prevented spatial memory deficits, extended life span, decreased microglial activation, and prevented synaptic loss [135, 136].

At the same time, although KA levels in CSF were not significantly different in our meta-analysis, most studies included in the systematic review reported an increase of CSF KA levels in AD dementia. Similarly, in previous studies with post-mortem brain tissue, KA levels were increased in several areas of individuals with AD dementia [45, 139] and Down syndrome (DS) [45, 139], whereas in one other study, no differences were reported in AD dementia patients [140]. These results suggest that peripheral levels of KA are lower in AD dementia, whereas central levels might be increased. These discrepancies may

be due to small sample sizes, age differences or not controlling for important covariates [8, 141] and require further research.

Lower peripheral levels of TRP, XA, and AA in AD dementia patients

Results from our meta-analysis suggest that TRP levels are lower in blood, but not in CSF of patients with AD dementia. In line with this, previous studies investigating TRP blood levels in AD dementia reported either a decrease or no differences, whereas studies in CSF were mostly non-significant. Additionally, most studies that investigated TRP levels in post-mortem brain tissue reported no differences in any of the brain areas investigated as well [84-89]. On top of its known role as a biochemical precursor for the kynurenine and serotonin pathways, studies have shown that TRP has antioxidant properties such as scavenging free radicals, reactive oxygen and chlorine species, and has the highest antiradical activity compared to other amino acids [142-144]. As such, lower peripheral levels of TRP, as demonstrated in our meta-analysis, potentially indicates less antioxidant activities in patients with AD dementia. Since inflammation activates the first and rate-limiting enzyme, indoleamine 2, 3-dioxygenase (IDO), which converts TRP to KYN and initiates the KP, this may have contributed, in part, to the lower TRP concentration in AD dementia patients.

According to our meta-analysis, AD dementia patients also had significantly lower blood levels of XA and AA compared to controls. Although XA and AA have not been investigated as widely as the other kynurenines, XA has been shown to be a double-edged sword owing to its anti- and sometimes pro-oxidant properties related to its metal chelating activities [145-147]. A recent population-based study by our group showed that, in individuals with type-2 diabetes, higher plasma levels of XA were associated with lower odds of cognitive impairment and better executive functioning and attention [148]. Additionally, in another population-based study, XA was associated with lower cardiovascular disease (CVD) mortality risk [149]. With respect to AA, studies have shown that this metabolite has anti-inflammatory properties due to its interaction with copper [150]. As a result, the synthetic AA analogue 3-methoxyanthranilate was put forward as a potential anti-inflammatory drug [151, 152]. Although further research is needed, the decreased levels of XA and AA in patients may signal diminished neuroprotective activity.

No differences in KYN, 3-HK, and KTR in AD dementia patients

Our meta-analysis indicated that levels of KYN, 3-HK, and KTR were not significantly different in AD dementia patients compared to controls. KYN and 3-HK are assumed to have neurodegenerative and excitotoxic properties, while KTR is used as a marker for IDO activity and inflammation [153]. IDO is activated by various inflammatory stimuli such as interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα) [11]. At the same time, 3-HK is an antioxidant and has been associated with lower odds of cognitive impairment in prediabetes and type-2 diabetes [148, 154]. Moreover, CSF levels of 3-HK were increased in patients with PD [155], while brain levels of 3-HK levels were increased in patients with HD [156].

Although results from our meta-analysis suggest that KTR was not significantly different between AD dementia patients and controls, previous studies suggest that IDO plays a role in the pathophysiology of AD dementia. For instance, IDO expression has been shown to be induced by $A\beta_{1-42}$ [157] and, similar to QA, was elevated in the hippocampus of AD dementia patients where it was associated with senile plaques [132]. Moreover, in patients with PD, IDO was higher in both serum and CSF and correlated with disease severity [158]. KTR was also associated with lower scores on cognitive tests in studies in healthy volunteers and in patients with AD dementia and ischemic stroke [13, 20, 62, 121, 123]. It is unclear why we found no differences in these metabolites, underscoring the need for further research.

Findings from meta-regression analysis

Several factors could explain inter-study differences in metabolite levels between groups. Results from our meta-regression showed 'analytical technique' and 'year of publication' explained heterogeneity of findings for KA and KTR. Although no previous studies specifically compared TRP and kynurenines between different types of analytical instruments, others have reported no statistically significant differences between HPLC and LC-MS/MS. Although both analytical instruments are liable and widely used, for metabolite analysis, LC-MS/MS has shown to be more accurate and sensitive compared to HPLC [159, 160]. As such, technological limitations at the time of conducting the studies may explain both heterogeneity factors, since metabolite measurements in earlier studies were likely performed with less sensitive equipment.

Additionally, the type of biomaterial was a source of heterogeneity as well. TRP measured in plasma or serum for instance had a significant influence on the estimates. This finding is in line with previous studies that reported differences between serum and plasma samples, and between the use of different anticoagulation tubes which is believed to affect metabolite concentrations as well [161, 162]. Lastly, our meta-regression also pointed to sex as a factor driving heterogeneity in KYN and KTR. As a result, these and other kynurenines should be examined in more detail, especially since most of them have been put forward as potential biomarkers.

Associations with age and other important confounders

Studies included in the systematic summary that investigated associations with age suggest that age is associated with lower blood levels of TRP and higher levels of KYN and KTR. As mentioned previously, KTR is used as a marker for IDO activity and inflammation [153]. IDO is one of the rate-limiting enzymes responsible for TRP degradation and is activated by various inflammatory stimuli such as IFNγ and TNFα. It is well known that aging is associated with alterations in immune system functioning and elevated levels of inflammatory markers, which likely explain these increases and degradation primarily through the KP, lowering TRP availability for serotonin synthesis in the brain [163]. In line with this notion, studies investigating associations of age with neopterin reported that higher levels were associated with age as well [15-17, 96, 100, 101, 112]. However, most studies included in this systematic review and meta-analysis did not correct for important confounders such as age. Since age is also the biggest risk factor of AD dementia, and most AD dementia patients were older compared to controls, this might also be a reason for the non-/differences observed in this study. As such, including age as a confounder in clinical studies is essential.

Other important factors that are known to be associated with kynurenine concentrations and/or cognitive functioning are sex, educational level, renal function, and lifestyle factors such as smoking habits, alcohol use, and body mass index (BMI) [103, 112, 118, 164-174]. Previous studies for instance reported higher blood levels of TRP, KYN, KA, XA, AA, and 3-HAA in men than in women [103, 112, 118, 164, 165]. Additionally, kynurenines were higher in individuals with reduced renal function [112] and renal disease [173, 174] as well. Unfortunately, if studies included in this systematic review adjusted their

analyses, they did so for age and sex, while other important factors were not measured or corrected for most of the time.

Limitations

The main limitation of our meta-review is the relatively small number of included studies, which underscores the need for more studies addressing the role of the KP in dementia and aging. This limited sample size was partially due to the fact that we had to exclude articles reporting the metabolite levels in median and interquartile range, unless the corresponding author was able to provide the raw data or mean and SD upon request. Additionally, although we performed an extensive search without filters and included a downstream snowballing approach, we may have missed potential articles. A recent study for instance has shown that a dual-reviewer abstract screening approach misses 3% of relevant studies [175]. Furthermore, the data included in our meta-analysis consisted of mean KP metabolite concentrations and SD, whereas most kynurenines are known to have skewed distributions. As such, most studies now report median and interquartile range. Finally, we limited publications to articles written in English and as such might have excluded other articles that would otherwise have met inclusion criteria.

There were also limitations in the original studies themselves. For instance, most studies included in this systematic review and meta-analysis did not correct for important confounders such as age. Since age is also the biggest risk factor of AD dementia, and most AD patients were older compared to controls, this might also be a reason for the non-/differences observed in this study. As such, including age as a confounder in clinical study is essential. Another limitation is that most studies used hospital controls. These controls may be neurologically normal, but may present other disorders which may influence kynurenines or dementia risks (in)directly since articles do not clearly state the full diagnosis of these controls. For example, studies have shown that CVD, diabetes and stroke have reported associations with kynurenines and cognition [149, 176-180]. Moreover, recent meta-analysis reviews confirmed that neuropsychiatric disorders such as depression and bipolar disorder influence kynurenines as well [181-183]. Additionally, in line with the previous point, some control individuals were from the memory clinic. Although they were categorized as neurologically healthy controls, they may in fact suffered from SCD. Patients with SCD have a higher risk of developing dementia compared to healthy individuals without

cognitive complaints. According to one meta-analysis, the conversion rates of SCD to MCI and dementia were 26.6% and 14.1%, respectively, over a period of 4 years [184]. Another limitation is that TRP is only available through food intake, thus fasting and non-fasting samples may influence the KP metabolite concentrations. Although, Carayol et al. (2015) reported that both TRP and KYN levels showed no significant difference between fasting and non-fasting serum samples, overall reproducibility was lower in non-fasting samples [185]. There were also inconsistent procedures in collecting ([non]fasted samples), measuring (analytical techniques) and analyzing (covariates of) KP metabolites. Lastly, most studies included in this review focused on AD dementia, and we do underscore the need for studies investigating different patient populations as well (e.g., VaD, LBD, FTD, and HD).

Future directions

There is a need for clinical studies that better control for confounders that (in)directly affect TRP and KP metabolites and the disorder of interest. This includes demographic, somatic and lifestyle factors (e.g., education level, BMI, kidney function, alcohol and tobacco use, cognitive and neuropsychiatric assessments, comorbidities, timing of the (non)fasted sampling and type of anticoagulation tubes). Likewise, future studies, if possible, should investigate different samples such as plasma, serum, and CSF simultaneously to better understand the dynamics of metabolite levels. Moreover, in order to better understand the role of KP, studies should investigate more downstream metabolites and relevant ratios in addition to TRP and KYN, and correlate them with pathology markers such as same-tissue amyloid or tau levels. Finally, although cross-sectional studies are informative, in order to fully understand potential specific and common pathophysiological mechanisms, it is vital to investigate kynurenines at different time points in longitudinal studies in individuals with different levels of cognitive impairment, different patient populations and patients with other comorbidities such as affective disorders.

Conclusion

Despite a large heterogeneity among clinical studies and a partial inconsistency in metabolite levels, the current review suggests that TRP and kynurenines are dysregulated in patients with dementia and cognitive impairment.

References

1. Winblad B, Amouyel P, Andrieu S, Ballard C, Brayne C, Brodaty H, et al. Defeating Alzheimer's disease and other dementias: a priority for European science and society. Lancet Neurol. 2016;15(5):455-532.
2. Association As. 2018 Alzheimer's disease facts and figures. Alzheimers & Dementia. 2018;14(3):367-425.
3. WHO. Global status report on the public health response to dementia: World Health Organization; 2021 [Available from: https://www.who.int/publications/i/item/9789240033245.
4. Galasko D, Montine TJ. Biomarkers of oxidative damage and inflammation in Alzheimer's disease. Biomark Med. 2010;4(1):27-36.
5. Wallin K, Bostrom G, Kivipelto M, Gustafson Y. Risk factors for incident dementia in the very old. Int Psychogeriatr. 2013;25(7):1135-43.
6. Demetrius LA, Magistretti PJ, Pellerin L. Alzheimer's disease: the amyloid hypothesis and the Inverse Warburg effect. Front Physiol. 2014;5:522.
7. Wu Y, Mai N, Zhong X, Wen Y, Zhou Y, Li H, et al. Kynurenine pathway changes in late-life depression with memory deficit. Psychiatry Res. 2018;269:45-9.
8. Sorgdrager FJH, Vermeiren Y, Van Faassen M, van der Ley C, Nollen EAA, Kema IP, et al. Age- and disease-specific changes of the kynurenine pathway in Parkinson's and Alzheimer's disease. Journal of Neurochemistry. 2019.
9. Rodrigues FB, Byrne LM, Lowe AJ, Tortelli R, Heins M, Flik G, et al. Kynurenine pathway metabolites in cerebrospinal fluid and blood as potential biomarkers in Huntington's disease. J Neurochem. 2021;158(2):539-53.
10. Zhang P, Huang J, Gou M, Zhou Y, Tong J, Fan F, et al. Kynurenine metabolism and metabolic syndrome in patients with schizophrenia. J Psychiatr Res. 2021;139:54-61.
11. Badawy AA. Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects. Int J Tryptophan Res. 2017;10:1178646917691938.
12. Zádori D, Veres G, Szalárdy L, Klivényi P, Vécsei L. Alzheimer's Disease: Recent Concepts on the Relation of Mitochondrial Disturbances, Excitotoxicity, Neuroinflammation, and Kynurenines. Journal of Alzheimer's disease : JAD. 2018;62(2):523-47.
13. Solvang SEH, Nordrehaug JE, Tell GS, Nygard O, McCann A, Ueland PM, et al. The kynurenine pathway and cognitive performance in community-dwelling older adults. The Hordaland Health Study. Brain, Behavior, and Immunity. 2019;75:155-62.
14. Kuster OC, Laptinskaya D, Fissler P, Schnack C, Zugel M, Nold V, et al. Novel Blood-Based Biomarkers of Cognition, Stress, and Physical or Cognitive Training in Older Adults at Risk of Dementia: Preliminary Evidence for a Role of BDNF, Irisin, and the Kynurenine Pathway. Journal of Alzheimer's Disease. 2017;59(3):1097-111.
15. Valdiglesias V, Sanchez-Flores M, Maseda A, Lorenzo-Lopez L, Marcos-Perez D, Lopez-Corton A, et al. Immune biomarkers in older adults: Role of physical activity. Journal of Toxicology and Environmental Health - Part A: Current Issues. 2017;80(13-15):605-20.
16. Capuron L, Schroecksnadel S, Feart C, Aubert A, Higueret D, Barberger-Gateau P, et al. Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: Role in neuropsychiatric symptoms. Biological Psychiatry. 2011;70(2):175-82.
17. Giil LM, Midttun O, Refsum H, Ulvik A, Advani R, Smith AD, et al. Kynurenine Pathway Metabolites in Alzheimer's Disease. Journal of Alzheimer's Disease. 2017;60(2):495-504.
18. Jacobs KR, Lim CK, Blennow K, Zetterberg H, Chatterjee P, Martins RN, et al. Correlation between plasma and CSF concentrations of kynurenine pathway metabolites in Alzheimer's disease and relationship to amyloid-β and tau. Neurobiology of Aging. 2019;80:11-20.
19. Whiley L, Chappell KE, D'Hondt E, Lewis MR, Jiménez B, Snowden SG, et al. Metabolic phenotyping reveals a reduction in the bioavailability of serotonin and kynurenine pathway metabolites in both the urine and serum of individuals living with Alzheimer's disease. Alzheimers Res Ther. 2021;13(1):20.

20. Willette AA, Pappas C, Hoth N, Wang Q, Klinedinst B, Willette SA, et al. Inflammation, Negative Affect, and Amyloid Burden in Alzheimer's Disease: Insights from the Kynurenine Pathway. Brain Behav Immun. 2021.
21. González-Sánchez M, Jiménez J, Narváez A, Antequera D, Llamas-Velasco S, Martín AH, et al. Kynurenic Acid Levels are Increased in the CSF of Alzheimer's Disease Patients. Biomolecules. 2020;10(4).
22. van der Velpen V, Teav T, Gallart-Ayala H, Mehl F, Konz I, Clark C, et al. Systemic and central nervous system metabolic alterations in Alzheimer's disease. Alzheimers Res Ther. 2019;11(1):93.
23. Page MJ, Moher D, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. BMJ. 2021;372:n160.
24. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71.
25. GA Wells BS, D O'Connell, J Peterson, V Welch, M Losos, P Tugwell. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. 2000.
26. Cannon JA, Moffitt P, Perez-Moreno AC, Walters MR, Broomfield NM, McMurray JJV, et al. Cognitive Impairment and Heart Failure: Systematic Review and Meta-Analysis. J Card Fail. 2017;23(6):464-75.
27. Cohen J. Statistical Power Analysis for the Behavioral Sciences. 2nd edition ed: Lawrence Erlbaum Associates; 1988.
28. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21(11):1539-58.
29. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. Bmj. 2003;327(7414):557-60.
30. Gavaghan DJ, Moore AR, McQuay HJ. An evaluation of homogeneity tests in meta-analyses in pain using simulations of individual patient data. Pain. 2000;85(3):415-24.
31. Higgins JPT TJ, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.2 (updated February 2021): Cochrane; 2021 [Available from: www.training.cochrane.org/handbook.
32. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315(7109):629-34.
33. Kaiser E, Schoenknecht P, Kassner S, Hildebrandt W, Kinscherf R, Schroeder J. Cerebrospinal fluid concentrations of functionally important amino acids and metabolic compounds in patients with mild cognitive impairment and Alzheimer's disease. Neurodegenerative Diseases. 2010;7(4):251-9.
34. Mashige F, Takai N, Shinozuka N, Wada H, Sakuma I, Ito A, et al. Development of a high-performance liquid chromatography system with multi- electrode electrochemical detectors for determination of levels of neurotransmitters in cerebrospinal fluid. Japanese Journal of Clinical Chemistry. 1993;22(3):147-55.
35. Hafstad Solvang SE, Nordrehaug JE, Aarsland D, Lange J, Ueland PM, McCann A, et al. Kynurenines, Neuropsychiatric Symptoms, and Cognitive Prognosis in Patients with Mild Dementia. Int J Tryptophan Res. 2019;12:1178646919877883.
36. Chouraki V, Preis SR, Yang Q, Beiser A, Li S, Larson MG, et al. Association of amine biomarkers with incident dementia and Alzheimer's disease in the Framingham Study. Alzheimer's and Dementia. 2017;13(12):1327-36.
37. Toledo JB, Arnold M, Kastenmuller G, Chang R, Baillie RA, Han X, et al. Metabolic network failures in Alzheimer's disease: A biochemical road map. Alzheimer's and Dementia. 2017;13(9):965-84.
38. Wennstrom M, Nielsen HM, Orhan F, Londos E, Minthon L, Erhardt S. Kynurenic Acid levels in cerebrospinal fluid from patients with Alzheimer's disease or dementia with lewy bodies. Int J Tryptophan Res. 2014;7:1-7.
39. Ibáñez C, Simó C, Barupal DK, Fiehn O, Kivipelto M, Cedazo-Mínguez A, et al. A new metabolomic workflow for early detection of Alzheimer's disease. J Chromatogr A. 2013;1302:65-71.

40. Kaddurah-Daouk R, Zhu H, Sharma S, Bogdanov M, Rozen SG, Matson W, et al. Alterations in metabolic pathways and networks in Alzheimer's disease. Translational Psychiatry. 2013;3 (no pagination).
41. Trushina E, Dutta T, Persson XM, Mielke MM, Petersen RC. Identification of altered metabolic pathways in plasma and CSF in mild cognitive impairment and Alzheimer's disease using metabolomics. PLoS One. 2013;8(5):e63644.
42. Czech C, Berndt P, Busch K, Schmitz O, Wiemer J, Most V, et al. Metabolite profiling of Alzheimer's disease cerebrospinal fluid. PLoS One. 2012;7(2):e31501.
43. Kaddurah-Daouk R, Rozen S, Matson W, Han X, Hulette CM, Burke JR, et al. Metabolomic changes in autopsy-confirmed Alzheimer's disease. Alzheimer's and Dementia. 2011;7(3):309-17.
44. Fonteh AN, Harrington RJ, Tsai A, Liao P, Harrington MG. Free amino acid and dipeptide changes in the body fluids from Alzheimer's disease subjects. Amino Acids. 2007;32(2):213-24.
45. Baran H, Jellinger K, Deecke L. Kynurenine metabolism in Alzheimer's disease. Journal of Neural Transmission. 1999;106(2):165-81.
46. Molina JA, Jiménez-Jiménez FJ, Vargas C, Gómez P, de Bustos F, Ortí-Pareja M, et al. Cerebrospinal fluid levels of non-neurotransmitter amino acids in patients with Alzheimer's disease. J Neural Transm (Vienna). 1998;105(2-3):279-86.
47. Tohgi H, Abe T, Takahashi S, Saheki M, Kimura M. Indoleamine concentrations in cerebrospinal fluid from patients with Alzheimer type and Binswanger type dementias before and after administration of citalopram, a synthetic serotonin uptake inhibitor. Journal of Neural Transmission - Parkinson's Disease and Dementia Section. 1995;9(2-3):121-31.
48. Heyes MP, Saito K, Crowley JS, Davis LE, Demitrack MA, Der M, et al. Quinolinic acid and kynurenine pathway metabolism in inflammatory and non-inflammatory neurological disease. Brain. 1992;115 (Pt 5):1249-73.
49. Martinez M, Frank A, Diez-Tejedor E, Hernanz A. Amino acid concentrations in cerebrospinal fluid and serum in Alzheimer's disease and vascular dementia. J Neural Transm Park Dis Dement Sect. 1993;6(1):1-9.
50. Tohgi H, Abe T, Takahashi S, Kimura M, Takahashi J, Kikuchi T. Concentrations of serotonin and its related substances in the cerebrospinal fluid in patients with Alzheimer type dementia. Neuroscience Letters. 1992;141(1):9-12.
51. Beal MF, Matson WR, Swartz KJ, Gamache PH, Bird ED. Kynurenine pathway measurements in Huntington's disease striatum: evidence for reduced formation of kynurenic acid. J Neurochem. 1990;55(4):1327-39.
52. Mourdian MM, Heyes MP, Pan JB, Heuser IJ, Markey SP, Chase TN, et al. No changes in central quinolinic acid levels in Alzheimer's disease. Neurosci Lett. 1989;105(1-2):233-8.
53. Janssens J, Vermeiren Y, van Faassen M, van der Ley C, Kema IP, De Deyn PP. Monoaminergic and Kynurenergic Characterization of Frontotemporal Dementia and Amyotrophic Lateral Sclerosis in Cerebrospinal Fluid and Serum. Neurochem Res. 2020;45(5):1191-201.
54. Lin CN, Huang CC, Huang KL, Lin KJ, Yen TC, Kuo HC. A metabolomic approach to identifying biomarkers in blood of Alzheimer's disease. Ann Clin Transl Neurol. 2019;6(3):537-45.
55. Atukeren P, Cengiz M, Yavuzer H, Gelisgen R, Altunoglu E, Oner S, et al. The efficacy of donepezil administration on acetylcholinesterase activity and altered redox homeostasis in Alzheimer's disease. Biomedicine and Pharmacotherapy. 2017;90:786-95.
56. Oxenkrug G, van der Hart M, Roeser J, Summergrad P. Peripheral Tryptophan - Kynurenine Metabolism Associated with Metabolic Syndrome is Different in Parkinson's and Alzheimer's Diseases. Endocrinol Diabetes Metab J. 2017;1(4).
57. Gonzalez-Dominguez R, Garcia-Barrera T, Gomez-Ariza JL. Metabolite profiling for the identification of altered metabolic pathways in Alzheimer's disease. Journal of Pharmaceutical and Biomedical Analysis. 2015;107:75-81.
58. González-Domínguez R, García-Barrera T, Gómez-Ariza JL. Application of a novel metabolomic approach based on atmospheric pressure photoionization mass spectrometry using flow injection analysis for the study of Alzheimer's disease. Talanta. 2015;131:480-9.

59. Gonzalez-Dominguez R, Garcia-Barrera T, Gomez-Ariza JL. Metabolomic study of lipids in serum for biomarker discovery in Alzheimer's disease using direct infusion mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis. 2014;98:321-6.
60. Gulaj E, Pawlak K, Bien B, Pawlak D. Kynurenine and its metabolites in Alzheimer's disease patients. Advances in Medical Sciences. 2010;55(2):204-11.
61. Hartai Z, Juhasz A, Rimanoczy A, Janaky T, Donko T, Dux L, et al. Decreased serum and red blood cell kynurenic acid levels in Alzheimer's disease. Neurochemistry International. 2007;50(2):308-13.
62. Widner B, Leblhuber F, Walli J, Tilz GP, Demel U, Fuchs D. Tryptophan degradation and immune activation in Alzheimer's disease. Journal of Neural Transmission. 2000;107(3):343-53.
63. Widner B, Leblhuber F, Walli J, Tilz GP, Demel U, Fuchs D. Degradation of tryptophan in neurodegenerative disorders. Adv Exp Med Biol. 1999;467:133-8.
64. Bonaccorso S, Lin A, Song C, Verkerk R, Kenis G, Bosmans E, et al. Serotonin-immune interactions in elderly volunteers and in patients with Alzheimer's disease (DAT): Lower plasma tryptophan availability to the brain in the elderly and increased serum interleukin-6 in DAT. Aging - Clinical and Experimental Research. 1998;10(4):316-23.
65. Fekkes D, van der Cammen TJ, van Loon CP, Verschoor C, van Harskamp F, de Koning I, et al. Abnormal amino acid metabolism in patients with early stage Alzheimer dementia. J Neural Transm (Vienna). 1998;105(2-3):287-94.
66. Basun H, Forssell LG, Almkvist O, Cowburn RF, Eklöf R, Winblad B, et al. Amino acid concentrations in cerebrospinal fluid and plasma in Alzheimer's disease and healthy control subjects. J Neural Transm Park Dis Dement Sect. 1990;2(4):295-304.
67. Watkins SE, Thomas DE, Clifford M, Tidmarsh SF. Plasma amino acids in patients with senile dementia and in subjects with Down's syndrome at an age vulnerable to Alzheimer changes. Journal of Mental Deficiency Research. 1989;33(2):159-66.
68. Li NJ, Liu WT, Li W, Li SQ, Chen XH, Bi KS, et al. Plasma metabolic profiling of Alzheimer's disease by liquid chromatography/mass spectrometry. Clinical Biochemistry. 2010;43(12):992-7.
69. Shao Y, Ouyang Y, Li T, Liu X, Xu X, Li S, et al. Alteration of Metabolic Profile and Potential Biomarkers in the Plasma of Alzheimer's Disease. Aging Dis. 2020;11(6):1459-70.
70. Tsuruoka M, Hara J, Hirayama A, Sugimoto M, Soga T, Shankle WR, et al. Capillary electrophoresis-mass spectrometry-based metabolome analysis of serum and saliva from neurodegenerative dementia patients. Electrophoresis. 2013;34(19):2865-72.
71. Santos ALM, Vitório JG, de Paiva MJN, Porto BLS, Guimarães HC, Canuto GAB, et al. Frontotemporal dementia: Plasma metabolomic signature using gas chromatography-mass spectrometry. J Pharm Biomed Anal. 2020;189:113424.
72. Rudman D, Mattson DE, Feller AG, Cotter R, Johnson RC. Fasting plasma amino acids in elderly men. Am J Clin Nutr. 1989;49(3):559-66.
73. Thomas DE, Chung AOKO, Dickerson JW, Tidmarsh SF, Shaw DM. Tryptophan and nutritional status of patients with senile dementia. Psychol Med. 1986;16(2):297-305.
74. Shaw DM, Tidmarsh SF, Karajgi BM, Sweeney EA, Williams S, Elameer M, et al. Pilot study of amino acids in senile dementia. Br J Psychiatry. 1981;139:580-2.
75. Rommer PS, Fuchs D, Leblhuber F, Schroth R, Greilberger M, Tafeit E, et al. Lowered levels of carbonyl proteins after Vitamin B supplementation in patients with mild cognitive impairment and Alzheimer's disease. Neurodegenerative Diseases. 2016;16(3-4):284-9.
76. Greilberger J, Fuchs D, Leblhuber F, Greilberger M, Wintersteiger R, Tafeit E. Carbonyl proteins as a clinical marker in Alzheimer's disease and its relation to tryptophan degradation and immune activation. Clinical Laboratory. 2010;56(9-10):441-8.
77. Ramos-Chavez LA, Roldan-Roldan G, Garcia-Juarez B, Gonzalez-Esquivel D, Perez de la Cruz G, Pineda B, et al. Low serum tryptophan levels as an indicator of global cognitive performance in nondemented women over 50 years of age. Oxidative Medicine and Cellular Longevity. 2018;2018 (no pagination).

78. Peña-Bautista C, Flor L, López-Nogueroles M, García L, Ferrer I, Baquero M, et al. Plasma alterations in cholinergic and serotonergic systems in early Alzheimer Disease: Diagnosis utility. Clin Chim Acta. 2020;500:233-40.
79. Cogo A, Mangin G, Maïer B, Callebert J, Mazighi M, Chabriat H, et al. Increased serum QUIN/KYNA is a reliable biomarker of post-stroke cognitive decline. Mol Neurodegener. 2021;16(1):7.
80. Liu M, Zhou K, Li H, Dong X, Tan G, Chai Y, et al. Potential of serum metabolites for diagnosing post-stroke cognitive impairment. Molecular bioSystems. 2015;11(12):3287-96.
81. de Leeuw FA, Peeters CFW, Kester MI, Harms AC, Struys EA, Hankemeier T, et al. Blood-based metabolic signatures in Alzheimer's disease. Alzheimers Dement (Amst). 2017;8:196-207.
82. Schwarz MJ, Guillemin GJ, Teipel SJ, Buerger K, Hampel H. Increased 3-Hydroxykynurenine serum concentrations differentiate Alzheimer's disease patients from controls. European Archives of Psychiatry and Clinical Neuroscience. 2013;263(4):345-52.
83. Graham SF, Chevallier OP, Elliott CT, Hölscher C, Johnston J, McGuinness B, et al. Untargeted metabolomic analysis of human plasma indicates differentially affected polyamine and L-arginine metabolism in mild cognitive impairment subjects converting to Alzheimer's disease. PLoS One. 2015;10(3):e0119452.
84. Baker GB, Reynolds GP. Biogenic amines and their metabolites in Alzheimer's disease: Noradrenaline, 5-hydroxytryptamine and 5-hydroxyindole-3-acetic acid depleted in hippocampus but not in substantia innominata. Neuroscience Letters. 1989;100(1-3):335-9.
85. Storga D, Vrecko K, Birkmayer JG, Reibnegger G. Monoaminergic neurotransmitters, their precursors and metabolites in brains of Alzheimer patients. Neurosci Lett. 1996;203(1):29-32.
86. Arai H, Kobayashi K, Ichimiya Y, Kosaka K, Iizuka R. Free amino acids in post-mortem cerebral cortices from patients with Alzheimer-type dementia. Neuroscience Research. 1985;2(6):486-90.
87. Arai H, Kobayashi K, Ichimiya Y, Kosaka K, Iizuka R. A preliminary study of free amino acids in the postmortem temporal cortex from Alzheimer-type dementia patients. Neurobiol Aging. 1984;5(4):319-21.
88. Paglia G, Stocchero M, Cacciatore S, Lai S, Angel P, Alam MT, et al. Unbiased Metabolomic Investigation of Alzheimer's Disease Brain Points to Dysregulation of Mitochondrial Aspartate Metabolism. Journal of Proteome Research. 2016;15(2):608-18.
89. Tarbit I, Perry EK, Perry RH, Blessed G, Tomlinson BE. Hippocampal free amino acids in Alzheimer's disease. J Neurochem. 1980;35(5):1246-9.
90. Xu J, Begley P, Church SJ, Patassini S, Hollywood KA, Jüllig M, et al. Graded perturbations of metabolism in multiple regions of human brain in Alzheimer's disease: Snapshot of a pervasive metabolic disorder. Biochim Biophys Acta. 2016;1862(6):1084-92.
91. Liang Q, Liu H, Li X, Zhang A-H. High-throughput metabolomics analysis discovers salivary biomarkers for predicting mild cognitive impairment and Alzheimer's disease. RSC Advances. 2016;6(79):75499-504.
92. Yilmaz A, Ugur Z, Bisgin H, Akyol S, Bahado-Singh R, Wilson G, et al. Targeted Metabolic Profiling of Urine Highlights a Potential Biomarker Panel for the Diagnosis of Alzheimer's Disease and Mild Cognitive Impairment: A Pilot Study. Metabolites. 2020;10(9).
93. Nik Mohd Fakhruddin NNI, Shahar S, Ismail IS, Ahmad Azam A, Rajab NF. Urine Untargeted Metabolomic Profiling Is Associated with the Dietary Pattern of Successful Aging among Malaysian Elderly. Nutrients. 2020;12(10).
94. Wu L, Han Y, Zheng Z, Peng G, Liu P, Yue S, et al. Altered Gut Microbial Metabolites in Amnestic Mild Cognitive Impairment and Alzheimer's Disease: Signals in Host-Microbe Interplay. Nutrients. 2021;13(1).
95. Kepplinger B, Baran H, Kronsteiner C, Reuss J. Increased Levels of Kynurenic Acid in the Cerebrospinal Fluid in Patients with Hydrocephalus. Neurosignals. 2019;27(1):1-11.
96. Bie J, Guest J, Guillemin GJ, Grant R. Central kynurenine pathway shift with age in women. Journal of Neurochemistry. 2016;136(5):995-1003.
97. Kepplinger B, Baran H, Kainz A, Ferraz-Leite H, Newcombe J, Kalina P. Age-related increase of kynurenic acid in human cerebrospinal fluid - IgG and beta2-microglobulin changes. Neurosignals. 2005;14(3):126-35.

98. Eklundh T, Eriksson M, Sjöberg S, Nordin C. Monoamine precursors, transmitters and metabolites in cerebrospinal fluid: a prospective study in healthy male subjects. Journal of Psychiatric Research. 1996;30(3):201-8.
99. Banki CM, Molnar G. The influence of age, height, and body weight on cerebrospinal fluid amine metabolites and tryptophan in women. Biol Psychiatry. 1981;16(8):753-62.
100. Sipahi H, Girgin G, Inanici F, Ariogul S, Sahin G, Baydar T. Tryptophan degradation and neopterin levels by aging. Pteridines. 2013;24(1):33-9.
101. Frick B, Schroecksnadel K, Neurauter G, Leblhuber F, Fuchs D. Increasing production of homocysteine and neopterin and degradation of tryptophan with older age. Clinical Biochemistry. 2004;37(8):684-7.
102. Chatterjee P, Cheong YJ, Bhatnagar A, Goozee K, Wu Y, McKay M, et al. Plasma metabolites associated with biomarker evidence of neurodegeneration in cognitively normal older adults. J Neurochem. 2020.
103. Darst BF, Koscik RL, Hogan KJ, Johnson SC, Engelman CD. Longitudinal plasma metabolomics of aging and sex. Aging (Albany NY). 2019;11(4):1262-82.
104. Proenza AM, Palou A, Roca P. Amino acid distribution in human blood. A significant pool of amino acids is adsorbed onto blood cell membranes. Biochem Mol Biol Int. 1994;34(5):971-82.
105. Armstrong MD, Stave U. A study of plasma free amino acid levels. III. Variations during growth and aging. Metabolism. 1973;22(4):571-8.
106. Urbańska EM, Luchowski P, Luchowska E, Pniewski J, Woźniak R, Chodakowska-Zebrowska M, et al. Serum kynurenic acid positively correlates with cardiovascular disease risk factor, homocysteine: a study in stroke patients. Pharmacol Rep. 2006;58(4):507-11.
107. Tohgi H, Takahashi S, Abe T. The effect of age on concentrations of monoamines, amino acids, and their related substances in the cerebrospinal fluid. Journal of Neural Transmission - Parkinson's Disease and Dementia Section. 1993;5(3):215-26.
108. Ferraro TN, Hare TA. Free and conjugated amino acids in human CSF: influence of age and sex. Brain Res. 1985;338(1):53-60.
109. Dunn WB, Lin W, Broadhurst D, Begley P, Brown M, Zelena E, et al. Molecular phenotyping of a UK population: defining the human serum metabolome. Metabolomics. 2015;11:9-26.
110. Collino S, Montoliu I, Martin FP, Scherer M, Mari D, Salvioli S, et al. Metabolic Signatures of Extreme Longevity in Northern Italian Centenarians Reveal a Complex Remodeling of Lipids, Amino Acids, and Gut Microbiota Metabolism. PLoS ONE. 2013;8(3).
111. Johnson LC, Martens CR, Santos-Parker JR, Bassett CJ, Strahler TR, Cruickshank-Quinn C, et al. Amino acid and lipid associated plasma metabolomic patterns are related to healthspan indicators with ageing. Clin Sci (Lond). 2018;132(16):1765-77.
112. Theofylaktopoulou D, Midttun O, Ulvik A, Ueland PM, Tell GS, Vollset SE, et al. A community-based study on determinants of circulating markers of cellular immune activation and kynurenines: the Hordaland Health Study. Clin Exp Immunol. 2013;173(1):121-30.
113. Demling J, Langer K, Mehr MQ. Age dependence of large neutral amino acid levels in plasma. Focus on Tryptophan. Advances in experimental medicine and biology. 1996;398:579-82.
114. Phipps DA, Powell C. Plasma tryptophan, age and depression. Age Ageing. 1985;14(2):71-5.
115. Pertovaara M, Raitala A, Lehtimäki T, Karhunen PJ, Oja SS, Jylhä M, et al. Indoleamine 2,3-dioxygenase activity in nonagenarians is markedly increased and predicts mortality. Mech Ageing Dev. 2006;127(5):497-9.
116. Westbrook R, Chung T, Lovett J, Ward C, Joca H, Yang H, et al. Kynurenines link chronic inflammation to functional decline and physical frailty. JCI Insight. 2020;5(16).
117. Xyda SE, Vuckovic I, Petterson XM, Dasari S, Lalia AZ, Parvizi M, et al. Distinct Influence of Omega-3 Fatty Acids on the Plasma Metabolome of Healthy Older Adults. J Gerontol A Biol Sci Med Sci. 2020;75(5):875-84.
118. Sarwar G, Botting HG, Collins M. A comparison of fasting serum amino acid profiles of young and elderly subjects. J Am Coll Nutr. 1991;10(6):668-74.

119. Ruiz-Ruiz S, Sanchez-Carrillo S, Ciordia S, Mena MC, Mendez-Garcia C, Rojo D, et al. Functional microbiome deficits associated with ageing: Chronological age threshold. Aging Cell. 2020;19(1).
120. Caballero B, Gleason RE, Wurtman RJ. Plasma amino acid concentrations in healthy elderly men and women. Am J Clin Nutr. 1991;53(5):1249-52.
121. Gold AB, Herrmann N, Swardfager W, Black SE, Aviv RI, Tennen G, et al. The relationship between indoleamine 2,3-dioxygenase activity and post-stroke cognitive impairment. Journal of Neuroinflammation. 2011;8 (no pagination).
122. Leblhuber F, Walli J, Jellinger K, Tilz GP, Widner B, Laccone F, et al. Activated immune system in patients with Huntington's disease. Clinical Chemistry and Laboratory Medicine. 1998;36(10):747-50.
123. Wissmann P, Geisler S, Leblhuber F, Fuchs D. Immune activation in patients with Alzheimer's disease is associated with high serum phenylalanine concentrations. Journal of the Neurological Sciences. 2013;329(1-2):29-33.
124. Park SA, Son SY, Lee AY, Park HG, Lee WL, Lee CH. Metabolite Profiling Revealed That a Gardening Activity Program Improves Cognitive Ability Correlated with BDNF Levels and Serotonin Metabolism in the Elderly. Int J Environ Res Public Health. 2020;17(2).
125. Sofic E, Halket J, Przyborowska A, Riederer P, Beckmann H, Sandler M, et al. Brain quinolinic acid in Alzheimer's dementia. Eur Arch Psychiatry Neurol Sci. 1989;239(3):177-9.
126. Vecsei L, Szalardy L, Fulop F, Toldi J. Kynurenines in the CNS: recent advances and new questions. Nat Rev Drug Discov. 2013;12(1):64-82.
127. Sharma R, Razdan K, Bansal Y, Kuhad A. Rollercoaster ride of kynurenines: steering the wheel towards neuroprotection in Alzheimer's disease. Expert Opin Ther Targets. 2018;22(10):849-67.
128. Chen Y, Guillemin GJ. Kynurenine pathway metabolites in humans: disease and healthy States. Int J Tryptophan Res. 2009;2:1-19.
129. Stone TW, Perkins MN. Quinolinic acid: a potent endogenous excitant at amino acid receptors in CNS. Eur J Pharmacol. 1981;72(4):411-2.
130. Bo L, Guojun T, Li G. An Expanded Neuroimmunomodulation Axis: sCD83-Indoleamine 2,3-Dioxygenase-Kynurenine Pathway and Updates of Kynurenine Pathway in Neurologic Diseases. Front Immunol. 2018;9:1363.
131. Kincses ZT, Toldi J, Vecsei L. Kynurenines, neurodegeneration and Alzheimer's disease. J Cell Mol Med. 2010;14(8):2045-54.
132. Guillemin GJ, Brew BJ, Noonan CE, Takikawa O, Cullen KM. Indoleamine 2,3 dioxygenase and quinolinic acid Immunoreactivity in Alzheimer's disease hippocampus. Neuropathology and Applied Neurobiology. 2005;31(4):395-404.
133. Guillemin GJ, Brew BJ, Noonan CE, Knight TG, Smythe G, Callen KM. Mass spectroscopic detection of quinolinic acid in microdissected Alzheimer's disease plaques. Inflamm Res. 2005;54:S200-S.
134. Rahman A, Ting K, Cullen KM, Braidy N, Brew BJ, Guillemin GJ. The excitotoxin quinolinic acid induces tau phosphorylation in human neurons. PLoS One. 2009;4(7):e6344.
135. Zwilling D, Huang SY, Sathyasaikumar KV, Notarangelo FM, Guidetti P, Wu HQ, et al. Kynurenine 3-monooxygenase inhibition in blood ameliorates neurodegeneration. Cell. 2011;145(6):863-74.
136. Carrillo-Mora P, Mendez-Cuesta LA, Perez-De La Cruz V, Fortoul-van Der Goes TI, Santamaria A. Protective effect of systemic L-kynurenine and probenecid administration on behavioural and morphological alterations induced by toxic soluble amyloid beta (25-35) in rat hippocampus. Behav Brain Res. 2010;210(2):240-50.
137. Gellert L, Fuzik J, Goblos A, Sarkozi K, Marosi M, Kis Z, et al. Neuroprotection with a new kynurenic acid analog in the four-vessel occlusion model of ischemia. Eur J Pharmacol. 2011;667(1-3):182-7.
138. Robotka H, Sas K, Agoston M, Rozsa E, Szenasi G, Gigler G, et al. Neuroprotection achieved in the ischaemic rat cortex with L-kynurenine sulphate. Life Sci. 2008;82(17-18):915-9.
139. Baran H, Cairns N, Lubec B, Lubec G. Increased kynurenic acid levels and decreased brain kynurenine aminotransferase I in patients with Down syndrome. Life Sci. 1996;58(21):1891-9.
140. Beal MF, Matson WR, Storey E, Milbury P, Ryan EA, Ogawa T, et al. Kynurenic acid concentrations are reduced in Huntington's disease cerebral cortex. J Neurol Sci. 1992;108(1):80-7.

141. Anderson G, Maes M. TRYCAT pathways link peripheral inflammation, nicotine, somatization and depression in the etiology and course of Parkinson's disease. CNS Neurol Disord Drug Targets. 2014;13(1):137-49.
142. Bitzer-Quintero OK, Dávalos-Marín AJ, Ortiz GG, Meza AR, Torres-Mendoza BM, Robles RG, et al. Antioxidant activity of tryptophan in rats under experimental endotoxic shock. Biomed Pharmacother. 2010;64(1):77-81.
143. Weiss G, Diez-Ruiz A, Murr C, Theur I, Fuchs D. Tryptophan Metabolites as Scavengers of Reactive Oxygen and Chlorine Species. Pteridines. 2002;13(4):140-3.
144. Pazos M, Andersen ML, Skibsted LH. Amino acid and protein scavenging of radicals generated by iron/hydroperoxide system: an electron spin resonance spin trapping study. J Agric Food Chem. 2006;54(26):10215-21.
145. Murakami K, Ito M, Yoshino M. Xanthurenic acid inhibits metal ion-induced lipid peroxidation and protects NADP-isocitrate dehydrogenase from oxidative inactivation. J Nutr Sci Vitaminol (Tokyo). 2001;47(4):306-10.
146. Hirai T, Fukushima K, Kumamoto K, Iwahashi H. Effects of some naturally occurring iron ion chelators on in vitro superoxide radical formation. Biol Trace Elem Res. 2005;108(1-3):77-85.
147. Murakami K, Haneda M, Yoshino M. Prooxidant action of xanthurenic acid and quinoline compounds: role of transition metals in the generation of reactive oxygen species and enhanced formation of 8-hydroxy-2'-deoxyguanosine in DNA. Biometals. 2006;19(4):429-35.
148. Bakker L, Ramakers I, van Boxtel MPJ, Schram MT, Stehouwer CDA, van der Kallen CJH, et al. Associations between plasma kynurenines and cognitive function in individuals with normal glucose metabolism, prediabetes and type 2 diabetes: the Maastricht Study. Diabetologia. 2021;64(11):2445-57.
149. Zuo H, Ueland PM, Ulvik A, Eussen SJ, Vollset SE, Nygård O, et al. Plasma Biomarkers of Inflammation, the Kynurenine Pathway, and Risks of All-Cause, Cancer, and Cardiovascular Disease Mortality: The Hordaland Health Study. Am J Epidemiol. 2016;183(4):249-58.
150. Gaubert S, Bouchaut M, Brumas V, Berthon G. Copper--ligand interactions and the physiological free radical processes. Part 3. Influence of histidine, salicylic acid and anthranilic acid on copper-driven Fenton chemistry in vitro. Free Radic Res. 2000;32(5):451-61.
151. Miche H, Brumas V, Berthon G. Copper(II) interactions with nonsteroidal antiinflammatory agents. II. Anthranilic acid as a potential. OH-inactivating ligand. J Inorg Biochem. 1997;68(1):27-38.
152. Halova-Lajoie B, Brumas V, Fiallo MM, Berthon G. Copper(II) interactions with non-steroidal anti-inflammatory agents. III--3-Methoxyanthranilic acid as a potential *OH-inactivating ligand: a quantitative investigation of its copper handling role in vivo. J Inorg Biochem. 2006;100(3):362-73.
153. Baumgartner R, Forteza MJ, Ketelhuth DFJ. The interplay between cytokines and the Kynurenine pathway in inflammation and atherosclerosis. Cytokine. 2019;122:154148.
154. Reyes Ocampo J, Lugo Huitron R, Gonzalez-Esquivel D, Ugalde-Muniz P, Jimenez-Anguiano A, Pineda B, et al. Kynurenines with neuroactive and redox properties: relevance to aging and brain diseases. Oxid Med Cell Longev. 2014;2014:646909.
155. Lewitt PA, Li J, Lu M, Beach TG, Adler CH, Guo L, et al. 3-hydroxykynurenine and other Parkinson's disease biomarkers discovered by metabolomic analysis. Mov Disord. 2013;28(12):1653-60.
156. Pearson SJ, Reynolds GP. Increased brain concentrations of a neurotoxin, 3-hydroxykynurenine, in Huntington's disease. Neurosci Lett. 1992;144(1-2):199-201.
157. Guillemin GJ, Smythe GA, Veas LA, Takikawa O, Brew BJ. A beta 1-42 induces production of quinolinic acid by human macrophages and microglia. Neuroreport. 2003;14(18):2311-5.
158. Widner B, Leblhuber F, Fuchs D. Increased neopterin production and tryptophan degradation in advanced Parkinson's disease. J Neural Transm (Vienna). 2002;109(2):181-9.
159. Unlu A, Onmaz DE, Abusoglu S, Abusoglu G. HPLC and LC-MS/MS measurement methods for the quantification of asymmetric dimethylarginine (ADMA) and related metabolites. Turkish Journal of Biochemistry. 2021;46(4):327-47.
160. Abdel-Hamid ME. Comparative LC–MS and HPLC analyses of selected antiepileptics and beta-blocking drugs. Il Farmaco. 2000;55(2):136-45.

161. Sotelo-Orozco J, Chen S-Y, Hertz-Picciotto I, Slupsky CM. A Comparison of Serum and Plasma Blood Collection Tubes for the Integration of Epidemiological and Metabolomics Data. Frontiers in Molecular Biosciences. 2021;8(650).
162. Yu Z, Kastenmüller G, He Y, Belcredi P, Möller G, Prehn C, et al. Differences between Human Plasma and Serum Metabolite Profiles. PLOS ONE. 2011;6(7):e21230.
163. Spalletta G, Bossu P, Ciaramella A, Bria P, Caltagirone C, Robinson RG. The etiology of poststroke depression: a review of the literature and a new hypothesis involving inflammatory cytokines. Molecular Psychiatry. 2006;11(11):984-91.
164. Deac OM, Mills JL, Shane B, Midttun Ø, Ueland PM, Brosnan JT, et al. Tryptophan catabolism and vitamin B-6 status are affected by gender and lifestyle factors in healthy young adults. J Nutr. 2015;145(4):701-7.
165. Tomioka H, Masuda J, Takada A, Iwanami A. Comparison of Plasma Levels of Tryptophan Metabolites between Healthy People and Patients of Bipolar Depression at Various Age and Gender. Journal of Biomedical Science and Engineering. 2020;13(6):120-9.
166. Ngandu T, von Strauss E, Helkala EL, Winblad B, Nissinen A, Tuomilehto J, et al. Education and dementia: what lies behind the association? Neurology. 2007;69(14):1442-50.
167. Stirland LE, O'Shea CI, Russ TC. Passive smoking as a risk factor for dementia and cognitive impairment: systematic review of observational studies. International psychogeriatrics. 2018;30(8):1177-87.
168. Anstey KJ, Peters R. Alcohol and dementia - risk or protective factor? Nat Rev Neurol. 2018;14(11):635-6.
169. Pawlak D, Tankiewicz A, Mysliwiec P, Buczko W. Tryptophan metabolism via the kynurenine pathway in experimental chronic renal failure. Nephron. 2002;90(3):328-35.
170. Seliger SL, Siscovick DS, Stehman-Breen CO, Gillen DL, Fitzpatrick A, Bleyer A, et al. Moderate renal impairment and risk of dementia among older adults: the Cardiovascular Health Cognition Study. J Am Soc Nephrol. 2004;15(7):1904-11.
171. Mangge H, Summers KL, Meinitzer A, Zelzer S, Almer G, Prassl R, et al. Obesity-related dysregulation of the tryptophan-kynurenine metabolism: role of age and parameters of the metabolic syndrome. Obesity (Silver Spring). 2014;22(1):195-201.
172. Cournot M, Marquie JC, Ansiau D, Martinaud C, Fonds H, Ferrieres J, et al. Relation between body mass index and cognitive function in healthy middle-aged men and women. Neurology. 2006;67(7):1208-14.
173. Saito K, Fujigaki S, Heyes MP, Shibata K, Takemura M, Fujii H, et al. Mechanism of increases in L-kynurenine and quinolinic acid in renal insufficiency. Am J Physiol Renal Physiol. 2000;279(3):F565-72.
174. Schefold JC, Zeden JP, Fotopoulou C, von Haehling S, Pschowski R, Hasper D, et al. Increased indoleamine 2,3-dioxygenase (IDO) activity and elevated serum levels of tryptophan catabolites in patients with chronic kidney disease: a possible link between chronic inflammation and uraemic symptoms. Nephrol Dial Transplant. 2009;24(6):1901-8.
175. Gartlehner G, Affengruber L, Titscher V, Noel-Storr A, Dooley G, Ballarini N, et al. Single-reviewer abstract screening missed 13 percent of relevant studies: a crowd-based, randomized controlled trial. J Clin Epidemiol. 2020;121:20-8.
176. Biessels GJ, Deary IJ, Ryan CM. Cognition and diabetes: a lifespan perspective. Lancet Neurol. 2008;7(2):184-90.
177. Darlington LG, Mackay GM, Forrest CM, Stoy N, George C, Stone TW. Altered kynurenine metabolism correlates with infarct volume in stroke. Eur J Neurosci. 2007;26(8):2211-21.
178. Oxenkrug GF. Increased Plasma Levels of Xanthurenic and Kynurenic Acids in Type 2 Diabetes. Mol Neurobiol. 2015;52(2):805-10.
179. Song P, Ramprasath T, Wang H, Zou M-H. Abnormal kynurenine pathway of tryptophan catabolism in cardiovascular diseases. Cell Mol Life Sci. 2017;74(16):2899-916.
180. Teixeira MM, Passos VMA, Barreto SM, Schmidt MI, Duncan BB, Beleigoli AMR, et al. Association between diabetes and cognitive function at baseline in the Brazilian Longitudinal Study of Adult Health (ELSA- Brasil). Scientific Reports. 2020;10(1):1596.

181. Marx W, McGuinness AJ, Rocks T, Ruusunen A, Cleminson J, Walker AJ, et al. The kynurenine pathway in major depressive disorder, bipolar disorder, and schizophrenia: a meta-analysis of 101 studies. Molecular Psychiatry. 2020.
182. Ogyu K, Kubo K, Noda Y, Iwata Y, Tsugawa S, Omura Y, et al. Kynurenine pathway in depression: A systematic review and meta-analysis. Neuroscience & Biobehavioral Reviews. 2018;90:16-25.
183. Hebbrecht K, Skorobogatov K, Giltay EJ, Coppens V, De Picker L, Morrens M. Tryptophan Catabolites in Bipolar Disorder: A Meta-Analysis. Frontiers in Immunology. 2021;12(1500).
184. Mitchell AJ, Beaumont H, Ferguson D, Yadegarfar M, Stubbs B. Risk of dementia and mild cognitive impairment in older people with subjective memory complaints: meta-analysis. Acta Psychiatr Scand. 2014;130(6):439-51.
185. Carayol M, Licaj I, Achaintre D, Sacerdote C, Vineis P, Key TJ, et al. Reliability of Serum Metabolites over a Two-Year Period: A Targeted Metabolomic Approach in Fasting and Non-Fasting Samples from EPIC. PloS one. 2015;10(8):e0135437-e.

Supplemental material

Supplementary Tables

Supplementary Figures

Supplementary Appendix

Table S1. Study details of all studies included in the systematic review

Study	Study design	Country	Biological sample	Groups (n)	Age	Female (%)	Medication free patients (spec.)
Arai (1984)[1]	C-C	JP	Post-mortem brain tissue	AD (4) CTRL (8)	59.5 ± 17.7 69.6 ± 11.8	unk	Yes *(neuroleptic treatment)*
Arai (1985)[2]	C-C	JP	Post-mortem brain tissue	AD (5) CTRL (8)	61.6 ± 16.1 69.6 ± 11.8	unk	Yes (neuroleptics, antidepressants, opiates)
Armstrong (1973)[3]	Cross.	US	Plasma	193	18-77	52.8	n/a
Atukeren (2017)[4]	C-C	TR	Serum	AD (14) CTRL (32)	78.9 ± 8.0 77.3 ± 6.7	42.9 56.3	Yes *(antibiotic NSAIDS, steroids)*
Baker (1989)[5]	C-C	UK	Post-mortem brain tissue	AD (12) CTRL (13) AD (24) CTRL (21)	80.6 ± 7.3 82.5 ± 7.1 81.0 ± 7.5 80.0 ± 7.4	66.67 69.23 70.83 76.19	Yes *(neuroactive drugs)*
Banki (1981)[6]	Cross.	HU	CSF plasma	32	41.5 ± 10.7	100	n/a
Baran (1999)[7]	C-C	AT	CSF Serum	AD (2) CTRL (5)	73.2 72.3 ± 7.8	unk	unk
Basun (1990)[8]	C-C	SE	Plasma	AD (22) CTRL (11)	74.0 ± 9.0 79.0 ± 2.0	59.1 54.5	unk
Beal (1990)[9]	C-C	US	CSF	AD (9) CTRL (50)	76.7 ± 7.2 43.8 ± 3.2	unk	unk
Bie (2016)[10]	Cross.	AU	CSF	49	20-40 40-60 60-80	100	n/a

Bonaccorso (1998)[11]	C-C	unk	Plasma	AD (15) CTRL (15) Younger (13) Older (13)	78.4 ± 10.3 75.6 ± 9.1 32.9 ± 8.1 78.3 ± 5.7	80.0 46.7 38.5 53.9	Yes *(psychotropic)*
Caballero (1991)[12]	Cross.	US	Plasma	Young, ≤ 40 (138) Old, ≥ 55 (74)	26 ± 5 71 ± 8	unk	n/a
Capuron (2011)[13]	Cross.	FR	Serum	Younger (unk) Older (unk)	< 80 ≥ 80	unk	n/a
Chatterjee (2020)[14]	Cross.	AU	Plasma	100	78.2 ± 5.5	68.0	n/a
Chouraki (2017)[15]	Pros.	US	Plasma	*At follow up:* Dem (93) AD (68) CTRL (1974)	55.3 ± 9.5 67.8 ± 6.1 unk	52.4 51.6 unk	unk
Cogo (2021)[16]	C-C	FR	Serum	PSCI (13) PSNCI (10)	69.4 ± 17.8 64.7 ± 13.3	38.5 40.0	unk
Collino (2013)[17]	Cross.	IT	Serum	Younger (18) Older (20) Cent. (12)	31.2 ± 5.0 73.1 ± 3.0 101.0 ± 2.0	50.0 50.0 83.3	n/a
Czech (2012)[18]	C-C	EU DE FR CE SE	CSF	AD (79) CTRL (51)	69.7 63.1 ± 7.7	55.7 52.9	Yes *(anticoagulants, anti-inflammatory, anti-depressives, medication for cognitive disorders, schizophrenia and anxiety)*

Table S1. (Continue)

Study	Study design	Country	Biological sample	Groups (n)	Age	Female (%)	Medication free patients (spec.)
Darst (2019)[19]	Pros.	US	Plasma	1212	60.8 ± 6.7	69.0	Yes *(all for 8h)*
de Leeuw (2017)[20]	C-C	NL	Plasma	AD (127)[a] SCD (121)[a]	65.1 (9.1) 62.7 (8.0)	50 46	No
Demling (1996)[21]	Cross.	DE	Plasma	Younger (55) Older (36)	27.4 69.0	58.2 50.0	n/a
Dunn (2015)[22]	Cross.	UK	Serum	Younger (unk) Older (unk)	< 50 > 64	unk	n/a
Eklundh (1996)[23]	Cross.	SE	CSF	14	32.2 ± 6.8	0.0	n/a
Fakhruddin (2020)[24]	C-C	MY	Urine	MCI (9) CTRL (9)	unk	unk	unk
Fekkes (1998)[25]	C-C	NL	Plasma	AD (14) CTRL (17)	73.6 ± 6.3 70.1 ± 1.3	71.4 0.0	Yes *(neuroleptics, anticonvulsive)*
Ferraro (1985)[26]	Cross.	US	CSF	Younger Older	≤ 40 > 40	unk	n/a
Fonteh (2007)[27]	C-C	US	CSF Plasma Urine	AD (8) CTRL (8)	77.9 ± 7.4 79.5 ± 5.5	50.0 50.0	No
Frick (2004)[28]	Cross.	AT	Serum	Younger (13) Older 1 (15) Older 2 (15)	48.5 ± 8.6 67.3 ± 2.7 80.1 ± 6.1	unk	n/a
Giil (2017)[29]	C-C	UK	Plasma	AD (42) CTRL (42)	78.5 ± 6.3 78.6 ± 6.8	unk	unk
Gold (2011)[30]	Cross.	CA	Plasma	IS (41)	72.3 ± 12.2	46.3	No
Gonzalez-Dominguez (2014)[31]	C-C	ES	Serum	AD (22) CTRL (18)	78.5 ± 5.0 70.7 ± 4.1	54.6 61.1	unk

Gonzalez-Dominguez (2015a)[32]	C-C	ES	Serum	AD (23) CTRL (21)	79.2 ± 5.9 72.1 ± 5.4	65.2 57.1	Yes *(all)*
Gonzalez-Dominguez (2015b)[33]	C-C	ES	Serum	AD (30) CTRL (30)	80.3 ± 5.0 73.5 ± 5.9	60.0 66.7	Yes *(all)*
González-Sánchez (2020)[34]	C-C	ES	CSF Plasma	Mod AD (20) Mild AD (41) MCI (24) FTD (8) CTRL (23)	73.3 ± 7.2 71.9 ± 8.1 72.0 ± 7.1 66.4 ± 5.2 64.7 ± 10.8	65.0 53.7 58.4 37.4 34.8	Yes *(med affecting cognition or motor function)*
Graham (2015)[35]	C-C	IE	Plasma	*At follow up:* AD (19) MCI (16) CTRL (37)	77.9 ± 4.4 72.4 ± 7.3 73.1 ± 8.9	63.2 50.0 51.4	unk
Greilberger (2010)[36]	C-C	AT	Plasma	AD/MCI (16) CTRL (15)	63.3 ± 13.7 62.8 ± 3.6	56.3 73.3	unk
Gulaj (2010)[37]	C-C	PL	Plasma	AD (34) CTRL (18)	78.8 ± 5.7 76.2 ± 7.3	70.6 72.2	unk
Hafstad Solvang (2019)[38]	C-C	NO	Serum	AD (90) LBD (65)	75.1 ± 7.8 75.1 ± 6.3	67.8 40.0	unk
Hafstad Solvang (2019)[39]	Cross.	NO	Plasma	2174	71[a]	55.2	n/a
Hartai (2007)[40]	C-C	HU	Plasma Red blood cells	AD (28) CTRL (31)	77.0 ± 6.3 73.0 ± 8.3	78.6 67.7	Yes *(med. Influencing Dopaminergic system or KP)*

Table S1. (Continue)

Study	Study design	Country	Biological sample	Groups (n)	Age	Female (%)	Medication free patients (spec.)
Heyes (1992)[41]	C-C	US CA	CSF	AD (39) CTRL (30) Younger (16) Older (30)	63.8 ± 1.2 59.1 ± 14.2 35.2 ± 8.4 59.1 ± 14.2	unk	Yes *(all)*
Ibáñez (2013)[42]	C-C	SE	CSF	AD (21) CTRL (21) MCI-AD (12) MCI-Stable (21)	69 ± 9.6 58 ± 8.9 63 ± 9.4 60 ± 8.9	71 57 50 33	unk
Jacobs (2019)[43]	C-C	SE	CSF Plasma	AD (20) SCD (18)	77.9 ± 7.5 73.1 ± 7.9	55.0 16.7	unk
Janssens (2020)[44]	C-C	BE	CSF Serum	FTD (39) CTRL (26)	67.4 ± 11.6 67.0 ± 8.0	48.7 46.2	unk
Johnson (2018)[45]	Cross.	US	Plasma	Younger (14) Older (29)	23 ± 3.7 61 ± 5.4	50.0 55.2	n/a
Kaddurah-Daouk (2011)[46]	C-C	US	CSF	AD (15) CTRL (15)	80.0 ± 1.1 82.0 ± 8.8	73.0 73.0	unk
Kaddurah-Daouk (2013)[47]	C-C	US	CSF	AD (40) MCI (36) CTRL (38)	69.0 69.9 69.5	75.0 52.8 66.8	unk
Kaiser (2010)[48]	C-C	DE	CSF	AD (14) MCI (13)	71.6 ± 8.8 67.2 ± 7.4	57.1 53.9	Yes *(all for ≥ 3 months)*

Kepplinger (2005)[49]	Cross.	AT	CSF Serum	Younger (17 Older (10)	35.4 ± 9.1 61.6 ± 10.4	41.2 50.0	n/a
Kepplinger (2019)[50]	Cross.	AT	CSF Serum	Younger (15) Older (15)	37.9 ± 10.5 65.9 ± 7.4	66.7 73.3	unk
Küster (2017)[51]	Cross.	DE	Serum	DEM (4) MCI (32) SCD (11)	71.2 ± 6.0	57.5	No
Leblhuber (1998)[52]	Cross.	AT	Serum	HD (12)	42.4 ± 11.7	33.3	Yes *(neuroleptics)*
Li (2010)[53]	C-C	CN	Plasma	AD (20) CTRL (20)	68 ± 10 70 ± 9	50.0 50.0	Yes *(all)*
Liang (2016)[54]	C-C	CN	Saliva	AD (660) MCI (583)	78.6 ± 5.7 78.9 ± 4.9	50.3 50.4	unk
Lin (2019)[55]	C-C	TW	Plasma	AD (15) MCI (10) CTRL (15)	76.9 ± 8.0 74.6 ± 8.5 66.8 ± 6.5	unk	unk
Liu (2015)[56]	C-C	CN	Serum	PSCI (30) PSNCI (30)	unk	unk	unk
Martinez (1993)[57]	C-C	ES	CSF Serum	AD (13) VaD (13) CTRL (15)	68.0 ± 6.0 71.0 ± 6.0 66.0 ± 8.0	69.2 46.2 46.7	Yes *(neuroleptics, antidepressants)*
Mashige (1993)[58]	C-C	JP	CSF	AD (8) VaD (3)	67.3 ± 15.1 75.0 ± 7.2	37.5 33.3	unk
Molina (1998)[59]	C-C	ES	CSF Plasma	AD (37) CTRL (32)	70.9 ± 8.5 67.9 ± 9.2	54.1 53.1	unk
Mourdian (1989)[60]	C-C	US	Post-mortem brain tissue CSF	AD (22) CTRL (21) AD (35) CTRL (23)	75.0 ± 9.4 73.0 ± 9.2 64.0 ± 5.9 65.0 ± 9.6	unk	unk

Table S1. (Continue)

Study	Study design	Country	Biological sample	Groups (n)	Age	Female (%)	Medication free patients (spec.)
Oxenkrug (2017)[61]	C-C	US	Serum	AD (20) CTRL (24)	unk	60.0 50.0	No
Paglia (2016)[62]	C-C	US	Post-mortem brain tissue	AD (21) CTRL (19)	82.4 ± 6.7 83.5 ± 6.4	57.1 36.8	unk
Park (2020)[63]	Cross.	KR	Serum	40	73.9 ± 5.1	65.0	n/a
Peña-Bautista (2020)[64]	C-C	ES	Plasma	MCI due to AD (25) CTRL (25)	70 (67-73)[a] 66 (62-70)[a]	60.0 36.0	unk
Pertovaara (2006)[65]	Cross.	FI	Serum	Younger (309) Older (284)	21-64 90-91	45.0 76.4	n/a
Phipps (1985)[66]	Cross.	UK	Plasma	Younger (84) Older (47)	17-69 70+	61.9 61.7	n/a
Proenza (1994)[67]	Cross.	ES	Plasma Red blood cells	40	44.8 ± 12.6	0.0	n/a
Ramos-Chavez (2018)[68]	C-C	MX	Serum	MCI (23) CTRL (54)	unk	unk	Yes *(immunosuppressive and immunomodulatory med)*
Rommer (2016)[69]	C-C	AT	Plasma	AD/MCI (16) CTRL (15)	63.3 ± 13.7 62.8 ± 3.6	56.3 73.3	unk
Rudman (1989)[70]	C-C	US	Plasma	Dem (17) CTRL (21)	73.0 75.0	0.0 0.0	unk
Ruiz-Ruiz (2020)[71]	Cross.	ES	Feces	Younger (10) Older (10)	35.4 ± 6.6 74.5 ± 4.3	50.0 70.0	n/a

Santos (2020)[72]	C-C	BR	Plasma	FTD (9) CTRL (15)	65.5 ± 9.5 67.7 ± 8.4	33.3 66.7	Yes *(anticoagulants, anti-inflammatory)*
Sarwar (1991)[73]	Cross.	CA	Serum	Younger (37) Older (30)	30-35 80-89	62.2 63.3	n/a
Schwarz (2013)[74]	C-C	DE	Serum	AD (20) SCD (19)	74.0 ± 7.6 59.5 ± 10.2	80.0 42.1	Yes *(anti-inflammatory)*
Shao (2020)[75]	C-C	CN	Plasma	AD (30) MCI (13) CTRL (43)	71.6 ± 8.8 67.9 ± 7.2 65.5 ± 7.9	66.7 38.5 41.9	unk
Shaw (1981)[76]	C-C	UK	Plasma	Dem (32) CTRL (70) Younger (46) Older (70)	77.1 70.1 42.2 70.1	unk	unk
Sipahi (2013)[77]	Cross.	TR	Serum	Younger (46) Older (48)	38.6 ± 6.8 72.5 ± 5.5	56.5 43.8	n/a
Sorgdrager (2019)[78]	C-C	BE	CSF Serum	AD (33) CTRL (39)	73.7 ± 6.0 71.3 ± 10.7	54.5 53.8	No
Storga (1996)[79]	C-C	AT	Post-mortem brain tissue	AD (8) CTRL (6)	61.8 ± 12.9 69.8 ± 5.4	37.5 50.0	No
Tarbit (1980)[80]	C-C	UK	Post-mortem brain tissue	AD (8) CTRL (7)	75 ± 22.6 78 ± 29.1	unk	unk
Theofylaktopoulou (2013)[81]	Cross.	NO	Plasma	Younger (3723) Older (3329)	45-46 70-72	55.4 55.9	n/a

Table S1. (Continue)

Study	Study design	Country	Biological sample	Groups (n)	Age	Female (%)	Medication free patients (spec.)
Thomas (1986)[82]	C-C	UK	Plasma	Dem (23) CTRL (23)	77.2 76.1	60.9 60.9	Yes *(meds interfering with vitamin metabolism or intestinal absorption)*
Tohgi (1992)[83]	C-C	JP	CSF	AD (14) CTRL (10)	68.4 ± 10.1 68.5 ± 6.1	unk	unk
Tohgi (1993)[84]	Cross.	JP	CSF	Younger (31) Older 1 (18) Older 2 (5)	33.8 ± 9.1 56.8 ± 4.3 68.2 ± 1.8	19.4 27.8 40.0	n/a
Tohgi (1995)[85]	C-C	JP	CSF	AD (15) CTRL (10)	68.0 ± 6.0 68.5 ± 6.1	unk	unk
Toledo (2017)[86]	Pros.	US Canada	Serum	*At baseline:* AD (175) MCI (356) CTRL (199)	75.6 75.1 75.3	51.4 64.6 50.3	No
Trushina (2013)[87]	C-C	US	CSF Plasma	AD (15) MCI (15) CTRL (15)	82.7 ± 4.2 80.4 ± 4.2 78.6 ± 3.5	20.0 27.0 33.3	No
Tsuruoka (2013)[88]	C-C	US	Serum Saliva	AD (3) FTD (4) LBD (3) CTRL (9)	64.3 ± 16.9 72.0 ± 2.9 75.3 ± 4.9 68.1 ± 13.7	0.0 0.0 33.3 100	unk

Urbańska (2006)[89]	Cross.	PL	Serum	26	68.4 ± 8.3	84.6	unk
Valdiglesias (2017)[90]	Cross.	ES	Serum	259	65 ≦	67.2	n/a
Van der Velpen (2019)[91]	C-C	CH	CSF Plasma	AD (40) CTRL (34)	74.9 ± 6.4 65.4 ± 6.2	60.0 67.7	unk
Watkins (1989)[92]	C-C	UK	Plasma	AD (22) CTRL (22)	77.3 76.0	68.2 68.2	Yes *(meds interfering with internal absorption)*
Wennström (2014)[93]	C-C	SE	CSF	AD (19) LBD (18) CTRL (20)	75.0 77.0 76.0	52.6 55.6 50.0	No
Westbrook (2020)[94]	Cross.	US	Serum	Younger (50) Older (116)	25.6 ± 6.2 77.6 ± 5.9	32.0 43.1	n/a
Whiley (2021)[95]	C-C	EU	Serum Urine	AD (103) MCI (165) CTRL (86)	76.5 ± 6.0 76.3 ± 6.0 75.9 ± 5.2	51.5 57.0 48.8	No
Widner (1999)[96]	C-C	AT	Serum	AD (24) CTRL (unk)	unk	unk	Yes *(neuroleptics)*
Widner (2000)[97]	C-C	AT	Serum	AD (21) CTRL (20) Younger (49) Older (20)	74.4 ± 5.4 73.4 ± 7.4 38.4 ± 12.0 73.4 ± 7.4	71.4 50.0 unk 50.0	Yes *(nootropics)*
Willette (2021)[98]	C-C	US CA	Serum	AD (112) MCI (396) CTRL (58)	74.8 ± 8.1 74.7 ± 7.4 75.1 ± 5.8	42.0 35.4 48.3	Yes (*SSRIs, cholinesterase inhibitors, NMDA antagonists)*
Wissmann (2013)[99]	Cross.	AT	Serum	AD (43)	81.7 ± 10.5	60.5	No

Table S1. (Continue)

Study	Study design	Country	Biological sample	Groups (n)	Age	Female (%)	Medication free patients (spec.)
Wu (2021)[100]	C-C	CN	Faeces	AD (27) MCI (22) CTRL (28)	74.2 ± 11.2 70.0 ± 11.3 74.3 ± 9.0	44.4 59.1 50.0	unk
Xu (2016)[101]	C-C	NZ	Post-mortem brain tissue	AD (9) CTRL (9)	70.3 ± 7.1 70.1 ± 6.7	44.4 44.4	unk
Xyda (2020)[102]	Cross.	US	Plasma	Younger (12) Older (12)	27 ± 5 76 ± 5	50.0 41.7	n/a
Yilmaz (2020)[103]	C-C	US	Urine	AD (20) MCI (10) CTRL (29)	79.9 ± 9.1 76.6 ± 9.4 79.1 ± 6.3	55.0 50.0 55.2	unk

Table S1. (Continue from beginning with different study details)

Study	In/out patients	Diagnostic criteria	Measured kynurenines	Method	Fasting	Storage Temp.	Raw data
Arai (1984)[1]	unk	unk	TRP	Hitachi 835 high-speed amino analyser	n/a	-80	Yes
Arai (1985)[2]	unk	unk	TRP	Hitachi 835 high-speed amino analyser	n/a	-80	No
Armstrong (1973)[3]	n/a	n/a	TRP	unk	Yes	unk	Yes
Atukeren (2017)[4]	unk	NINCDS ADRDA	N-f-KYN KYN	Spectro fluorometric	Yes	-80	Yes

Baker (1989)[5]	unk	unk	TRP	HPLC	unk	unk	Yes
Banki (1981)[6]	n/a	n/a	TRP	Fluorometric	Yes	-20	Yes
Baran (1999)[7]	unk	unk	KYN KA 3-HK	unk	unk	unk	Yes
Basun (1990)[8]	Out	DSM-III	TRP	unk	Yes	-70	Yes
Beal (1990)[9]	Both	unk	KA	HPLC	unk	unk	Yes
Bie (2016)[10]	n/a	n/a	TRP KYN KA 3-HK 3-HAA PIC QA KTR PIC/QA Neopterin	GC-MS	unk	-20 to -80	Yes
Bonaccorso (1998)[11]	unk	DSM-III-R	TRP	HPLC	Yes	-75	Yes
Caballero (1991)[12]	n/a	n/a	TRP	Fluorometrically	Yes	-30	Yes
Capuron (2011)[13]	n/a	n/a	TRP KYN Neopterin	HPLC	Yes	-80	Yes
Chatterjee (2020)[14]	n/a	n/a	TRP KYN	UHPLC HRAM-MS	Yes	-80	No

Table S1. (Continue)

Study	In/out patients	Diagnostic criteria	Measured kynurenines	Method	Fasting	Storage Temp.	Raw data
Chouraki (2017)[15]	unk	DSM-IV NINCDS ADRDA	TRP KYN KA XA AA 3-HAA QA	LC-MS	Yes	-80	Yes
Cogo (2021)[16]	In	Test battery (MMSE, MoCA, WAIS-IV, FAB, TMT, Rey figure, Stroop)	TRP KYN KA QA KTR QA/KA	HPLC Mass fragmentography	n/a	n/a	Yes
Collino (2013)[17]	n/a	n/a	TRP	LC -MS/MS	Yes	-80	Yes
Czech (2012)[18]	unk	DSM-IV NINCDS ADRDA	TRP KYN	GC-MS LC -MS/MS	unk	unk	No
Darst (2019)[19]	n/a	n/a	TRP KYN	UPLC -MS/MS	Yes	-80	No
de Leeuw (2017)[20]	Out	NINCDS-ADRDA NIA-AA	TRP KYN	UPLC -MS/MS	No	-80	No
Demling (1996)[21]	n/a	n/a	TRP	HPLC	Yes	unk	Yes
Dunn (2015)[22]	n/a	n/a	TRP	UPLC-MS GC-MS	unk	-80	No
Eklundh (1996)[23]	n/a	n/a	TRP	HPLC	Yes	-70	No

Fakhruddin (2020)[24]	Out	unk	TRP KYN	unk	unk	-80	No
Fekkes (1998)[25]	Out	DSM-III-R NINCDS ADRDA	TRP	HPLC	unk	-80	Yes
Ferraro (1985)[26]	n/a	n/a	TRP	HPLC/ fluorometric analyser	unk	-80	Yes
Fonteh (2007)[27]	unk	NINCDS- ADRDA	TRP	LC-MS/MS	unk	-80	No
Frick (2004)[28]	n/a	n/a	TRP KYN KTR Neopterin	HPLC	unk	-20	Yes
Giil (2017)[29]	unk	CamCog, CERAD	TRP KYN 3-HK KA XA AA 3-HAA QA KTR Neopterin	LC-MS/MS	No	-80	Yes
Gold (2011)[30]	unk	NINCDS WHO- MONICA	TRP KYN KTR	HPLC	Yes	-80	Yes
Gonzalez- Dominguez (2014)[31]	unk	NINCDS ADRDA	KYN	DI-MS	Yes	-80	No

Table S1. (Continue)

Study	In/out patients	Diagnostic criteria	Measured kynurenines	Method	Fasting	Storage Temp.	Raw data
Gonzalez-Dominguez (2015a)[32]	Out	NINCDS ADRDA	TRP	GC-MS	Yes	-80	Yes
Gonzalez-Dominguez (2015b)[33]	Out	NINCDS ADRDA	PIC	FIA-APPI -QTOF-MS	unk	-80	Yes
González-Sánchez (2020)[34]	unk	NIA-AA, Biomarker profile, imaging	TRP KA KA/TRP	ELISA	unk	-80	Yes
Graham (2015)[35]	unk	NINCDS-ADRDA Petersen	TRP N-f-KYN 3-HK	LC-QTOF -MS	unk	-80	No
Greilberger (2010)[36]	unk	NINCDS ADRDA	TRP KYN KTR Neopterin	RP-HPLC	Yes	-70	Yes
Gulaj (2010)[37]	unk	DSM-IV	TRP KYN 3-HK KA AA QA KTR 3-HK/KYN KA/KYN AA/KYN QA/3-HK	HPLC	unk	-40	Yes

Hafstad Solvang (2019)[38]	unk	NINCDS ADRDA	TRP KYN 3-HK KA XA AA PIC QA KTR KA/KYN Neopterin	LC-MS/MS	No	-80	Yes
Hafstad Solvang (2019)[39]	n/a	n/a	TRP KYN 3-HK KA XA AA 3-HAA PIC QA KTR Neopterin	LC-MS/MS	No	-80	Yes
Hartai (2007)[40]	unk	DSM-IV NINCDS ADRDA	KYN KA	HPLC	unk	unk	Yes
Heyes (1992)[41]	unk	unk	TRP KYN KA QA QA/KA	unk	No	unk	Yes
Ibáñez (2013)[42]	unk	DSM-IV NINCDS ADRDA	TRP	RP-UHPLC-MS HILIC UHPLC-MS	Yes	-80	Yes

Table S1. (Continue)

Study	In/out patients	Diagnostic criteria	Measured kynurenines	Method	Fasting	Storage Temp.	Raw data
Jacobs (2019)[43]	Out	unk	TRP KYN 3-HK KA AA 3-HAA PIC QA KTR 3-HK/KYN 3-HAA/AA PIC/QA QA/KA Neopterin	UHPLC HPLC, GC-MS	unk	-80	Yes
Janssens (2020)[44]	Out	n/a	TRP KYN 3-HK KA XA AA QA PIC KTR 3-HK/XA	LC-MS/MS	n/a	-80	No
Johnson (2018)[45]	n/a	n/a	TRP	LC-MS/MS	Yes	-80	Yes
Kaddurah-Daouk (2011)[46]	Out	CERAD	TRP KYN 3-HAA TRP/KYN	LC-ECA	unk	-80	Yes

Kaddurah-Daouk (2013)[47]	Out	NINCDS ADRDA	TRP KYN KTR	LC-ECA	unk	unk	Yes
Kaiser (2010)[48]	Out	NINCDS ADRDA	TRP	HPLC	Yes	-80	Yes
Kepplinger (2005)[49]	n/a	n/a	KA	HPLC/ fluorometric analyser	unk	-30	Yes
Kepplinger (2019)[50]	n/a	n/a	TRP KYN KA	HPLC/ fluorometric analyser	unk	-40	Yes
Küster (2017)[51]	Out	unk	TRP KYN 3-HK KA QA	LC-MS/MS	No	-80	No
Leblhuber (1998)[52]	unk	Molecular genetics, autopsy	TRP KYN KTR Neopterin	HPLC ELISA	unk	-20	No
Li (2010)[53]	Out	unk	TRP	UPLC-MS	unk	-80	No
Liang (2016)[54]	Out	unk	TRP	FUPLC-MS	unk	-80	No
Lin (2019)[55]	unk	DSM-IV NINCDS ADRDA	KTR	LC-MS/MS	Yes	unk	Yes
Liu (2015)[56]	In	MoCA	TRP KYN	UHPLC-QTOF-MS	Yes	-80	No
Martinez (1993)[57]	unk	NINCDS ADRDA	TRP	HPLC	Yes	-40	Yes
Mashige (1993)[58]	unk	unk	TRP	HPLC	unk	-80	Yes

Table S1. (Continue)

Study	In/out patients	Diagnostic criteria	Measured kynurenines	Method	Fasting	Storage Temp.	Raw data
Molina (1998)[59]	unk	DSM-IV NINCDS ADRDA	TRP	Ion-exchange chromatography	Yes	-30	Yes
Mourdian (1989)[60]	unk	NINCDS ADRDA	KTR QA	NCI GC-MS	Yes	-70	Yes
Oxenkrug (2017)[61]	unk	MMSE	TRP KYN 3-HK KA XA AA KTR	HPLC-MS	unk	-80	Yes
Paglia (2016)[62]	unk	NIA-Reagan	TRP	UPLC-HILIC-MS	n/a	unk	Yes
Park (2020)[63]	n/a	n/a	TRP KYN AA	GC-TOF-MS	Yes	-80	No
Peña-Bautista (2020)[64]	Out	NIA-AA, Biomarker profile	TRP	UPLC-MS/MS	unk	-80	Yes
Pertovaara (2006)[65]	n/a	n/a	TRP KYN KTR	RP-HPLC	unk	unk	Yes
Phipps (1985)[66]	n/a	n/a	TRP	unk	unk	unk	Yes

Proenza (1994)[67]	n/a	n/a	TRP	HPLC	Yes	n/a	Yes
Ramos-Chavez (2018)[68]	Out	Test battery	TRP KYN 3-HK KA KTR 3-HK/TRP KA/TRP	HPLC/ fluorometric analyser	unk	-70	Yes
Rommer (2016)[69]	unk	NINCDS ADRDA	TRP KYM KTR Neopterin	RP-HPLC	Yes	-70	Yes
Rudman (1989)[70]	In	unk	TRP	HPLC	Yes	-20	Yes
Ruiz-Ruiz (2020)[71]	n/a	n/a	TRP	LC-MS	unk	-80	No
Santos (2020)[72]	Out	NIA-AA Biomarker profile	TRP	GC-MS	Yes	-80	No
Sarwar (1991)[73]	n/a	n/a	TRP	LC	Yes	-70	Yes
Schwarz (2013)[74]	Out	NINCDS ADRDA	TRP KYN 3-HK KA PIC QA 3-HK/TRP PIC/TRP	HPLC GC-MS	unk	-80	Yes
Shao (2020)[75]	unk	NINCDS ADRDA Petersen	TRP	UPLC	Yes	-80	No

Table S1. (Continue)

Study	In/out patients	Diagnostic criteria	Measured kynurenines	Method	Fasting	Storage Temp.	Raw data
Shaw (1981)[76]	In	Hare scale (1978)	TRP	unk	Yes	unk	Yes
Sipahi (2013)[77]	n/a	n/a	TRP KYN KTR Neopterin	RP-HPLC ELISA	unk	-20	Yes
Sorgdrager (2019)[78]	Out	NINCDS ADRDA	TRP KYN 3-HK KA XA QA KTR KA/QA XA/3-HK	LC-MS/MS	unk	-80	Yes
Storga (1996)[79]	unk	unk	TRP	HPLC	n/a	-70	No
Tarbit (1980)[80]	In	unk	TRP	Rank-Hilger Chromospek amino acid analyser	n/a	-20	Yes
Theofylaktopoulou (2013)[81]	n/a	n/a	TRP KYN 3-HK KA XA AA 3-HAA KTR Neopterin	LC-MS/MS	No	-80	Yes

Thomas (1986)[82]	In	History, Clinical characteristics and test performance	TRP	Ultra-filtration	Yes	-20	Yes
Tohgi (1992)[83]	unk	DSM III-R Hachinski NINCDS ADRDA CT/ MRI	TRP KYN 3-HK KTR 3-HK/TRP	HPLC	Yes	-80	Yes
Tohgi (1993)[84]	n/a	n/a	TRP KYN	HPLC	unk	-80	Yes
Tohgi (1995)[85]	unk	DSM III-R Hachinski NINCDS ADRDA CT/ MRI	TRP KYN 3-HK KYN/3-HK	HPLC	Yes	-80	Yes
Toledo (2017)[86]	Out	NINCDS ADRDA PET/ MRI	TRP KYN	UPLC-MS/MS	Yes	unk	No
Trushina (2013)[87]	Out	DSM-IV NINCDS ADRDA	TRP	LC-MS	Yes	-80	No
Tsuruoka (2013)[88]	unk	NINCDS ADRDA	TRP	CE-TOF-MS	unk	-80	Yes
Urbańska (2006)[89]	unk	Anamnesis, neurological examination CT	KA	HPLC/ Fluorometric analyser	Yes	-72	Yes
Valdiglesias (2017)[90]	n/a	n/a	TRP KYN KTR Neopterin	HPLC ELISA	unk	unk	Yes

Table S1. (Continue)

Study	In/out patients	Diagnostic criteria	Measured kynurenines	Method	Fasting	Storage Temp.	Raw data
Van der Velpen (2019)[91]	Out	CDR Biomarker profile, CT/ MRI	TRP KYN 3-HK KA AA QA	UHPLC-MS	unk	unk	No
Watkins (1989)[92]	In	History, clinical characteristics and test performance	TRP	unk	Yes	-20	Yes
Wennström (2014)[93]	Out	DSM-IV NINCDS ADRDA	KA	RP-HPLC	unk	-80	Yes
Westbrook (2020)[94]	n/a	n/a	TRP KYN 3-HK KA XA AA QA KTR	HPLC	unk	-80	No
Whiley (2021)[95]	Out	DSM-IV NINCDS ADRDA	TRP KYN 3-HK KA XA 3-HAA QA PIC KTR	UHPLC-MS/MS UHPLC-QTOF-MS	unk	-80	Yes

Widner (1999)[96]	unk	unk	TRP KYN KTR Neopterin	HPLC ELISA	unk	-20	No
Widner (2000)[97]	Out	NINCDS ADRDA	TRP KYN KTR	HPLC	unk	unk	Yes
Willette (2021)[98]	Out	NINCDS ADRDA	TRP KYN KTR	LC-MS	unk	unk	Yes
Wissmann (2013)[99]	unk	NINCDS ADRDA PET/ MRI	KTR Neopterin	RP-HPLC ELISA	unk	unk	No
Wu (2021)[100]	Out	DSM-IV NINCDS ADRDA	KYN KA	UPLC-MS	Yes	unk	No
Xu (2016)[101]	unk	CERAD Braak	TRP	GC-MS	unk	-80	No
Xyda (2020)[102]	n/a	n/a	TRP KYN	UPLC-MS	Yes	-80	Yes
Yilmaz (2020)[103]	Out	NINCDS ADRDA	TRP	UPLC	Yes	-80	Yes

unk unknown, *n/a* not applicable, [a]Age in median (IQR). Abbreviations: C-C, Case-control; Cross, Cross-sectional; Pros, Prospective; AD, Alzheimer's dementia; CTRL, Controls; Dem, Dementia; IS, Ischemic stroke patients; MCI, Mild Cognitive Impairment; HD, Huntington's disease; PSCI, Post-stroke cognitive impairment; PSNCI, Post-stroke no cognitive impairment; SCD, Subjective Cognitive Decline; LBD, Lewy Body Dementia; Cent, Centenarians; NINCDS ADRDA, National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association; NIA-AA, National Institute on Aging – Alzheimer's Association; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; WHO-MONICA, World Health Organization-Multinational Monitoring of Trends and Determinants in Cardiovascular Disease; FAB, Frontal Assessment Battery; MMSE, Mini Mental State Examination; CamCog, Cambridge Cognition examination; MoCA, Montreal Cognitive Assessment; WAIS, Wechsler Adult Intelligence Scale; ELISA, Enzyme-linked immunosorbent assay; GC, Gas chromatography; LC, Liquid chromatography; HPLC, High-performance liquid chromatography; UPLC/UHPLC, Ultra high performance liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; QTOF, Quadrupole time of flight; HILIC, Hydrophilic interaction liquid chromatography; NCI, Negative chemical ionization; FIA, Flow injection analysis; ECA, Electrochemical array; HRAM, High resolution accurate mass; APPI, Atmospheric pressure photo ionization; JP, Japan; TR, Turkey; HU, Hungary; US, United States; UK, United Kingdom; AT, Austria; SE, Sweden; AU, Australia; FR, France; IT, Italy; DE, Germany; CH, Switzerland; NL, Netherlands; MY, Malaysia; CA, Canada; ES,

Spain; IE, Ireland; PL, Poland; NO, Norway; BE, Belgium; CN, China; TW, Taiwan; KR, Republic of Korea; FI, Finland; MX, Mexico; BR, Brazil; NZ, New Zealand; EU, European Union.

Table S2. Differences in CSF KP metabolite ratios and neopterin between cases and controls

Study	Cases			Controls			Cov. in analyses	Ratios									Neop
	N	age	% female	N	age	% female		TRP/ KYN	HK/ TRP	KYN/ HK	PIC/ QA	QA/ KA	KA/ QA	KA/ TRP	HAA/ AA	HK/ XA	XA/ HK	
Alzheimer's dementia																		
González-Sánchez (2020)[34]	Mod (20)	73.3 ± 7.2	65.0	23	64.7 ± 10.8	34.8	None	-	-	-	-	-	-	ns	-	-	-	-
	Mild (41)	71.9 ± 8.1	53.7					-	-	-	-	-	-	↑	-	-	-	-
Jacobs (2019)[43]	20	77.9 ± 7.5	55.0	18	73.1 ± 7.9	16.7	Sex	-	-	-	ns	↓	-	-	ns	-	-	ns
Sorgdrager (2019)[78]	33	73.7 ± 6.0	54.5	39	71.3 ± 10.7	53.8	Age, sex	-	-	-	-	-	↓	-	-	-	ns	-
Kaddurah-Daouk (2011)[46]	15	80.0 ± 1.1	73.0	15	82.0 ± 8.8	73.0	clinical/ demographic measures, compound ratios	ns	-	-	-	-	-	-	-	-	-	-
Tohgi (1995)[85]	15	68.0 ± 6.0	unk	10	68.5 ± 6.1	unk	None	-	-	↑	-	-	-	-	-	-	-	-
Heyes (1992)[41]	39	63.8 ± 1.2	unk	30	59.1 ± 14.2	unk	None	-	-	-	-	ns	-	-	-	-	-	-
Tohgi (1992)[83]	14	68.4 ± 10.1	unk	10	68.5 ± 6.1	unk	None	-	↓	-	-	-	-	-	-	-	-	-

Frontotemporal dementia																						
González-Sánchez (2020)[34]	8	66.4 ± 5.2	37.4	23	64.7 ± 10.8	34.8	None	-	-	-	-	-	-	ns	-	-	-	-
Janssens (2020) [44]	39	67.4 ± 11.6	48.7	25	67.3 ± 8.1	37.9	None	-	-	-	-	-	-	-	-	ns	-	-
Mild cognitive impairment																						
González-Sánchez (2020)[34]	24	72.0 ± 7.1	58.4	23	64.7 ± 10.8	34.8	None	-	-	-	-	-	-	ns	-	-	-	-

↑ Higher in cases, ↓ lower in cases, *ns* non-significant, *unk* unknown, - metabolite not measured. If not stated otherwise, controls consisted of neurologically healthy individuals. Abbreviations: Mod, Moderate; TRP, Tryptophan; KYN, Kynurenine; KA, Kynurenic acid; AA, Anthranilic acid; HK, 3-hydroxykynurenine; HAA, 3-hydroxyanthranilic acid; XA, Xanthurenic acid; QA, Quinolinic acid; PIC, Picolinic acid; Neop, Neopterin.

Table S3. Differences in plasma and serum KP metabolite ratios and neopterin

Study	Cases			Controls			Blood	Cov. in analyses
	N	Age	% female	N	Age	% female		
Alzheimer's disease dementia								
Sorgdrager (2019)[78]	33	73.7 ± 6.0	54.5	39	71.3 ± 10.7	53.8	S	Age, sex
Schwarz (2013)[74]	20	74.0 ± 7.6	80.0	SCD (19)	59.5 ± 10.2	42.1	S	Age, sex
Widner (1999)[96]	24	unk	unk	unk	unk	unk	S	None
González-Sánchez (2020)[34]	Mod (20)	73.3 ± 7.2	65.0	23	64.7 ± 10.8	34.8	P	None
	Mild (41)	71.9 ± 8.1	53.7					
Jacobs (2019)[43]	20	77.9 ± 7.5	55.0	18	73.1 ± 7.9	16.7	P	Sex
Giil (2017)[29]	42	78.5 ± 6.3	unk	42	78.6 ± 6.8	unk	P	Age, sex, creatinine
Gulaj (2010)[37]	34	78.8 ± 5.7	70.6	18	76.2 ± 7.3	72.2	P	None
Frontotemporal dementia								
Janssens (2020) [44]	39	67.4 ± 11.6	48.7	26	67.0 ± 8.0	46.2	S	None
Mild cognitive impairment + Alzheimer's disease dementia								
Rommer (2016)[69]	16	63.3 ± 13.7	56.3	15	62.8 ± 3.6	73.3	P	None
Greilberger (2010)[36]	16	63.3 ± 13.7	56.3	15	62.8 ± 3.6	73.3	P	None
Mild cognitive impairment								
Ramos-Chavez (2018)[68]	23	unk	unk	54	unk	unk	S	Age, TRP
González-Sánchez (2020)[34]	24	72.0 ± 7.1	58.4	23	64.7 ± 10.8	34.8	P	None
Post-stroke cognitive impairment								
Cogo (2021)[16]	13	69.4 ± 17.8	38.5	PSNCI (10)	64.7 ± 13.3	40.0	S	None

↑ Higher in cases, ↓ lower in cases, *ns* non-significant, *unk* unknown, - metabolite not measured. If not stated decline; PSCNI, Post-stroke no cognitive impairment; S, Serum; P, Plasma; TRP, Tryptophan; KYN, 3-hydroxyanthranilic acid; XA, Xanthurenic acid; QA, Quinolinic acid; PIC, Picolinic acid; KTR, Kynurenine-

between cases and controls

Ratios													
HK/ KYN	**AA/ KYN**	**HAA/ AA**	**PIC/ QA**	**QA/ KA**	**KA/ QA**	**KA/ KYN**	**XA/ HK**	**QA/ HK**	**KA/ TRP**	**HK/ TRP**	**PIC/ TRP**	**HK/ XA**	**Neop**
-	-	-	-	-	ns	-	↓	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	↑	ns	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	↑
-	-	-	-	-	-	-	-	-	ns	-	-	-	-
-	-	-	-	-	-	-	-	-	ns	-	-	-	-
ns	-	ns	ns	ns	-	-	-	-	-	-	-	-	ns
-	-	-	-	-	-	-	-	-	-	-	-	-	ns
↓	↓	-	-	-	-	↓	-	↑	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	ns	-
-	-	-	-	-	-	-	-	-	-	-	-	-	ns
-	-	-	-	-	-	-	-	-	-	-	-	-	ns
-	-	-	-	-	-	-	-	-	↑	↑	-	-	-
-	-	-	-	-	-	-	-	-	ns	-	-	-	-
-	-	-	-	↑	-	-	-	-	-	-	-	-	-

otherwise, controls consisted of neurologically healthy individuals. Abbreviations: SCD, Subjective cognitive
Kynurenine; KA, Kynurenic acid; AA, Anthranilic acid; HK, 3-hydroxykynurenine; HAA,
Tryptophan ratio; Neop, Neopterin.

Table S4. Differences in TRP and KP metabolites between cases and controls in

Study	Cases			Controls		
	N	Age	Female (%)	N	Age	Female (%)
Red blood cells						
Hartai (2007)[40]	AD (28)	77.0 ± 6.3	78.6	31	73.0 ± 8.3	67.7
Saliva						
Liang (2016)[54]	AD (660)	78.6 ± 5.7	50.3	MCI (583)	78.9 ± 4.9	50.4
Tsuruoka (2013)[88]	AD (3)	64.3 ± 16.9	0.0	9	68.1 ± 13.7	100
	FTD (4)	72.0 ± 2.9	0.0			
	LBD (3)	75.3 ± 4.9	33.3			
Urine						
Whiley (2021)[95]	AD (103)	76.5 ± 6.0	51.5	86	75.9 ± 5.2	48.8
	MCI (165)	76.3 ± 6.0	57.0			
Fakhruddin (2020)[24]	MCI (9)	unk	unk	9	unk	unk
Yilmaz (2020)[103]	AD (20)	79.9 ± 9.1	55.0	29	79.1 ± 6.3	55.2
	MCI (10)	76.6 ± 9.4	50.0			
Fonteh (2007)[27]	AD (8)	77.9 ± 7.4	50.0	8	79.5 ± 5.5	50.0
Feces						
Wu (2021)[100]	AD (27)	74.2 ± 11.2	44.4	28	74.3 ± 9.0	50.0
	MCI (22)	70.0 ± 11.3	59.1			

other biological materials

Covariate in analyses	Metabolites									
	TRP	**KYN**	**3-HK**	**KA**	**XA**	**AA**	**3-HAA**	**QA**	**PIC**	**KTR**
None	-	ns	-	↓	-	-	-	-	-	-
None	↑	-	-	-	-	-	-	-	-	-
None	ns	-	-	-	-	-	-	-	-	-
	ns	-	-	-	-	-	-	-	-	-
	ns	-	-	-	-	-	-	-	-	-
None	↓	ns	ns	↓	↓	-	ns	-	-	↓
	ns	ns	ns	↓	↓	-	ns	-	-	↓
None	↓	↓	-	-	-	-	-	-	-	-
None	ns	-	-	-	-	-	-	-	-	-
	ns	-	-	-	-	-	-	-	-	-
None	ns	-	-	-	-	-	-	-	-	-
None	-	ns	-	ns	-	-	-	-	-	-
	-	ns	-	ns	-	-	-	-	-	-

Table S4. (Continue)

Study	Cases			Controls		
	N	Age	Female (%)	N	Age	Female (%)
Post-mortem brain tissue						
Paglia (2016)[62]	AD (21) Frontal cortex	82.4 ± 6.7	57.1	19 Frontal cortex	83.5 ± 6.4	36.8
Tarbit (1980)[80]	AD (8) Hippocampus	75.0 ± 22.6	unk	7 Hippocampus	78.0 ± 29.1	unk
Xu (2016)[101]	AD (9) Hippocampus Entorhinal cortex Middle temporal gyrus Sensory cortex Motor cortex Cingulate gyrus Cerebellum	70.3 ± 7.1	44.4	9 Hippocampus Entorhinal cortex Middle temporal gyrus Sensory cortex Motor cortex Cingulate gyrus Cerebellum	70.1 ± 6.7	44.4
Storga (1996)[79]	AD (8) Globus pallidus Putamen Nucleus amygdalae Nucleus caudatus Substantia nigra Gyrus cingula Raphe	61.8 ± 12.9	37.5	6 Globus pallidus Putamen Nucleus amygdalae Nucleus caudatus Substantia nigra Gyrus cingula Raphe	69.8 ± 5.4	50.0
Baker (1989)[5]	AD Hippocampus (12) Substantia innominata (24)	80.6 ± 7.3 81.0 ± 7.5	66.7 70.8	 Hippocampus (13) Substantia innominata (21)	82.5 ± 7.1 80.0 ± 7.4	69.2 76.2
Mourdian (1989)[60]	AD Frontal (A4) (12) Frontal (A9) (10) Parietal (A39) (4) Temporal (A22) (9) Temporal (A38) (11) Occipital (A17) (11) Hippocampus (9) Caudate (18)	75.0 ± 9.4	unk	Frontal (A4) (6) Frontal (A9) (11) Parietal (A39) (10) Temporal (A22) (14) Temporal (A38) (10) Occipital (A17) (16) Hippocampus (11) Caudate (10)	73.0 ± 9.2	unk

Cov. in analyses	Metabolites									
	TRP	**KYN**	**3-HK**	**KA**	**XA**	**AA**	**3-HAA**	**QA**	**PIC**	**KTR**
None	ns	-	-	-	-	-	-	-	-	-
None	ns	-	-	-	-	-	-	-	-	-
None	↑ ↑ ↑ ns ↑ ↑ ns	-	-	-	-	-	-	-	-	-
None	ns ns ns ns ns ns ns	-	-	-	-	-	-	-	-	-
None	ns ns	-	-	-	-	-	-	-	-	-
None	-	-	-	-	-	-	-	ns ns ns ns ns ns ns ns	-	-

Table S4. (Continue)

Study	Cases			Controls		
	N	Age	Female (%)	N	Age	Female (%)
Post-mortem brain tissue						
Arai (1985)[2]	AD (5) Superior frontal Orbital Cingulate Inferior temporal (4) Insular	61.6 ± 16.1	unk	5 Superior frontal Orbital Cingulate Inferior temporal (4) Insular	69.6 ± 11.8	unk
Arai (1984)[1]	AD (4) Temporal	59.5 ± 17.7	unk	8 Temporal	69.6 ± 11.8	unk

↑ Sig. higher in cases, ↓ Sig. lower in cases, *ns* non-significant, *unk* unknown, - metabolite not measured. If
Abbreviations: IQR, interquartile range; AD, Alzheimer's disease dementia; FTD, Frontotemporal dementia; PSCI, post-stroke cognitive impairment; PSNCI, post-stroke no cognitive impairment; P, Plasma; S Serum; AA, Anthranilic acid; HK, 3-hydroxykynurenine; HAA, 3-hydroxyanthranilic acid; XA, Xanthurenic acid;

Cov. in analyses	Metabolites									
	TRP	**KYN**	**3-HK**	**KA**	**XA**	**AA**	**3-HAA**	**QA**	**PIC**	**KTR**
None	ns ns ns ns ns	-	-	-	-	-	-	-	-	-
None	ns	-	-	-	-	-	-	-	-	-

not stated otherwise, controls consisted of neurologically healthy individuals. [a]Age in median (IQR).
LBD, Lewy body dementia; MCI, mild cognitive impairment; Dem, dementia; CTRL, control;
med, Medication; supp, Supplements; TRP, Tryptophan; KYN, Kynurenine; KA, Kynurenic acid;
QA, Quinolinic acid; PIC, Picolinic acid; KTR, Kynurenine-Tryptophan ratio; Neop, Neopterin.

Table S5. Associations between TRP and KP metabolites and age

Study	N	Age	Female (%)	Range	Covariate in analyses	Metabolites									
						TRP	KYN	3-HK	KA	XA	AA	3-HAA	QA	PIC	KTR
CSF															
Kepplinger (2019)[50]	30	51.9	70.0	18-80	None	↑	↑	-	↑	-	-	-	-	-	-
Sorgdrager (2019)[78]	105	72.7	49.5	unk	Age*disease	ns	↑	ns	↑	ns	-	-	↑	-	-
Bie (2016)[10]	49	unk	100	0-98	None	↓	ns	↓	ns	-	-	-	↑	↑	↑
Wennström (2014)[93]	20	76.0	50.0	71-84	None	-	-	-	ns	-	-	-	-	-	-
Kepplinger (2005)[49]	27	45.1 ± 15.6	44.4	25-74	None	-	-	-	↑	-	-	-	-	-	-
Eklundh (1996)[23]	14	32.2 ± 6.8	0.0	22-45	Height, weight, tapping-time, atmospheric pressure, storage time, neuraxis distance	↑↓	-	-	-	-	-	-	-	-	-
Heyes (1992)[41]	46	50.8	unk	unk	None	ns	ns	-	ns	-	-	-	↑	-	-
Martinez (1993)[57]	15	66.0 ± 8.0	46.7	unk	None	ns	-	-		-	-	-	-	-	-
Beal (1990)[9]	50	43.8 ± 3.2	unk	unk	None	-	-	-	↑	-	-	-	-	-	-

Mourdian (1989)[60]	29	unk	unk	22-81	None	-	-	-		-	-	-	ns	-	-
Banki (1981)[6]	32	41.5 ± 10.7	100	unk	None	ns	-	-	-	-	-	-	-	-	-
Serum															
Kepplinger (2019)[50]	30	51.9	70.0	18-80	None	ns	ns		ns	-	-	-	-	-	-
Sorgdrager (2019)[78]	105	72.7	49.5	unk	Age*disease	ns	↑	ns	↑	ns	-	-	↑	-	-
Valdiglesias (2017)[90]	259	unk	67.2	65 ≦	Sex, smoking habits	↓	↑	-	-	-	-	-	-	-	↑
Küster (2017)[51]	47	71.2 ± 6.0	57.5	60-88	None	-	ns	ns	ns	-	-	-	ns	-	-
Sipahi (2013)[77]	94	55.9 ± 19.4	50.0	24-88	None	↓	↑	-	-	-	-	-	-	-	↑
Capuron (2011)[13]	284	79.9 ± 4.5	69.4	unk	Sex, BMI	↓	↑	-	-	-	-	-	-	-	↑
Urbańska (2006)[89]	26	68.4 ± 8.3	84.6	unk	None	-	-	-	↑	-	-	-	-	-	-
Kepplinger (2005)[49]	27	45.1 ± 15.6	44.4	25-74	None	-	-	-	ns	-	-	-	-	-	-
Frick (2004)[28]	43	66.3	48.8	34-93	None	↓	-	-	-	-	-	-	-	-	↑
Martinez (1993)[57]	15	66.0 ± 8.0	46.7	unk	None	ns	-	-	-	-	-	-	-	-	-

Table S5. (Continue)

Study	N	Age	Female (%)	Range	Covariate in analyses	Metabolites									
						TRP	KYN	3-HK	KA	XA	AA	3-HAA	QA	PIC	KTR
Plasma															
Chatterjee (2020)[14]	100	78.2 ± 5.5	68.0	65-90	None	↓	ns	-	-	-	-	-	-	-	-
Shao (2020)[75]	43	65.5 ± 7.9	41.9	unk	None	↓	-	-	-	-	-	-	-	-	-
Darst (2019)[19]	1212	60.8 ± 6.7	69.0	unk	Sex, race, BMI, sample storage time, cholesterol lowering medication	↓	↑	-	-	-	-	-	-	-	-
Giil (2017)[29]	130	unk	46.9	unk	Sex, diagnosis, creatine	ns	↑	ns	ns	↓	↑	ns	↑	-	↑
Hartai (2007)[40]	31	73.0 ± 8.3	67.7	unk	None	-	ns	-	ns	-	-	-	-	-	-
Bonaccorso (1998)[11]	31	55.2 ± 22.2	54.8	22-91	None	ns	-	-	-	-	-	-	-	-	-
Proenza (1994)[67]	40	44.8 ± 12.6	0.0	20-64	None	↓	-	-	-	-	-	-	-	-	-
Basun (1990)[8]	11	79.0 ± 2.0	54.5	77-84	None	ns	-	-	-	-	-	-	-	-	-
Banki (1981)[6]	32	41.5 ± 10.7	100	unk	None	ns	-	-	-	-	-	-	-	-	-

Armstrong (1973)[3]	193	unk	52.8	18-77	None	ns	-	-	-	-	-	-	-	-	-

↑ Sig. positive association, ↓ Sig. negative association, *ns* non-significant, *unk* unknown, ↑↓ non-linear association, - metabolite not measured. Abbreviations: TRP, Tryptophan; KYN, Kynurenine; KA, Kynurenic acid; AA, Anthranilic acid; HK, 3-hydroxykynurenine; HAA, 3-hydroxyanthranilic acid; XA, Xanthurenic acid; QA, Quinolinic acid; PIC, Picolinic acid; KTR, Kynurenine-Tryptophan ratio.

Table S6. Differences in TRP and KP metabolites between younger and older

Study	Younger				Older			
	N	Age	Female (%)	Range	N	Age	Female (%)	Range
CSF								
Kepplinger (2019)[50]	15	37.9 ± 10.5	66.7	18-50	15	65.9 ± 7.4	73.3	51-80
Kepplinger (2005)[49]	17	35.4 ± 9.1	41.2	25-50	10	61.6 ± 10.4	50.0	50-74
Tohgi (1993)[84]	31	33.8 ± 9.1	19.4	<50	18	56.8 ± 4.3	27.8	50-64
Heyes (1992)[41]	16	35.2 ± 8.4	unk	unk	30	59.1 ± 14.2	unk	unk
Ferraro (1985)[26]	unk	< 40	unk	unk	unk	> 40	unk	unk
Serum								
Westbrook (2020)[94]	50	25.6 ± 6.2	32.0	20-30	116	77.6 ± 5.9	43.1	70-93
Kepplinger (2019)[50]	15	37.9 ± 10.5	66.7	18-50	15	65.9 ± 7.4	73.3	51-80
Dunn (2015)[22]	unk	< 50	unk	19-50	unk	> 64	unk	64-81
Sipahi (2013)[77]	46	38.6 ± 6.8	56.5	24-63	48	72.5 ± 5.5	43.8	65-88
Collino (2013)[17]	18	31.2 ± 5.0	50.0	25-40	20	73.1 ± 3.0	50.0	68-76
Capuron (2011)[13]	unk	unk	unk	<80	unk	unk	unk	80≦
Pertovaara (2006)[65]	309	45.0[a]	45.0	21-64	284	unk	76.4	90-91
Kepplinger (2005)[49]	17	35.4 ± 9.1	41.2	25-50	10	61.6 ± 10.4	50.0	50-74
Frick (2004)[28]	13	48.5 ± 8.6	unk	34-57	15	67.3 ± 2.7	unk	61-71
Widner (2000)[97]	49	38.4 ± 12.0	unk	20-63	20	73.4 ± 7.4	50.0	57-91
Sarwar (1991) [73]	37	unk	62.2	30-35	30	unk	63.3	80-89
Plasma								
Xyda (2020)[102]	12	27.0 ± 5.0	50.0	18-35	12	76.0 ± 5.0	58.3	65-85

groups

Covariate in analyses	Metabolites								
	TRP	**KYN**	**3-HK**	**KA**	**XA**	**AA**	**3-HAA**	**QA**	**KTR**
None	ns	↑	-	↑	-	-	-	-	-
None	-	-	-	↑	-	-	-	-	-
None	ns	ns	-	-	-	-	-	-	-
None	-	-	-	-	-	-	-	↑	-
Sex	ns	-	-	-	-	-	-	-	-
None	ns	↑	ns	ns	ns	ns	-	ns	↑
None	ns	ns	-	↑	-	-	-	-	-
Sex	↓	-	-	-	-	-	-	-	-
None	↓	↑	-	-	-	-	-	-	↑
None	↓	-	-	-	-	-	-	-	-
Sex, BMI	ns	↑	-	-	-	-	-	-	↑
None	ns	↑	-	-	-	-	-	-	↑
None	-	-	-	ns	-	-	-	-	-
None	ns	ns	-	-	-	-	-	-	ns
None	↓	ns	-	-	-	-	-	-	↑
Sex, age*sex	↓	-	-	-	-	-	-	-	-
None	ns	ns	-	-	-	-	-	-	-

Table S6. (Continue)

Study	Younger				Older			
	N	Age	Female (%)	Range	N	Age	Female (%)	Range
Plasma								
Johnson (2018)[45]	14	23.0 ± 3.7	50.0	18-30	29	61.0 ± 5.4	55.2	45-74
Theofylakto poulou (2013)[81]	3723	unk	55.4	45-46	3329	unk	55.9	70-72
Bonaccorso (1998)[11]	13	32.9 ± 8.1	38.5	22-45	13	78.3 ± 5.7	53.9	70-91
Demling (1996)[21]	55	27.4	58.2	18-38	36	69.0	50.0	60-84
Caballero (1991)[12]	F (72)	25.0 ± 5.0	100	unk	F (42)	72.0 ± 8.0	100	unk
	M (68)	26.0 ± 4.0	0.0	unk	M (32)	71.0 ± 7.0	0.0	unk
Phipps (1985)[66]	84	unk	61.9	17-69	47	unk	61.7	70≦
Shaw (1981)[76]	46	42.2	unk	unk	70	70.1	unk	unk
Feces								
Ruiz-Ruiz (2019)[71]	10	35.4 ± 6.6	50.0	27-44	10	74.5 ± 4.3	70.0	68-81

↑ Sig. higher in older group, ↓ Sig. lower in older group, *ns* non-significant, *unk* unknown, - metabolite not
AA, Anthranilic acid; HK, 3-hydroxykynurenine; HAA, 3-hydroxyanthranilic acid; XA, Xanthurenic acid;

Covariate in analyses	Metabolites								
	TRP	KYN	3-HK	KA	XA	AA	3-HAA	QA	KTR
None	↓	-	-	-	-	-	-	-	-
Sex, eGFR, BMI, physical activity, smoking	↓	↑	↑	↑	↓	↑	↑	-	↑
Sex	ns	-	-	-	-	-	-	-	-
None	↓	-	-	-	-	-	-	-	-
None	ns	-	-	-	-	-	-	-	-
	↓	-	-	-	-	-	-	-	-
None	↓	-	-	-	-	-	-	-	-
Sex	↓	-	-	-	-	-	-	-	-
None	↓	-	-	-	-	-	-	-	-

measured. [a] Median. Abbreviations: TRP, Tryptophan; KYN, Kynurenine; KA, Kynurenic acid; QA, Quinolinic acid; PIC, Picolinic acid; KTR, Kynurenine-Tryptophan ratio.

Table S7. Associations between KP metabolite ratios and neopterin and age

Study	N	Age	Female (%)	Range	Tissue	Cov. in analyses	PIC/QA	Neopterin
Bie (2016)[10]	49	unk	100	0-98	CSF	None	ns	↑
Valdiglesias (2017)[90]	259	unk	67.2	65 ≦	S	Sex, smoking habits	-	↑
Sipahi (2013)[77]	94	55.9 ± 19.4	50.0	24-88	S	None	-	↑
Capuron (2011)[13]	284	79.9 ± 4.5	69.4	unk	S	Sex, BMI	-	↑
Frick (2004)[28]	43	66.3	48.8	34-93	S	None	-	↑
Giil (2017)[29]	130	unk	46.9	unk	P	Sex, diagnosis, creatine	-	↑

↑ Positive association, ↓ negative association, *ns* non-significant, *unk* unknown, - ratio not measured. Abbreviations: CSF, Cerebrospinal fluid; S, Serum; P, Plasma; QA, Quinolinic acid; PIC, Picolinic acid.

Table S8. Differences in CSF, plasma and serum neopterin between younger and older groups

Study	Younger				Older				Tissue	Cov. in analyses	Neopterin
	N	Age	Female (%)	Range	N	Age	Female (%)	Range			
Sipahi (2013)[77]	46	38.6 ± 6.8	56.5	24-63	48	72.5 ± 5.5	43.8	65-88	S	None	ns
Capuron (2011)[13]	unk	unk	unk	<80	unk	unk	unk	80≦	S	Sex, BMI	↑
Frick (2004)[28]	13	48.5 ± 8.6	unk	34-57	15	67.3 ± 2.7	unk	61-71	S	None	ns
Theofylakto poulou (2013)[81]	3723	unk	55.4	45-46	3329	unk	55.9	70-72	P	Sex, eGFR, BMI, physical activity, smoking	↑

↑ Higher in older group, ↓ lower in older group, *ns* non-significant, *unk* unknown. Abbreviations: P, Plasma; S, Serum.

Table S9. Associations between TRP and KP metabolites and MMSE scores in cases and controls

Study	Group		Tissue	Covariate in analyses	Metabolites									
	N	Age			TRP	KYN	3-HK	KA	XA	AA	3-HAA	PIC	QA	KTR
Shao (2020)[75]	AD (30)	71.6 ± 8.8	P	None	ns	-	-	-	-	-	-	-	-	-
Sorgdrager (2019)[78]	AD (33)	73.7 ± 6.0	CSF	None	ns	ns	ns	ns	ns	-	-	-	ns	-
Sorgdrager (2019)[78]	AD (33)	73.7 ± 6.0	S	None	↑	↑	ns	ns	ns	-	-	-	↑	-
Wissmann (2013)[99]	AD (43)	81.7 ± 10.5	S	None	-	-	-	-	-	-	-	-	-	↓
Gulaj (2010)[37]	AD (34)	78.8 ± 5.7	P	None	ns	ns	ns	↑	-	ns	-	-	ns	ns
Widner (2000)[97]	AD (21)	74.4 ± 5.4	S	None	ns	↓	-	-	-	-	-	-	-	↓
Whiley (2021)[95]	AD (103) MCI (165) CTRL (86)	77.0 ± 61.3	S	None	ns	ns	-	-	↑	-	-	-	-	-
Hafstad Solvang (2019)[38]	AD (90) LBD (65)	75.1 ± 7.3	S	Age, time, age*time, smoking, AD*LBD, AD*LBD*time, eGFR, PLP	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Wennström (2014)[93]	AD (19) LBD (18) CTRL (20)	76.0	CSF	None	-	-	-	ns	-	-	-	-	-	-

Table S9. (Continue)

Study	Group		Tissue	Covariate in analyses	Metabolites									
	N	Age			TRP	KYN	3-HK	KA	XA	AA	3-HAA	PIC	QA	KTR
Widner (1999)[96]	AD (24) HD (12) CTRL (unk)	unk	S	None	↑	ns	-	-	-	-	-	-	-	ns
Tohgi (1992)[83]	AD (14) CTRL (10)	68.4	CSF	None	ns	ns	ns	-	-	-	-	-	-	ns
Basun (1990)[8]	AD (22) CTRL (11)	unk	P	None	ns	-	-	-	-	-	-	-	-	-
Leblhuber (1998)[52]	HD (12)	42.4 ± 11.7	S	None	↑	ns	-	-	-	-	-	-	-	ns
Gold (2011)[30]	IS (41)	72.3 ± 12.2	P	Age	-	-	-	-	-	-	-	-	-	↓
Park (2020)[63]	CTRL (40)	73.9 ± 5.1	S	None	ns	ns	-	-	-	ns	-	-	-	-
Willette (2021)[98]	AD (112) MCI (396) CTRL (58)	unk	S	Age, sex. education	-	-	-	-	-	-	-	-	-	ns

↑ Sig. positive association, ↓ Sig. negative association, *ns* non-significant, *unk* unknown, - metabolite not measured. Abbreviations: AD, Alzheimer's dementia; MCI, Mild Cognitive Impairment; CTRL, Control; LBD, Lewy-body dementia; HD, Huntington's disease; IS, Ischemic stroke patients; CSF, Cerebrospinal fluid; P, Plasma; S, Serum; eGFR, estimated Glomerular Filtration Rate; PLP, Pyridoxal phosphate; TRP, Tryptophan; KYN, Kynurenine; KA, Kynurenic acid; AA, Anthranilic acid; HK, 3-hydroxykynurenine; HAA, 3-hydroxyanthranilic acid; XA, Xanthurenic acid; QA, Quinolinic acid; PIC, Picolinic acid; KTR, Kynurenine-Tryptophan ratio.

Table S10. Associations between TRP and KP metabolites and other cognitive scores in cases and controls

Study	Group		Tissue	Covariate in analyses	Cognitive test	Metabolites									
	N	Age				TRP	KYN	3-HK	KA	XA	AA	3-HAA	PIC	QA	KTR
Chatterjee (2020)[14]	CTRL (100)	78.2 ± 5.5	P	Age, sex, APOE ε4 status, BMI	Global cognition[a]	ns	ns	-	-	-	-	-	-	-	-
Gulaj (2010)[37]	AD (34)	78.8 ± 5.7	P	None	CDT	ns	ns	ns	ns		ns	-	-	↓	ns
Mourdian (1989)[60]	AD (35)	64.0 ± 5.9	CSF	None	DRS	-	-	-	-	-	-	-	-	ns	-
					WMS	-	-	-	-	-	-	-	-	ns	-
Toledo (2017)[86]	AD (175) MCI (356) CTRL (199)	75.3	S	Age, gender, education, APOE ε4 status	ADAS-Cog13	ns	ns	-	-	-	-	-	-	-	-
Basun (1990)[8]	AD (22) CTRL (11)	unk	P	None	Memory (CDR)	ns	-	-	-	-	-	-	-	-	-
					Orientation (CDR)	ns	-	-	-	-	-	-	-	-	-
Küster (2017)[51]	DEM (4) MCI (32) SCD (11)	71.2 ± 6.0	S	Age, education, alcohol consumption	Global cognition[b]	-	ns	ns	ns	-	-	-	-	ns	-
					Memory compound	-	ns	ns	ns	-	-	-	-	ns	-
					Executive function compound	-	ns	ns	ns	-	-	-	-	↓	-

Table S10. (Continue)

Study	Group		Tissue	Covariate in analyses	Cognitive test	Metabolites									
	N	Age				TRP	KYN	3-HK	KA	XA	AA	3-HAA	PIC	QA	KTR
Hafstad Solvang (2019)[39]	CTRL (2174)	71.0 (med)	P	Age, sex, BMI, education, eGFR, smoking, diabetes, hypertension, myocardial infarction, stroke, PLP, CRP, NSAIDS	COWAT	ns	ns	ns	ns	ns	ns	ns	ns	ns	↓
					DST	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
					KOLT	ns	ns	ns	ns	ns	ns	ns	ns	ns	↓
Willette (2021)[98]	AD (112) MCI (396) CTRL (58)	unk	S	Age, sex. education	RAVLT Trials 1-5	-	-	-	-	-	-	-	-	-	↓
					Short delay memory	-	-	-	-	-	-	-	-	-	↓
					Items forgotten during long delay	-	-	-	-	-	-	-	-	-	↑
					Memory factor	-	-	-	-	-	-	-	-	-	↓

↑ Sig. positive association, ↓ Sig. negative association, *ns* non-significant, *unk* unknown, - metabolite not measured. [a]Composite score of verbal and visual episodic memory, working memory and executive functioning. [b]Average of two component scores (memory functions and attention/ executive functions) as determined by principal component analysis. Abbreviations: CTRL, Control; AD, Alzheimer's dementia; MCI, Mild Cognitive Impairment; DEM, Dementia; SCD, Subjective Cognitive Decline; CSF, Cerebrospinal fluid; P, Plasma; S, Serum; eGFR estimated Glomerular Filtration Rate; PLP, Pyridoxal phosphate; CRP, C-reactive protein; NSAIDS, Non-Steroidal Anti-inflammatory Drugs; CDT, Clock Drawing Test; DRS, Mattis Dementia Rating Scale; WMS, Wechsler Memory Scale; ADAS-Cog13, Alzheimer's Disease Assessment Scale cognitive subscale; CDR, Clinical Dementia Rating Scale; COWAT, Controlled Oral Word Association Test; DST, Digit Symbol Test; KOLT, Kendrick Object Learning Test; RAVLT, Rey Auditory Verbal Learning Test; TRP, Tryptophan; KYN, Kynurenine; KA, Kynurenic acid; AA, Anthranilic acid; HK, 3-hydroxykynurenine; HAA, 3-hydroxyanthranilic acid; XA, Xanthurenic acid; QA, Quinolinic acid; PIC, Picolinic acid; KTR, Kynurenine-Tryptophan ratio.

Table S11. Associations between KP metabolite ratios or neopterin and cognitive scores in samples of patients and controls

Study	Group		Tissue	Covariate in analyses	Cognitive test	Ratios					Neopterin
	N	Age				HK/ KYN	AA/ KYN	KA/ KYN	QA/ HK	HK/ TRP	
Hafstad Solvang (2019)[39]	CTRL (2174)	71.0[a]	P	Age, sex, BMI, education, eGFR, smoking, diabetes, hypertension, myocardial infarction, stroke, PLP, CRP, NSAIDS	COWAT	-	-	-	-	-	↓
					DST	-	-	-	-	-	ns
					KOLT	-	-	-	-	-	↓
Hafstad Solvang (2019)[38]	AD (90) LBD (65)	75.1 ± 7.31	S	Age, time, age*time, smoking, AD*LBD, AD*LBD*time, eGFR, PLP	MMSE	-	-	ns	-	-	ns
Wissmann (2013)[99]	AD (43)	81.7 ± 10.5	S	None	MMSE	-	-	-	-	-	↓
					CDT	-	-	-	-	-	↓
Gulaj (2010)[37]	AD (34)	78.8 ± 5.7	P	None	MMSE	ns	ns	↑	ns	-	-
					CDT	ns	ns	ns	ns	-	-
Widner (1999)[96]	AD (24) HD (12) CTRL (unk)	unk	S	None	MMSE	-	-	-	-	-	ns
Leblhuber (1998)[52]	HD (12)	42.4 ± 11.7	S	None	MMSE	-	-	-	-	-	ns
Tohgi (1992)[83]	AD (14) CTRL (10)	68.4	CSF	None	MMSE	-	-	-	-	ns	-

↑ Positive association, ↓ negative association, *ns* non-significant, *unk* unknown, - metabolite not measured. [a]Age in median. Abbreviations: AD, Alzheimer's dementia; CTRL, Control; CSF, Cerebrospinal fluid; P, Plasma; S, Serum; eGFR, estimated Glomerular Filtration Rate; PLP, Pyridoxal phosphate; CRP, C-reactive protein; NSAIDS, Non-Steroidal Anti-inflammatory Drugs; MMSE, Mini Mental State Examination; CDT, Clock Drawing Test; COWAT, Controlled Oral Word Association Test; DST, Digit Symbol Test; KOLT, Kendrick Object Learning Test; TRP, Tryptophan; KYN, Kynurenine; HK, 3-hydroxykynurenine; KA, Kynurenic acid; AA, Anthranilic acid; QA, Quinolinic acid.

Table S12. Effect sizes and Egger's bias coefficients of AD dementia and SCD studies

		Effect size		Heterogeneity		Publication bias	
	N	**SMD (95% CI)[a]**	**p-value**	**I^2 (%)**	**p-value**	**Egger's bias coefficient**	**p-value**
Tryptophan							
Overall	3	-0.45 (-0.82, -0.08)	**0.018**	0.0	0.607	-103.12	0.081
CSF	1	-	-	-	-	-	-
Blood	2	-	-	-	-	-	-
Plasma	1	-	-	-	-	-	-
Serum	1	-	-	-	-	-	-
Kynurenine							
Overall	3	0.21 (-0.16, 0.57)	0.271	0.0	0.630	-28.11	0.868
CSF	1	-	-	-	-	-	-
Blood	2	-	-	-	-	-	-
Plasma	1	-	-	-	-	-	-
Serum	1	-	-	-	-	-	-
3-Hydroxykynurenine							
Overall	3	0.63 (0.25, 1.02)	**0.001**	83.5	**0.002**	28.74	0.064
CSF	1	-	-	-	-	-	-
Blood	2	-	-	-	-	-	-
Plasma	1	-	-	-	-	-	-
Serum	1	-	-	-	-	-	-
Kynurenic acid							
Overall	3	0.38 (0.01, 0.75)	**0.047**	67.1	**0.048**	36.96	0.101
CSF	1	-	-	-	-	-	-
Blood	2	-	-	-	-	-	-
Plasma	1	-	-	-	-	-	-
Serum	1	-	-	-	-	-	-
Picolinic acid							
Overall	3	0.45 (0.08, 0.82)	**0.017**	0.0	0.862	13.41	0.893
CSF	1	-	-	-	-	-	-
Blood	2	-	-	-	-	-	-
Plasma	1	-	-	-	-	-	-

Serum	1	-	-	-	-	-	-
Quinolinic acid							
Overall	3	-0.16 (-0.53, 0.20)	0.384	13.5	0.315	76.64	0.767
CSF	1	-	-	-	-	-	-
Blood	2	-	-	-	-	-	-
Plasma	1	-	-	-	-	-	-
Serum	1	-	-	-	-	-	-

[a]SMD > 0 trend towards higher in AD, SMD < 0 trend towards lower in AD. Statistically significant *p*-values are in bold. Abbreviations: SMD, Standardized mean difference; 95% CI, 95% confidence interval; CSF, Cerebrospinal fluid.

Table S13. Effect sizes and Egger's bias coefficients of MCI and control studies

		Effect size		**Heterogeneity**		**Publication bias**	
	N	**SMD (95% CI)[a]**	**p-value**	**I^2 (%)**	**p-value**	**Egger's bias coefficient**	**p-value**
Tryptophan							
Overall	5	-0.20 (-0.40, 0.01)	0.057	62.0	**0.032**	-2.25	0.329
CSF	1	-	-	-	-	-	-
Blood	4	-0.24 (-0.45, -0.02)	**0.031**	68.0	**0.025**	-3.12	0.235
Plasma	2	-	-	-	-	-	-
Serum	2	-	-	-	-	-	-
Kynurenine							
Overall	3	-0.02 (-0.23, 0.20)	0.891	77.8	**0.011**	3.15	0.660
CSF	1	-	-	-	-	-	-
Blood	2	-	-	-	-	-	-
Plasma	0	-	-	-	-	-	-
Serum	2	-	-	-	-	-	-

Table S13. (Continue)

		Effect size		Heterogeneity		Publication bias	
	N	**SMD (95% CI)[a]**	**p-value**	**I^2 (%)**	**p-value**	**Egger's bias coefficient**	**p-value**
Kynurenic acid							
Overall	3	0.23 (-0.11, 0.58)	0.188	83.7	**0.002**	5.94	0.724
CSF	1	-	-	-	-	-	-
Blood	2	-	-	-	-	-	-
Plasma	1	-	-	-	-	-	-
Serum	1	-	-	-	-	-	-
KTR							
Overall	4	0.07 (-0.14, 0.28)	0.512	85.6	**< 0.001**	0.80	0.868
CSF	1	-	-	-	-	-	-
Blood	3	-0.07 (-0.30, 0.17)	0.579	86.1	**0.001**	-0.04	0.995
Plasma	0	-	-	-	-	-	-
Serum	3	-0.07 (-0.30, 0.17)	0.579	86.1	**0.001**	-0.04	0.995

[a]SMD > 0 trend towards higher in MCI, SMD < 0 trend towards lower in MCI. Statistically significant *p*-values are in bold. Abbreviations: SMD, Standardized mean difference; 95% CI, 95% confidence interval; CSF, Cerebrospinal fluid; KTR, Kynurenine-Tryptophan ratio.

Table S14. Quality assessment of data included in the systematic review

Study	Selection				Comparability		Exposure			Total
	1	2	3	4	1a	1b	1	2	3	
Case-control data										
Arai (1984)[1]	1	0	0	0	1	1	1	1	0	5
Arai (1985)[2]	1	0	0	0	1	1	1	1	0	5
Atukeren (2017)[4]	1	0	0	0	1	1	1	1	0	5
Baker (1989)[5]	1	0	0	1	1	1	1	1	0	6
Baran (1999)[7]	0	0	0	0	1	0	0	1	0	2
Basun (1990)[8]	1	0	1	1	1	0	1	1	0	6
Beal (1990)[9]	1	0	0	1	0	0	1	1	0	4
Bonaccorso (1998)[11]	1	0	0	1	1	1	1	1	0	6
Cogo (2021) [16]	0	0	0	0	0	0	1	1	0	2
Czech (2012)[18]	1	0	0	0	1	0	1	0	0	3
de Leeuw (2017)[20]	1	0	0	0	1	1	1	1	0	5
Fakhruddin (2020)[24]	1	0	1	1	1	0	0	1	0	5
Fekkes (1998)[25]	1	1	0	1	1	0	1	0	0	5
Fonteh (2007)[27]	1	0	0	0	1	1	1	1	0	5
Giil (2017)[29]	1	1	1	0	1	1	1	1	0	7
Gonzalez-Dominguez (2014)[31]	1	0	0	0	1	1	1	0	0	4
Gonzalez-Dominguez (2015a)[32]	1	1	0	0	0	0	1	1	0	4
Gonzalez-Dominguez (2015b)[33]	1	0	0	0	0	0	1	1	0	3
González-Sánchez (2020)[34]	1	1	1	1	0	0	1	0	0	5
Graham (2015)[35]	1	0	1	0	1	0	1	1	0	5
Greilberger (2010)[36]	1	1	0	0	1	0	1	0	0	4
Gulaj (2010)[37]	1	0	0	0	1	1	1	1	0	5
Hartai (2007)[40]	1	0	0	0	1	1	1	0	0	4
Heyes (1992)[41]	0	0	1	0	0	0	0	0	0	1
Ibáñez (2013)[42]	1	0	0	0	0	0	1	1	0	3
Jacobs (2019)[43]	1	1	0	0	1	0	1	1	0	5
Janssens (2020)[44]	0	0	0	0	1	0	1	1	0	3
Kaddurah-Daouk (2011)[46]	1	0	0	0	1	1	1	1	0	5
Kaddurah-Daouk (2013)[47]	1	0	0	0	1	1	1	1	0	5
Li (2010)[53]	0	0	0	0	1	1	1	1	0	4
Liang (2016)[54]	1	0	0	0	0	0	1	1	0	3
Lin (2019)[55]	1	0	0	0	0	0	1	1	0	3
Liu (2015)[56]	1	0	0	1	0	0	1	1	0	4
Martinez (1993)[57]	1	0	0	1	1	0	1	1	0	5

Table S14. (Continue)

Study	Selection				Comparability		Exposure			Total
	1	2	3	4	1a	1b	1	2	3	
Case-control data										
Molina (1998)[59]	1	1	0	0	1	1	1	0	0	5
Mourdian (1989)[60]	1	0	0	0	1	0	1	0	0	3
Oxenkrug (2017)[61]	0	0	0	0	1	1	0	0	0	2
Paglia (2016)[62]	1	0	0	0	1	1	0	0	0	3
Peña-Bautista (2020)[64]	1	0	0	0	1	1	1	1	0	5
Ramos-Chavez (2018)[68]	1	0	0	1	1	1	1	1	0	6
Rommer (2016)[69]	1	1	0	0	1	0	1	0	0	4
Rudman (1989)[70]	1	0	1	0	1	0	0	0	0	3
Santos (2020)[72]	1	0	1	0	1	1	1	0	0	5
Schwarz (2013)[74]	1	1	0	0	1	1	1	1	0	6
Shao (2020)[75]	1	0	0	0	0	1	1	0	0	3
Shaw (1981)[76]	0	0	0	0	0	1	0	0	0	1
Sorgdrager (2019)[78]	1	0	0	0	1	1	1	0	0	4
Storga (1996)[79]	1	0	0	1	0	0	1	1	0	4
Tarbit (1980)[80]	0	0	0	0	0	0	1	0	0	1
Thomas (1986)[82]	1	0	1	0	1	1	1	1	0	6
Tohgi (1992)[83]	1	0	0	0	1	0	1	0	0	3
Tohgi (1995)[85]	1	0	0	0	1	0	1	0	0	3
Trushina (2013)[87]	1	0	1	0	0	0	1	1	0	4
Tsuruoka (2013)[88]	1	1	0	0	1	0	1	0	0	4
Van der Velpen (2019)[91]	1	1	1	0	0	0	1	1	0	5
Watkins (1989)[92]	1	0	1	0	1	1	1	1	0	6
Wennström (2014)[93]	1	1	1	0	1	0	1	1	0	6
Whiley (2021)[95]	1	0	0	0	1	0	1	1	0	4
Widner (1999)[96]	1	0	0	0	0	0	0	1	0	2
Widner (2000)[97]	1	0	0	0	1	0	1	0	0	3
Willette (2021)[98]	1	0	0	0	1	1	1	1	0	5
Wu (2021)[100]	1	0	1	0	1	1	1	1	0	6
Xu (2016)[101]	1	0	0	0	1	1	1	0	0	4
Yilmaz (2020)[103]	1	0	1	0	1	1	1	1	0	6

Table S14. (Continue)

Study	Selection				Comparability		Exposure			Total
	1	2	3	4	1a	1b	1	2	3	
Different patient populations [a]										
Hafstad Solvang (2019)[38]	1	0	1	0	1	1	1	1	0	6
Kaiser (2010)[48]	1	0	1	0	0	0	1	1	0	4
Mashige (1993)[58]	0	0	0	0	0	0	1	1	0	2
Cross-sectional data [a]										
Armstrong (1973)[3]	1	0	1	1	0	0	1	1	1	6
Banki (1981)[6]	0	0	1	1	0	0	1	1	1	5
Bie (2016)[10]	1	0	1	1	0	0	1	1	1	6
Caballero (1991)[12]	0	0	1	1	0	0	1	1	1	5
Capuron (2011)[13]	1	1	1	1	1	1	1	1	0	8
Chatterjee (2020)[14]	1	0	1	1	0	1	1	1	1	7
Collino (2013)[17]	1	0	1	1	0	0	1	1	0	5
Demling (1996)[21]	0	0	0	1	0	0	1	1	0	3
Dunn (2015)[22]	1	0	1	1	0	0	1	1	1	5
Eklundh (1996)[23]	0	0	1	0	0	1	1	1	1	4
Ferraro (1985)[26]	0	0	0	0	0	0	0	0	1	1
Frick (2004)[28]	0	0	0	1	0	0	1	1	1	4
Gold (2011)[30]	1	0	1	1	1	1	1	1	0	7
Hafstad Solvang (2019)[39]	1	1	1	1	1	1	1	1	1	9
Johnson (2018)[45]	1	0	1	1	0	0	1	1	1	6
Kepplinger (2005)[49]	1	0	1	1	0	0	1	1	0	5
Kepplinger (2019)[50]	1	0	1	1	0	0	1	1	1	6
Küster (2017)[51]	1	0	1	1	0	0	1	0	0	4
Leblhuber (1998)[52]	1	0	0	1	0	0	1	1	1	5
Park (2020)[63]	1	0	1	1	0	0	1	1	1	6
Pertovaara (2006)[65]	1	0	1	1	0	0	1	1	1	6
Phipps (1985)[66]	0	0	0	0	0	0	1	1	0	2
Proenza (1994)[67]	1	0	1	1	0	0	1	1	1	6
Ruiz-Ruiz (2020)[71]	0	0	1	1	0	0	1	0	0	3
Sarwar (1991)[73]	1	0	0	1	0	0	1	1	1	5
Sipahi (2013)[77]	0	0	0	1	0	0	1	1	1	4
Theofylaktopoulou (2013)[81]	1	1	1	1	1	1	1	1	1	9
Tohgi (1993)[84]	0	0	1	1	0	0	1	1	1	5
Urbańska (2006)[89]	1	0	0	1	1	1	1	1	1	7
Valdiglesias (2017)[90]	0	0	1	1	1	1	1	1	1	7

Table S14. (Continue)

Study	Selection				Comparability		Exposure			Total
	1	2	3	4	1a	1b	1	2	3	
Cross-sectional data [a]										
Westbrook (2020)[94]	1	0	1	1	0	0	1	1	1	6
Wissmann (2013)[99]	0	0	1	1	0	0	1	1	1	5
Xyda (2020)[102]	1	0	1	1	0	0	1	1	1	6
Cohort data										
Chouraki (2017)[15]	1	1	1	1	1	1	1	1	1	9
Darst (2019)[19]	1	1	1	n/a	1	1	1	n/a	0	6
Toledo (2017)[86]	1	1	1	0	1	1	1	1	0	7

[a]Assessed by using an adapted scale of the NOS for case-control studies, see appendix S3 and S4.
Abbreviations: *n/a* not applicable.

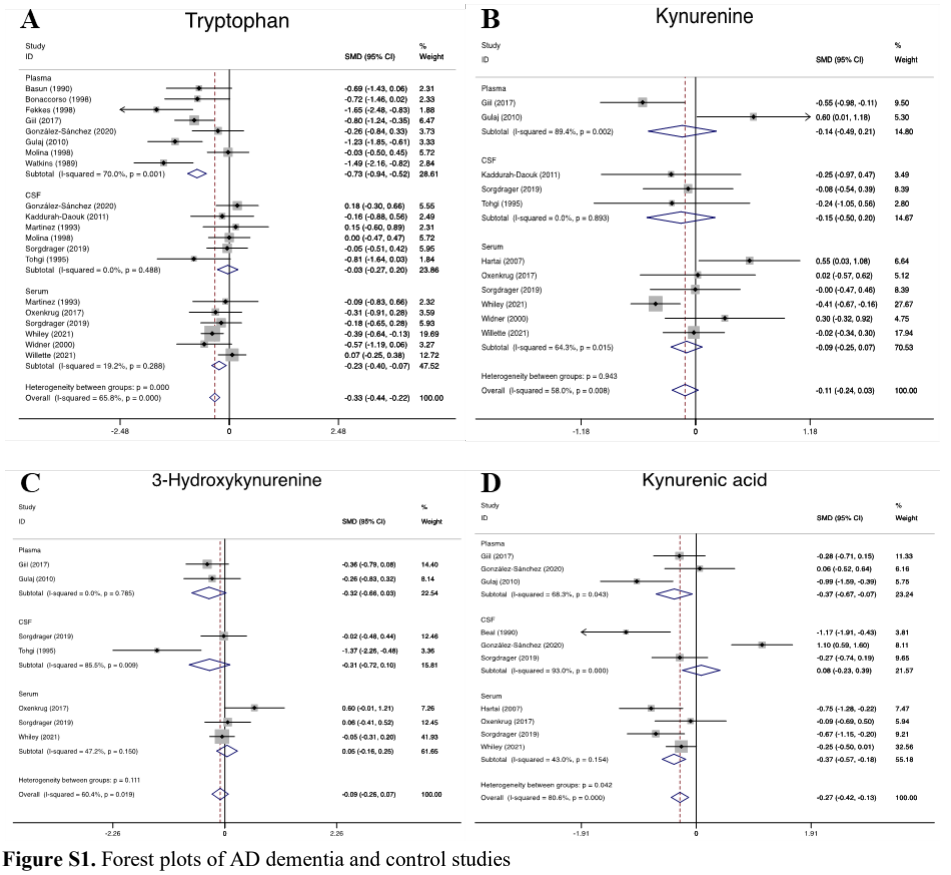
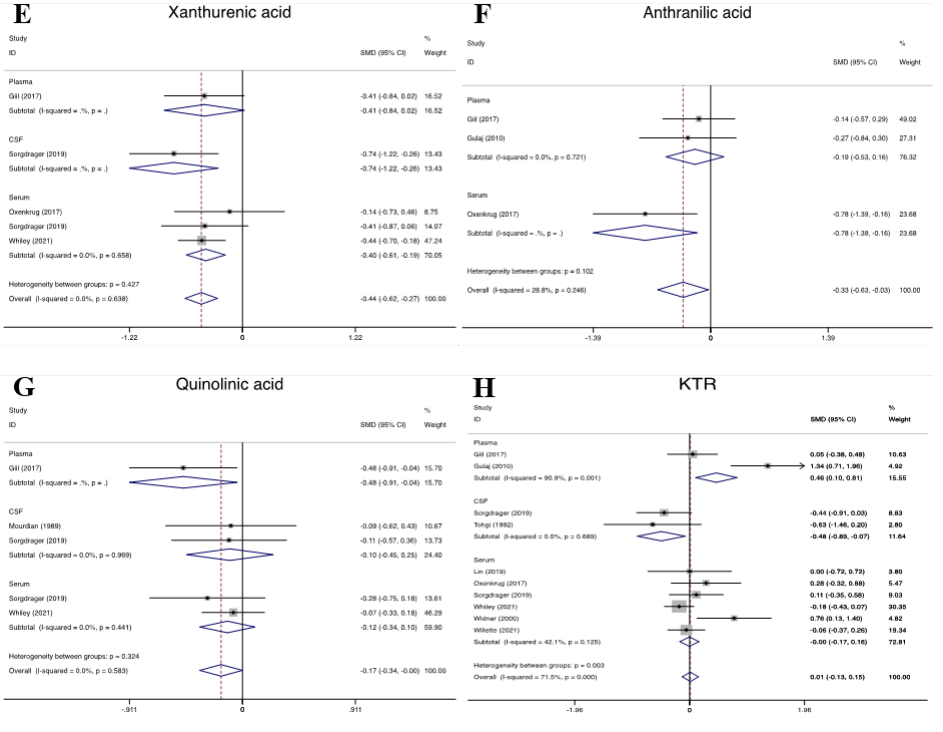


Figure S1. Forest plots of AD dementia and control studies


E
Xanthurenic acid
Study
ID
SMD (95% CI)
%
Weight
Plasma
Gill (2017)
-0.41 (-0.84, 0.02) 16.52
Subtotal (I-squared = .%, p = .)
-0.41 (-0.84, 0.02) 16.52
CSF
Sorgdrager (2019)
-0.74 (-1.22, -0.26) 13.43
Subtotal (I-squared = .%, p = .)
-0.74 (-1.22, -0.26) 13.43
Serum
Oxenkrug (2017)
-0.14 (-0.73, 0.46) 8.75
Sorgdrager (2019)
-0.41 (-0.87, 0.06) 14.07
Whiley (2021)
-0.44 (-0.70, -0.18) 47.24
Subtotal (I-squared = 0.0%, p = 0.658)
-0.40 (-0.61, -0.19) 70.05
Heterogeneity between groups: p = 0.427
Overall (I-squared = 0.0%, p = 0.638)
-0.44 (-0.62, -0.27) 100.00
-1.22
0
1.22
F
Anthranilic acid
Study
ID
SMD (95% CI)
%
Weight
Plasma
Gill (2017)
-0.14 (-0.57, 0.29) 49.02
Gulaj (2010)
-0.27 (-0.84, 0.30) 27.31
Subtotal (I-squared = 0.0%, p = 0.721)
-0.19 (-0.53, 0.16) 76.32
Serum
Oxenkrug (2017)
-0.78 (-1.39, -0.16) 23.68
Subtotal (I-squared = .%, p = .)
-0.78 (-1.39, -0.16) 23.68
Heterogeneity between groups: p = 0.102
Overall (I-squared = 28.8%, p = 0.246)
-0.33 (-0.63, -0.03) 100.00
-1.39
0
1.39
G
Quinolinic acid
Study
ID
SMD (95% CI)
%
Weight
Plasma
Gill (2017)
-0.48 (-0.91, -0.04) 15.70
Subtotal (I-squared = .%, p = .)
-0.48 (-0.91, -0.04) 15.70
CSF
Mourdian (1989)
-0.09 (-0.62, 0.43) 10.67
Sorgdrager (2019)
-0.11 (-0.57, 0.36) 13.73
Subtotal (I-squared = 0.0%, p = 0.969)
-0.10 (-0.45, 0.25) 24.40
Serum
Sorgdrager (2019)
-0.28 (-0.75, 0.18) 13.61
Whiley (2021)
-0.07 (-0.33, 0.18) 46.29
Subtotal (I-squared = 0.0%, p = 0.441)
-0.12 (-0.34, 0.10) 59.90
Heterogeneity between groups: p = 0.324
Overall (I-squared = 0.0%, p = 0.583)
-0.17 (-0.34, -0.00) 100.00
-.911
0
.911
H
KTR
Study
ID
SMD (95% CI)
%
Weight
Plasma
Gill (2017)
0.05 (-0.38, 0.48) 10.63
Gulaj (2010)
1.34 (0.71, 1.96) 4.92
Subtotal (I-squared = 90.9%, p = 0.001)
0.46 (0.10, 0.81) 15.55
CSF
Sorgdrager (2019)
-0.44 (-0.91, 0.03) 8.83
Tohgi (1992)
-0.63 (-1.46, 0.20) 2.80
Subtotal (I-squared = 0.0%, p = 0.689)
-0.48 (-0.89, -0.07) 11.64
Serum
Lin (2019)
0.00 (-0.72, 0.72) 3.80
Oxenkrug (2017)
0.28 (-0.32, 0.88) 5.47
Sorgdrager (2019)
0.11 (-0.35, 0.58) 9.03
Whiley (2021)
-0.18 (-0.43, 0.07) 30.35
Widner (2000)
0.76 (0.13, 1.40) 4.82
Willette (2021)
-0.06 (-0.37, 0.26) 19.34
Subtotal (I-squared = 42.1%, p = 0.125)
-0.00 (-0.17, 0.16) 72.81
Heterogeneity between groups: p = 0.003
Overall (I-squared = 71.5%, p = 0.000)
0.01 (-0.13, 0.15) 100.00
-1.96
0
1.96

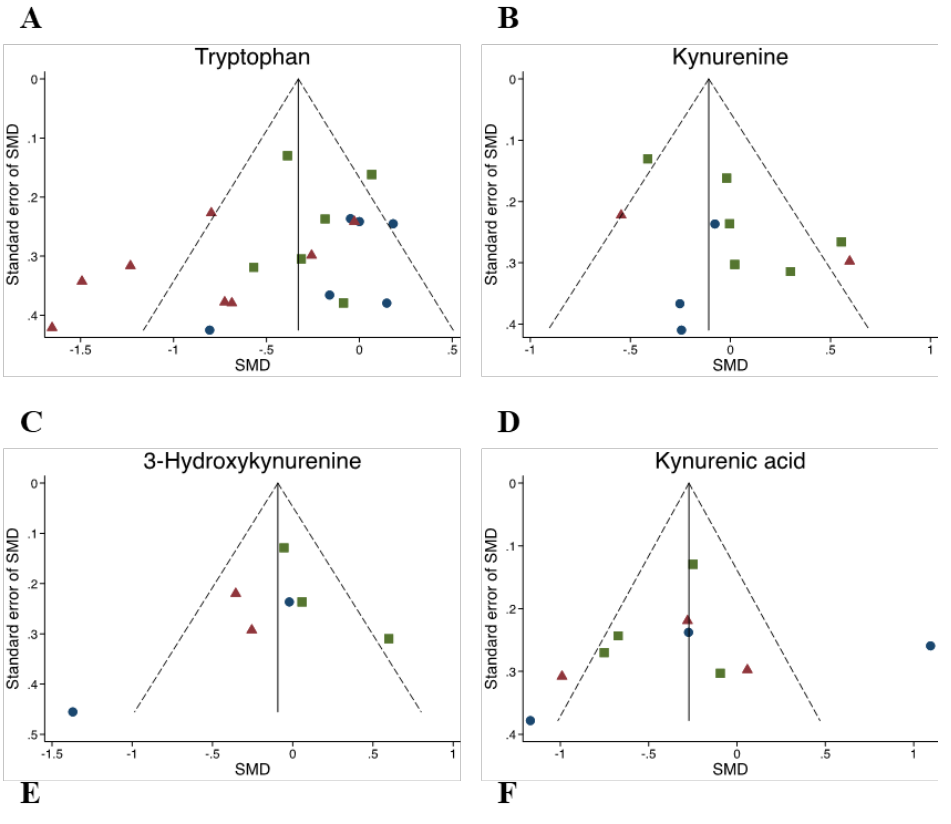
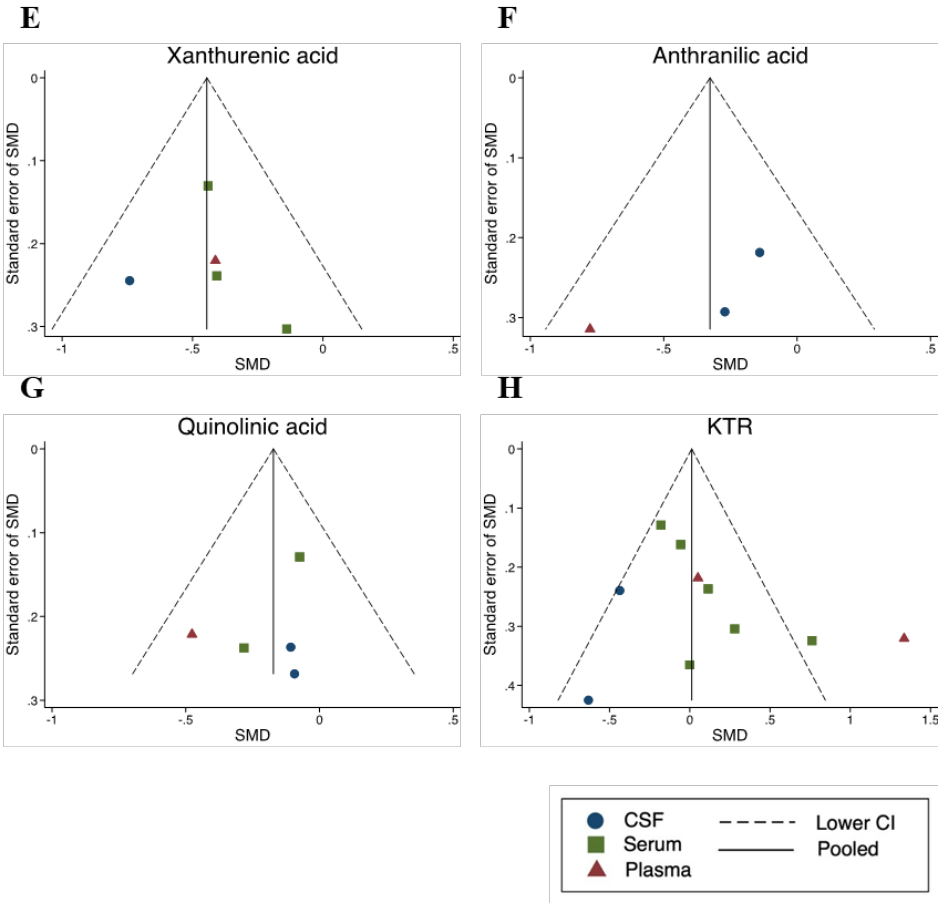


Figure S2. Funnel plots of AD dementia and control studies


E
Xanthurenic acid
F
Anthranilic acid
G
Quinolinic acid
H
KTR
Standard error of SMD
SMD
CSF
Serum
Plasma
Lower CI
Pooled

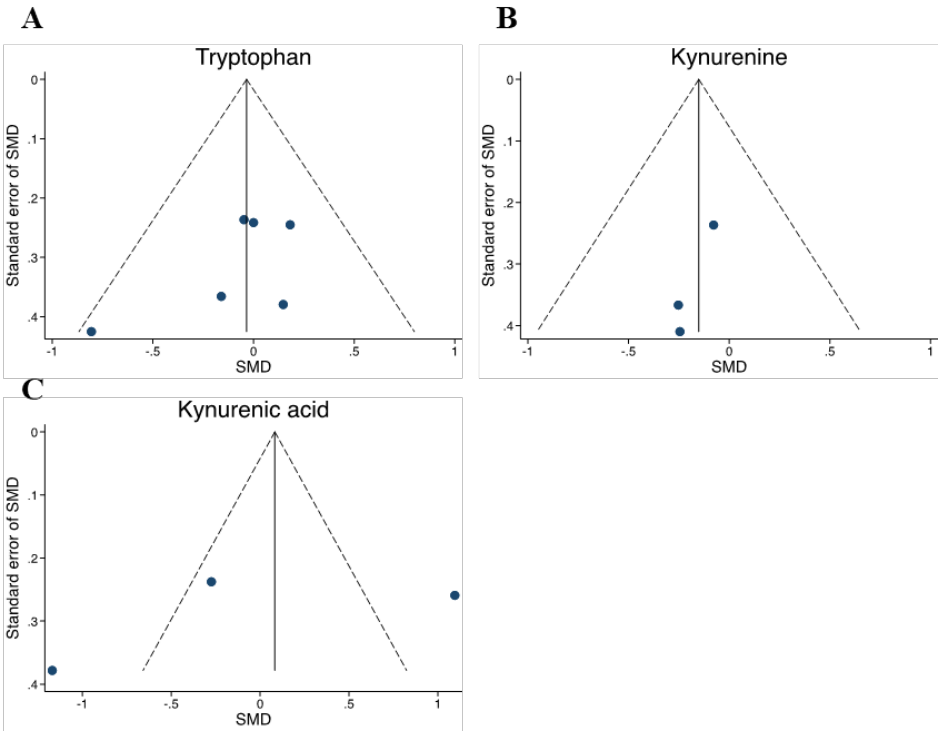


Figure S3. Funnel plots of AD dementia and control studies, separately in CSF.

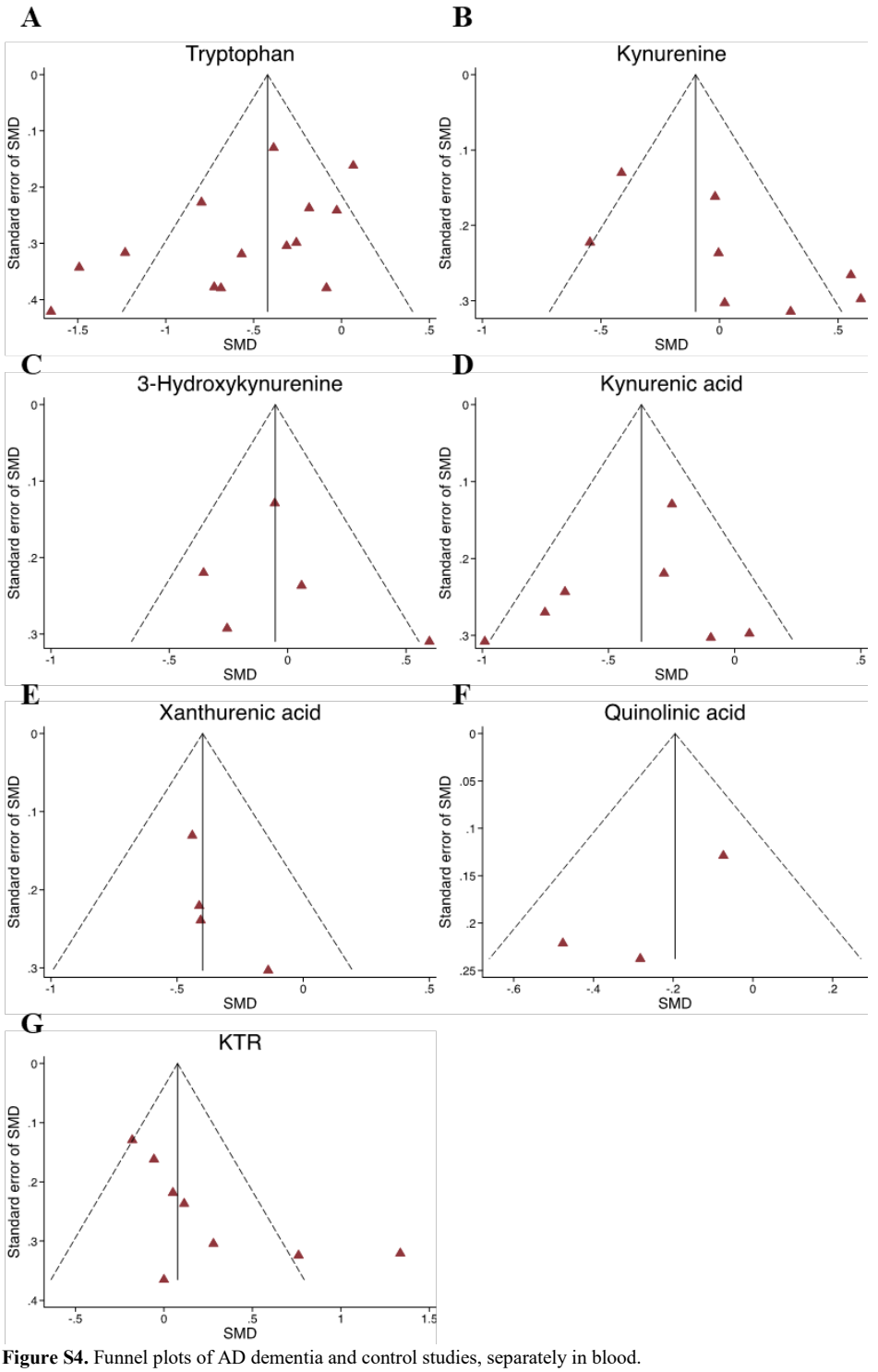


Figure S4. Funnel plots of AD dementia and control studies, separately in blood.

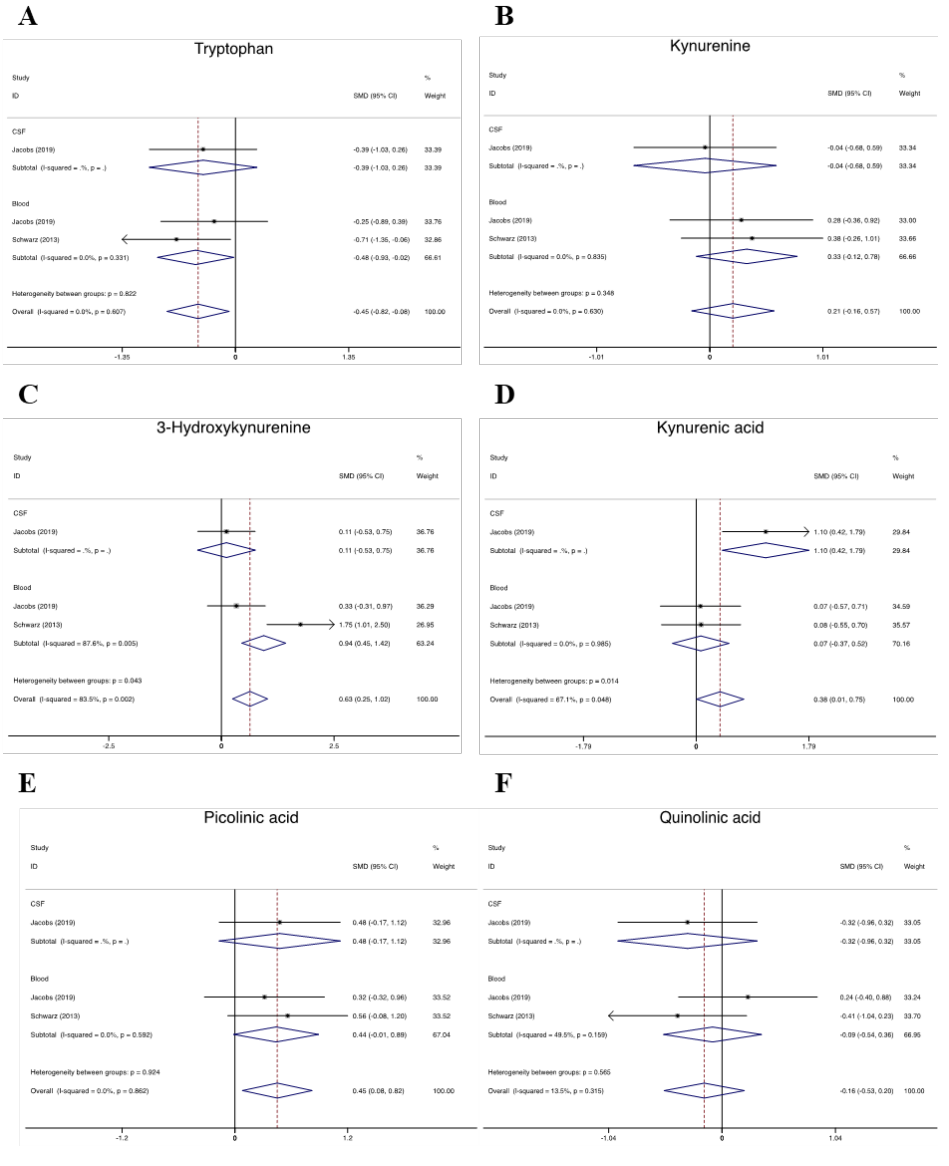


Figure S5. Forest plots of AD dementia and SCD studies

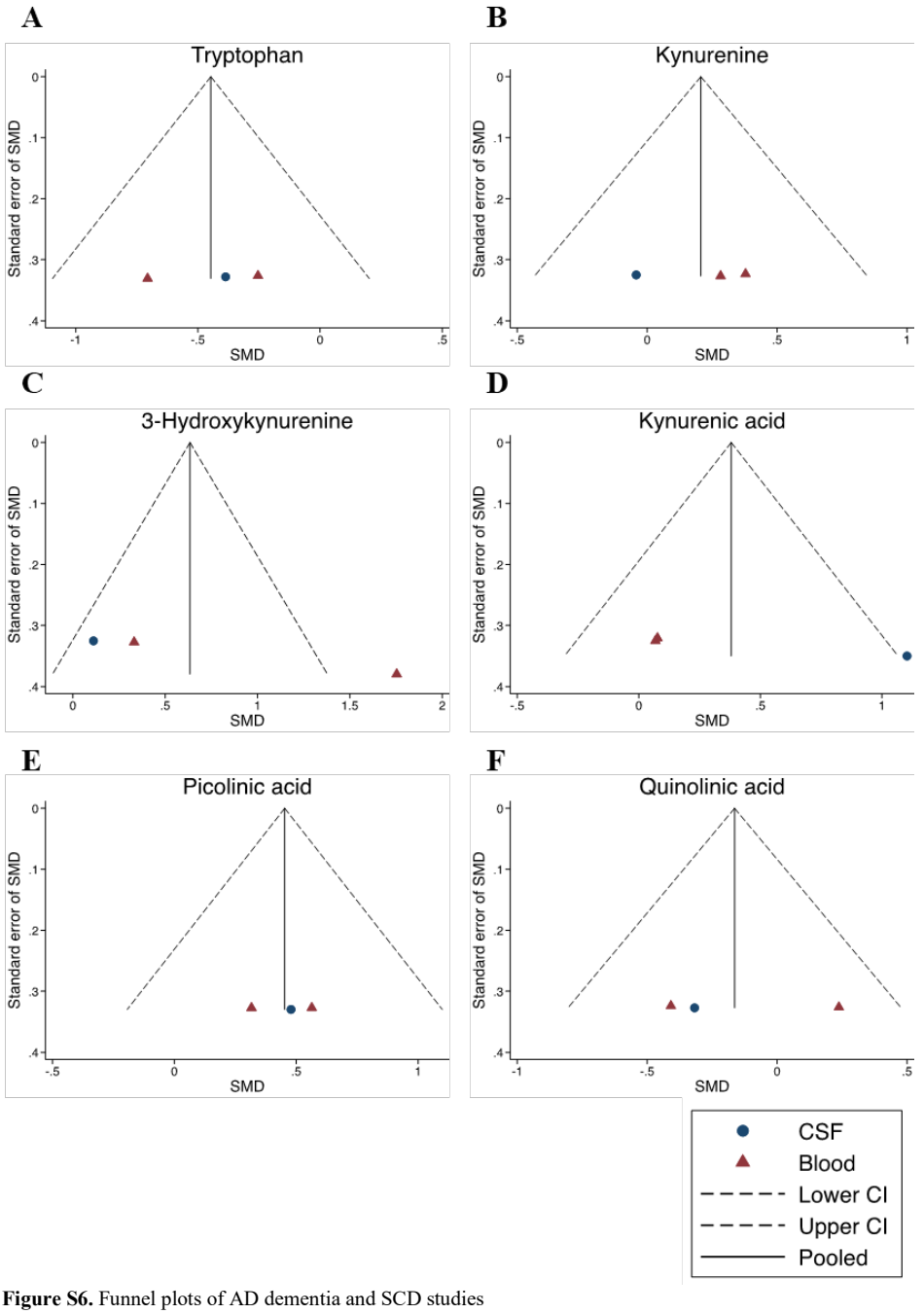


Figure S6. Funnel plots of AD dementia and SCD studies

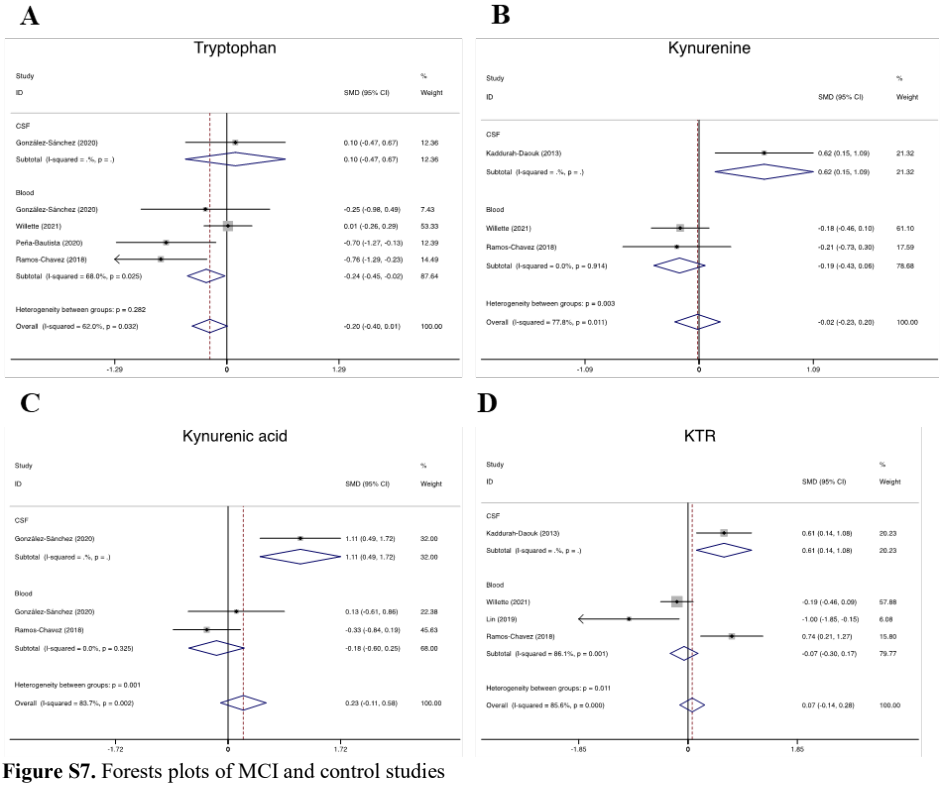


Figure S7. Forests plots of MCI and control studies

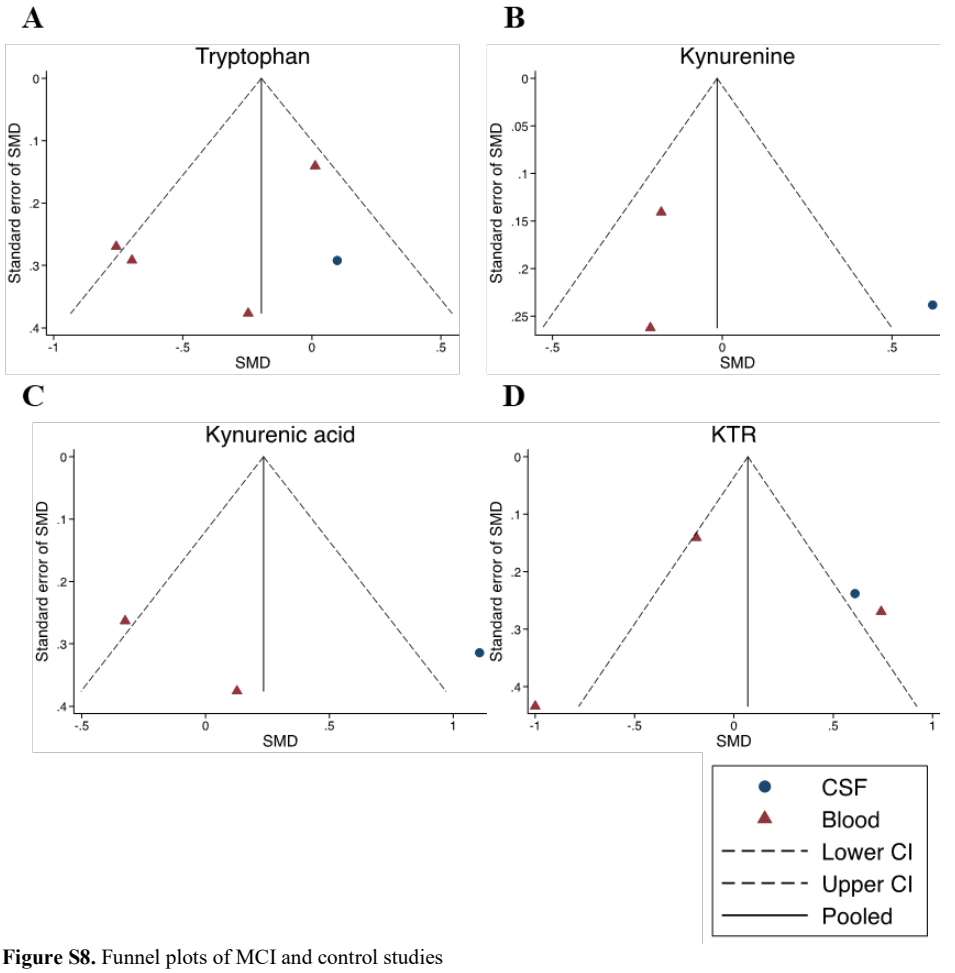


Figure S8. Funnel plots of MCI and control studies

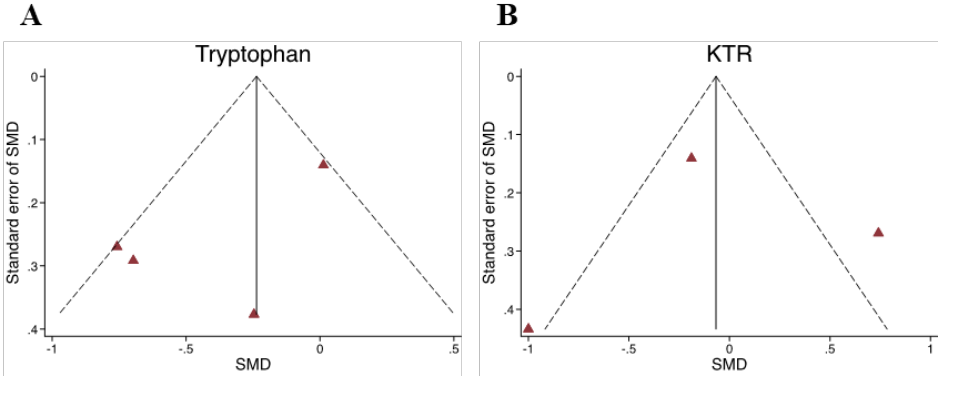


Figure S9. Funnel plots of MCI and control studies, separately in blood

Appendix S1. Data extraction form

Study Characteristics

Study ID *(surname of first author and year first full report of study was published e.g. Smith 2001)*	
Title	

Methods

Type of study *(please tick)*	☐ Case-control ☐ Cross-sectional ☐ Prospective ☐ Retrospective ☐ Other, namely _____
Participant characteristics *(overall)*	N: Mean age: Age range: N (%) Female:
Population description *(specify the groups studied (e.g. inpatient, outpatient) and/or* *from which study participants are drawn)*	
Person measuring *(e.g. psychiatrist, researcher, study nurse. If multiple persons were involved, specify tasks per person)*	

Appendix S1. (Continue)

Diagnosis *(please tick)*	☐ Normal aging/ cognition ☐ Cognitive impairment • ☐ Subjective cognitive impairment (SCD) • ☐ Mild cognitive impairment (MCI) • ☐ Cognitive impairment-no dementia (CIND) • ☐ Vascular cognitive impairment (VCI) • ☐ ___ standard deviation below comparison group ☐ Dementia • ☐ All-cause dementia • ☐ Alzheimer's disease dementia • ☐ Vascular dementia • ☐ Huntington's disease • ☐ Creutzfeldt-Jakob disease • ☐ Lewy body dementia • ☐ Down syndrome • ☐ Frontotemporal dementia • ☐ Mixed dementia • ☐ Normal pressure hydrocephalus • ☐ Posterior cortical atrophy • ☐ Parkinson's disease • ☐ Korsakoff syndrome • ☐ Unknown

Appendix S1. (Continue)

Diagnosis of dementia *(DSM-IV, ICD-10, research criteria, unknown)* **or definition of cognitive impairment** *(e.g. Peterson criteria, 2 standard deviations below control group)*			
Comparator(s)/ control *(please tick)*	☐ Control (no neurological disorder) ☐ Different stages/ severity of dementia, namely _____ ☐ Different ages of controls, namely _____		
Characteristics *(per group)*	Group: N: Mean age: Range: N (%) Female:	Group: N: Mean age: Range: N (%) Female:	Group: N: Mean age: Range: N (%) Female:
Comorbidities? *(please tick)*	☐ Yes, namely _____ ☐ No ☐ Unclear		
Setting *(including location and social context)*			
Inclusion criteria			
Exclusion criteria			

Appendix S1. (Continue)

Method of recruitment of participants *(e.g. phone, mail, clinic patients)*	
Exclusions *(Number of exclusions and reason for exclusion)*	
Repeated assessment *(please tick)*	☐ Yes, namely _____ ☐ No ☐ Unclear
Neuropsychological test used *(include sub-domains, e.g. TMT-A, TMT-B, immediate recall CVLT, delayed recall CVLT, delayed recognition CVLT)*	

Appendix S1. (Continue)

Exposure

Measured metabolites	☐ Tryptophan ☐ N-formyl kynurenine ☐ Kynurenine ☐ 3-hydroxykynurenine ☐ Kynurenic acid ☐ Xanthurenic acid ☐ Anthranilic acid ☐ 3-hydroxyanthranilic acid ☐ Cinnabarinic acid ☐ Picolinic acid ☐ Quinolinic acid ☐ Other, namely _____
Measurement technique	

Appendix S1. (Continue)

Biomaterial(s) *(please tick)*	☐ Blood ☐ Plasma ☐ Serum ☐ CSF ☐ Urine ☐ Faecal material ☐ Saliva ☐ Post-mortem tissues ☐ Peripheral blood mononuclear cell ☐ Human primary neuron ☐ Human induced pluripotent stem cell
Metabolite *(fill in separate form per metabolite)*	
Intra assay variations **Inter assay variations**	
Biomaterial(s)	
Outcome *(e.g. cognitive impairment, dementia, depression)*	
Concentrations	
Statistical methods used *(e.g. logistic regression, mixed effects regression, Cox regression)*	

Appendix S1. (Continue)

Interaction analyses? *(please tick)*	☐ Yes, namely _____ ☐ No ☐ Unclear
Adjustment for potential confounders? *(please tick)*	☐ Yes, namely _____ ☐ No ☐ Unclear
Other findings	

Other information

Key conclusions of study authors	
Correspondence for further study information	
Notes:	

Appendix S2. Newcastle-Ottawa quality assessment scale adapted for studies with different patient populations.

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories, with the exception for 'Assessment for outcome' from the Outcome category. A maximum of two stars can be given for Comparability.

Selection (Maximum 4 stars)

Case 1:

1. Is the case definition adequate?

a) Yes, with independent validation *****
b) Yes, e.g. record linkage or based on self-reports
c) No description

2. Sample size, response rate, and comparability between respondent and non-respondents

a) Consecutive or obviously representative series of cases *****
b) Potential for selection biases or not stated

Case 2:

1. Is the case definition adequate?

a) Yes, with independent validation *****
b) Yes, e.g. record linkage or based on self-reports
c) No description

2. Sample size, response rate, and comparability between respondent and non-respondents

a) Consecutive or obviously representative series of cases *****
b) Potential for selection biases or not stated

Appendix S2. (Continue)

Comparability (Maximum 2 stars)

Comparability of cohorts on the basis of the design or analysis
a) Study controls for the most important factor *****
b) Study controls for any additional factor *****
c) No control for any important factor

Outcome (Maximum 3 stars)

1. Ascertainment of exposure
a) Secure record (e.g. surgical records) *****
b) Structured interview where blind to case/control status *****
c) Interview not blinded to case/control status
d) Written self-report or medical record only
e) No description

2. Same method of ascertainment for both groups?
a) Yes *****
b) No

3. Non-Response rate
a) Same rate for both groups *****
b) Non respondents described
c) Rate different and no designation

Appendix S3. Newcastle-Ottawa quality assessment scale adapted for cross-sectional studies.

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories, with the exception for 'Assessment for outcome' from the Outcome category. A maximum of two stars can be given for Comparability.

Selection (Maximum 4 stars)

1. Representativeness of the exposed cohort

a) Truly representative of the general population (random sampling) *****
b) Somewhat representative of general population (non-random sampling) *****
c) Selected group of users (e.g. nurses, volunteers)
d) No description of the sampling strategy

2. Sample size, response rate, and comparability between respondent and non-respondents

a) Sample size is justified, response rate AND the comparability between respondents and non-respondents characteristics are described *****
b) Sample size is justified and the response rate OR the comparability between respondents and non-respondents characteristics is described *****
c) Sample size is justified, but no description of the response rate and the characteristics of the responders and non-responders
d) Sample size is not justified, and there is no description of the response rate or the characteristics of the responders and non-responders

3. Selection of the non-exposed cohort

a) Drawn from the same community as the exposed cohort *****
b) Drawn from a different source
c) No description of the derivation of the non-exposed cohort

4. Ascertainment of exposure

a) Validated measurement tool *****
b) Non-validated measurement tool, but the tool is available or described *****
c) Self-report
d) No description

Appendix S3. (Continue)

Comparability (Maximum 2 stars)

Comparability of cohorts on the basis of the design or analysis

a) Study controls for the most important factor *****

b) Study controls for any additional factor *****

c) No control for any important factor

Outcome (Maximum 3 stars)

1. Assessment of outcome

a) Independent blind assessment ******

b) Record linkage ******

c) Self-report

d) No description

2. Statistical test

a) The statistical test used to analyse the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (p value) *****

b) The statistical test is not appropriate, not described or incomplete

Supplementary References

1. Arai H, Kobayashi K, Ichimiya Y, Kosaka K, Iizuka R. A preliminary study of free amino acids in the postmortem temporal cortex from Alzheimer-type dementia patients. Neurobiol Aging. 1984;5(4):319-21.
2. Arai H, Kobayashi K, Ichimiya Y, Kosaka K, Iizuka R. Free amino acids in post-mortem cerebral cortices from patients with Alzheimer-type dementia. Neuroscience Research. 1985;2(6):486-90.
3. Armstrong MD, Stave U. A study of plasma free amino acid levels. III. Variations during growth and aging. Metabolism. 1973;22(4):571-8.
4. Atukeren P, Cengiz M, Yavuzer H, Gelisgen R, Altunoglu E, Oner S, et al. The efficacy of donepezil administration on acetylcholinesterase activity and altered redox homeostasis in Alzheimer's disease. Biomedicine and Pharmacotherapy. 2017;90:786-95.
5. Baker GB, Reynolds GP. Biogenic amines and their metabolites in Alzheimer's disease: Noradrenaline, 5-hydroxytryptamine and 5-hydroxyindole-3-acetic acid depleted in hippocampus but not in substantia innominata. Neuroscience Letters. 1989;100(1-3):335-9.
6. Banki CM, Molnar G. The influence of age, height, and body weight on cerebrospinal fluid amine metabolites and tryptophan in women. Biol Psychiatry. 1981;16(8):753-62.
7. Baran H, Jellinger K, Deecke L. Kynurenine metabolism in Alzheimer's disease. Journal of Neural Transmission. 1999;106(2):165-81.
8. Basun H, Forssell LG, Almkvist O, Cowburn RF, Eklöf R, Winblad B, et al. Amino acid concentrations in cerebrospinal fluid and plasma in Alzheimer's disease and healthy control subjects. J Neural Transm Park Dis Dement Sect. 1990;2(4):295-304.
9. Beal MF, Matson WR, Swartz KJ, Gamache PH, Bird ED. Kynurenine pathway measurements in Huntington's disease striatum: evidence for reduced formation of kynurenic acid. J Neurochem. 1990;55(4):1327-39.
10. Bie J, Guest J, Guillemin GJ, Grant R. Central kynurenine pathway shift with age in women. Journal of Neurochemistry. 2016;136(5):995-1003.
11. Bonaccorso S, Lin A, Song C, Verkerk R, Kenis G, Bosmans E, et al. Serotonin-immune interactions in elderly volunteers and in patients with Alzheimer's disease (DAT): Lower plasma tryptophan availability to the brain in the elderly and increased serum interleukin-6 in DAT. Aging - Clinical and Experimental Research. 1998;10(4):316-23.
12. Caballero B, Gleason RE, Wurtman RJ. Plasma amino acid concentrations in healthy elderly men and women. American Journal of Clinical Nutrition. 1991;53(5):1249-52.
13. Capuron L, Schroecksnadel S, Feart C, Aubert A, Higueret D, Barberger-Gateau P, et al. Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: Role in neuropsychiatric symptoms. Biological Psychiatry. 2011;70(2):175-82.
14. Chatterjee P, Cheong YJ, Bhatnagar A, Goozee K, Wu Y, McKay M, et al. Plasma metabolites associated with biomarker evidence of neurodegeneration in cognitively normal older adults. J Neurochem. 2020.
15. Chouraki V, Preis SR, Yang Q, Beiser A, Li S, Larson MG, et al. Association of amine biomarkers with incident dementia and Alzheimer's disease in the Framingham Study. Alzheimer's and Dementia. 2017;13(12):1327-36.
16. Cogo A, Mangin G, Maïer B, Callebert J, Mazighi M, Chabriat H, et al. Increased serum QUIN/KYNA is a reliable biomarker of post-stroke cognitive decline. Mol Neurodegener. 2021;16(1):7.
17. Collino S, Montoliu I, Martin FP, Scherer M, Mari D, Salvioli S, et al. Metabolic Signatures of Extreme Longevity in Northern Italian Centenarians Reveal a Complex Remodeling of Lipids, Amino Acids, and Gut Microbiota Metabolism. PLoS ONE. 2013;8(3).
18. Czech C, Berndt P, Busch K, Schmitz O, Wiemer J, Most V, et al. Metabolite profiling of Alzheimer's disease cerebrospinal fluid. PLoS One. 2012;7(2):e31501.
19. Darst BF, Koscik RL, Hogan KJ, Johnson SC, Engelman CD. Longitudinal plasma metabolomics of aging and sex. Aging (Albany NY). 2019;11(4):1262-82.

20. de Leeuw FA, Peeters CFW, Kester MI, Harms AC, Struys EA, Hankemeier T, et al. Blood-based metabolic signatures in Alzheimer's disease. Alzheimers Dement (Amst). 2017;8:196-207.
21. Demling J, Langer K, Mehr MQ. Age dependence of large neutral amino acid levels in plasma. Focus on Tryptophan. Advances in experimental medicine and biology. 1996;398:579-82.
22. Dunn WB, Lin W, Broadhurst D, Begley P, Brown M, Zelena E, et al. Molecular phenotyping of a UK population: defining the human serum metabolome. Metabolomics. 2015;11:9-26.
23. Eklundh T, Eriksson M, Sjöberg S, Nordin C. Monoamine precursors, transmitters and metabolites in cerebrospinal fluid: a prospective study in healthy male subjects. Journal of Psychiatric Research. 1996;30(3):201-8.
24. Nik Mohd Fakhruddin NNI, Shahar S, Ismail IS, Ahmad Azam A, Rajab NF. Urine Untargeted Metabolomic Profiling Is Associated with the Dietary Pattern of Successful Aging among Malaysian Elderly. Nutrients. 2020;12(10).
25. Fekkes D, van der Cammen TJ, van Loon CP, Verschoor C, van Harskamp F, de Koning I, et al. Abnormal amino acid metabolism in patients with early stage Alzheimer dementia. J Neural Transm (Vienna). 1998;105(2-3):287-94.
26. Ferraro TN, Hare TA. Free and conjugated amino acids in human CSF: influence of age and sex. Brain Res. 1985;338(1):53-60.
27. Fonteh AN, Harrington RJ, Tsai A, Liao P, Harrington MG. Free amino acid and dipeptide changes in the body fluids from Alzheimer's disease subjects. Amino Acids. 2007;32(2):213-24.
28. Frick B, Schroecksnadel K, Neurauter G, Leblhuber F, Fuchs D. Increasing production of homocysteine and neopterin and degradation of tryptophan with older age. Clinical Biochemistry. 2004;37(8):684-7.
29. Giil LM, Midttun O, Refsum H, Ulvik A, Advani R, Smith AD, et al. Kynurenine Pathway Metabolites in Alzheimer's Disease. Journal of Alzheimer's Disease. 2017;60(2):495-504.
30. Gold AB, Herrmann N, Swardfager W, Black SE, Aviv RI, Tennen G, et al. The relationship between indoleamine 2,3-dioxygenase activity and post-stroke cognitive impairment. Journal of Neuroinflammation. 2011;8 (no pagination).
31. Gonzalez-Dominguez R, Garcia-Barrera T, Gomez-Ariza JL. Metabolomic study of lipids in serum for biomarker discovery in Alzheimer's disease using direct infusion mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis. 2014;98:321-6.
32. Gonzalez-Dominguez R, Garcia-Barrera T, Gomez-Ariza JL. Metabolite profiling for the identification of altered metabolic pathways in Alzheimer's disease. Journal of Pharmaceutical and Biomedical Analysis. 2015;107:75-81.
33. González-Domínguez R, García-Barrera T, Gómez-Ariza JL. Application of a novel metabolomic approach based on atmospheric pressure photoionization mass spectrometry using flow injection analysis for the study of Alzheimer's disease. Talanta. 2015;131:480-9.
34. González-Sánchez M, Jiménez J, Narváez A, Antequera D, Llamas-Velasco S, Martín AH, et al. Kynurenic Acid Levels are Increased in the CSF of Alzheimer's Disease Patients. Biomolecules. 2020;10(4).
35. Graham SF, Chevallier OP, Elliott CT, Hölscher C, Johnston J, McGuinness B, et al. Untargeted metabolomic analysis of human plasma indicates differentially affected polyamine and L-arginine metabolism in mild cognitive impairment subjects converting to Alzheimer's disease. PLoS One. 2015;10(3):e0119452.
36. Greilberger J, Fuchs D, Leblhuber F, Greilberger M, Wintersteiger R, Tafeit E. Carbonyl proteins as a clinical marker in Alzheimer's disease and its relation to tryptophan degradation and immune activation. Clinical Laboratory. 2010;56(9-10):441-8.
37. Gulaj E, Pawlak K, Bien B, Pawlak D. Kynurenine and its metabolites in Alzheimer's disease patients. Advances in Medical Sciences. 2010;55(2):204-11.
38. Hafstad Solvang SE, Nordrehaug JE, Aarsland D, Lange J, Ueland PM, McCann A, et al. Kynurenines, Neuropsychiatric Symptoms, and Cognitive Prognosis in Patients with Mild Dementia. Int J Tryptophan Res. 2019;12:1178646919877883.

39. Solvang SEH, Nordrehaug JE, Tell GS, Nygard O, McCann A, Ueland PM, et al. The kynurenine pathway and cognitive performance in community-dwelling older adults. The Hordaland Health Study. Brain, Behavior, and Immunity. 2019;75:155-62.
40. Hartai Z, Juhasz A, Rimanoczy A, Janaky T, Donko T, Dux L, et al. Decreased serum and red blood cell kynurenic acid levels in Alzheimer's disease. Neurochemistry International. 2007;50(2):308-13.
41. Heyes MP, Saito K, Crowley JS, Davis LE, Demitrack MA, Der M, et al. Quinolinic acid and kynurenine pathway metabolism in inflammatory and non-inflammatory neurological disease. Brain. 1992;115 (Pt 5):1249-73.
42. Ibáñez C, Simó C, Barupal DK, Fiehn O, Kivipelto M, Cedazo-Mínguez A, et al. A new metabolomic workflow for early detection of Alzheimer's disease. J Chromatogr A. 2013;1302:65-71.
43. Jacobs KR, Lim CK, Blennow K, Zetterberg H, Chatterjee P, Martins RN, et al. Correlation between plasma and CSF concentrations of kynurenine pathway metabolites in Alzheimer's disease and relationship to amyloid-β and tau. Neurobiology of Aging. 2019;80:11-20.
44. Janssens J, Vermeiren Y, van Faassen M, van der Ley C, Kema IP, De Deyn PP. Monoaminergic and Kynurenergic Characterization of Frontotemporal Dementia and Amyotrophic Lateral Sclerosis in Cerebrospinal Fluid and Serum. Neurochem Res. 2020;45(5):1191-201.
45. Johnson LC, Martens CR, Santos-Parker JR, Bassett CJ, Strahler TR, Cruickshank-Quinn C, et al. Amino acid and lipid associated plasma metabolomic patterns are related to healthspan indicators with ageing. Clin Sci (Lond). 2018;132(16):1765-77.
46. Kaddurah-Daouk R, Rozen S, Matson W, Han X, Hulette CM, Burke JR, et al. Metabolomic changes in autopsy-confirmed Alzheimer's disease. Alzheimer's and Dementia. 2011;7(3):309-17.
47. Kaddurah-Daouk R, Zhu H, Sharma S, Bogdanov M, Rozen SG, Matson W, et al. Alterations in metabolic pathways and networks in Alzheimer's disease. Translational Psychiatry. 2013;3 (no pagination).
48. Kaiser E, Schoenknecht P, Kassner S, Hildebrandt W, Kinscherf R, Schroeder J. Cerebrospinal fluid concentrations of functionally important amino acids and metabolic compounds in patients with mild cognitive impairment and Alzheimer's disease. Neurodegenerative Diseases. 2010;7(4):251-9.
49. Kepplinger B, Baran H, Kainz A, Ferraz-Leite H, Newcombe J, Kalina P. Age-related increase of kynurenic acid in human cerebrospinal fluid - IgG and beta2-microglobulin changes. Neurosignals. 2005;14(3):126-35.
50. Kepplinger B, Baran H, Kronsteiner C, Reuss J. Increased Levels of Kynurenic Acid in the Cerebrospinal Fluid in Patients with Hydrocephalus. Neurosignals. 2019;27(1):1-11.
51. Kuster OC, Laptinskaya D, Fissler P, Schnack C, Zugel M, Nold V, et al. Novel Blood-Based Biomarkers of Cognition, Stress, and Physical or Cognitive Training in Older Adults at Risk of Dementia: Preliminary Evidence for a Role of BDNF, Irisin, and the Kynurenine Pathway. Journal of Alzheimer's Disease. 2017;59(3):1097-111.
52. Leblhuber F, Walli J, Jellinger K, Tilz GP, Widner B, Laccone F, et al. Activated immune system in patients with Huntington's disease. Clinical Chemistry and Laboratory Medicine. 1998;36(10):747-50.
53. Li NJ, Liu WT, Li W, Li SQ, Chen XH, Bi KS, et al. Plasma metabolic profiling of Alzheimer's disease by liquid chromatography/mass spectrometry. Clinical Biochemistry. 2010;43(12):992-7.
54. Liang Q, Liu H, Li X, Zhang A-H. High-throughput metabolomics analysis discovers salivary biomarkers for predicting mild cognitive impairment and Alzheimer's disease. RSC Advances. 2016;6(79):75499-504.
55. Lin CN, Huang CC, Huang KL, Lin KJ, Yen TC, Kuo HC. A metabolomic approach to identifying biomarkers in blood of Alzheimer's disease. Ann Clin Transl Neurol. 2019;6(3):537-45.
56. Liu M, Zhou K, Li H, Dong X, Tan G, Chai Y, et al. Potential of serum metabolites for diagnosing post-stroke cognitive impairment. Molecular bioSystems. 2015;11(12):3287-96.
57. Martinez M, Frank A, Diez-Tejedor E, Hernanz A. Amino acid concentrations in cerebrospinal fluid and serum in Alzheimer's disease and vascular dementia. J Neural Transm Park Dis Dement Sect. 1993;6(1):1-9.
58. Mashige F, Takai N, Shinozuka N, Wada H, Sakuma I, Ito A, et al. Development of a high-performance liquid chromatography system with multi- electrode electrochemical detectors for determination

of levels of neurotransmitters in cerebrospinal fluid. Japanese Journal of Clinical Chemistry. 1993;22(3):147-55.
59. Molina JA, Jiménez-Jiménez FJ, Vargas C, Gómez P, de Bustos F, Ortí-Pareja M, et al. Cerebrospinal fluid levels of non-neurotransmitter amino acids in patients with Alzheimer's disease. J Neural Transm (Vienna). 1998;105(2-3):279-86.
60. Mourdian MM, Heyes MP, Pan JB, Heuser IJ, Markey SP, Chase TN, et al. No changes in central quinolinic acid levels in Alzheimer's disease. Neurosci Lett. 1989;105(1-2):233-8.
61. Oxenkrug G, van der Hart M, Roeser J, Summergrad P. Peripheral Tryptophan - Kynurenine Metabolism Associated with Metabolic Syndrome is Different in Parkinson's and Alzheimer's Diseases. Endocrinol Diabetes Metab J. 2017;1(4).
62. Paglia G, Stocchero M, Cacciatore S, Lai S, Angel P, Alam MT, et al. Unbiased Metabolomic Investigation of Alzheimer's Disease Brain Points to Dysregulation of Mitochondrial Aspartate Metabolism. Journal of Proteome Research. 2016;15(2):608-18.
63. Park SA, Son SY, Lee AY, Park HG, Lee WL, Lee CH. Metabolite Profiling Revealed That a Gardening Activity Program Improves Cognitive Ability Correlated with BDNF Levels and Serotonin Metabolism in the Elderly. Int J Environ Res Public Health. 2020;17(2).
64. Peña-Bautista C, Flor L, López-Nogueroles M, García L, Ferrer I, Baquero M, et al. Plasma alterations in cholinergic and serotonergic systems in early Alzheimer Disease: Diagnosis utility. Clin Chim Acta. 2020;500:233-40.
65. Pertovaara M, Raitala A, Lehtimäki T, Karhunen PJ, Oja SS, Jylhä M, et al. Indoleamine 2,3-dioxygenase activity in nonagenarians is markedly increased and predicts mortality. Mech Ageing Dev. 2006;127(5):497-9.
66. Phipps DA, Powell C. Plasma tryptophan, age and depression. Age Ageing. 1985;14(2):71-5.
67. Proenza AM, Palou A, Roca P. Amino acid distribution in human blood. A significant pool of amino acids is adsorbed onto blood cell membranes. Biochem Mol Biol Int. 1994;34(5):971-82.
68. Ramos-Chavez LA, Roldan-Roldan G, Garcia-Juarez B, Gonzalez-Esquivel D, Perez de la Cruz G, Pineda B, et al. Low serum tryptophan levels as an indicator of global cognitive performance in nondemented women over 50 years of age. Oxidative Medicine and Cellular Longevity. 2018;2018 (no pagination).
69. Rommer PS, Fuchs D, Leblhuber F, Schroth R, Greilberger M, Tafeit E, et al. Lowered levels of carbonyl proteins after Vitamin B supplementation in patients with mild cognitive impairment and Alzheimer's disease. Neurodegenerative Diseases. 2016;16(3-4):284-9.
70. Rudman D, Mattson DE, Feller AG, Cotter R, Johnson RC. Fasting plasma amino acids in elderly men. Am J Clin Nutr. 1989;49(3):559-66.
71. Ruiz-Ruiz S, Sanchez-Carrillo S, Ciordia S, Mena MC, Mendez-Garcia C, Rojo D, et al. Functional microbiome deficits associated with ageing: Chronological age threshold. Aging Cell. 2020;19(1).
72. Santos ALM, Vitório JG, de Paiva MJN, Porto BLS, Guimarães HC, Canuto GAB, et al. Frontotemporal dementia: Plasma metabolomic signature using gas chromatography-mass spectrometry. J Pharm Biomed Anal. 2020;189:113424.
73. Sarwar G, Botting HG, Collins M. A comparison of fasting serum amino acid profiles of young and elderly subjects. J Am Coll Nutr. 1991;10(6):668-74.
74. Schwarz MJ, Guillemin GJ, Teipel SJ, Buerger K, Hampel H. Increased 3-Hydroxykynurenine serum concentrations differentiate Alzheimer's disease patients from controls. European Archives of Psychiatry and Clinical Neuroscience. 2013;263(4):345-52.
75. Shao Y, Ouyang Y, Li T, Liu X, Xu X, Li S, et al. Alteration of Metabolic Profile and Potential Biomarkers in the Plasma of Alzheimer's Disease. Aging Dis. 2020;11(6):1459-70.
76. Shaw DM, Tidmarsh SF, Karajgi BM, Sweeney EA, Williams S, Elameer M, et al. Pilot study of amino acids in senile dementia. Br J Psychiatry. 1981;139:580-2.
77. Sipahi H, Girgin G, Inanici F, Ariogul S, Sahin G, Baydar T. Tryptophan degradation and neopterin levels by aging. Pteridines. 2013;24(1):33-9.

78. Sorgdrager FJH, Vermeiren Y, Van Faassen M, van der Ley C, Nollen EAA, Kema IP, et al. Age- and disease-specific changes of the kynurenine pathway in Parkinson's and Alzheimer's disease. Journal of Neurochemistry. 2019.
79. Storga D, Vrecko K, Birkmayer JG, Reibnegger G. Monoaminergic neurotransmitters, their precursors and metabolites in brains of Alzheimer patients. Neurosci Lett. 1996;203(1):29-32.
80. Tarbit I, Perry EK, Perry RH, Blessed G, Tomlinson BE. Hippocampal free amino acids in Alzheimer's disease. J Neurochem. 1980;35(5):1246-9.
81. Theofylaktopoulou D, Midttun O, Ulvik A, Ueland PM, Tell GS, Vollset SE, et al. A community-based study on determinants of circulating markers of cellular immune activation and kynurenines: the Hordaland Health Study. Clin Exp Immunol. 2013;173(1):121-30.
82. Thomas DE, Chung AOKO, Dickerson JW, Tidmarsh SF, Shaw DM. Tryptophan and nutritional status of patients with senile dementia. Psychol Med. 1986;16(2):297-305.
83. Tohgi H, Abe T, Takahashi S, Kimura M, Takahashi J, Kikuchi T. Concentrations of serotonin and its related substances in the cerebrospinal fluid in patients with Alzheimer type dementia. Neuroscience Letters. 1992;141(1):9-12.
84. Tohgi H, Takahashi S, Abe T. The effect of age on concentrations of monoamines, amino acids, and their related substances in the cerebrospinal fluid. Journal of Neural Transmission - Parkinson's Disease and Dementia Section. 1993;5(3):215-26.
85. Tohgi H, Abe T, Takahashi S, Saheki M, Kimura M. Indoleamine concentrations in cerebrospinal fluid from patients with Alzheimer type and Binswanger type dementias before and after administration of citalopram, a synthetic serotonin uptake inhibitor. Journal of Neural Transmission - Parkinson's Disease and Dementia Section. 1995;9(2-3):121-31.
86. Toledo JB, Arnold M, Kastenmuller G, Chang R, Baillie RA, Han X, et al. Metabolic network failures in Alzheimer's disease: A biochemical road map. Alzheimer's and Dementia. 2017;13(9):965-84.
87. Trushina E, Dutta T, Persson XM, Mielke MM, Petersen RC. Identification of altered metabolic pathways in plasma and CSF in mild cognitive impairment and Alzheimer's disease using metabolomics. PLoS One. 2013;8(5):e63644.
88. Tsuruoka M, Hara J, Hirayama A, Sugimoto M, Soga T, Shankle WR, et al. Capillary electrophoresis-mass spectrometry-based metabolome analysis of serum and saliva from neurodegenerative dementia patients. Electrophoresis. 2013;34(19):2865-72.
89. Urbańska EM, Luchowski P, Luchowska E, Pniewski J, Woźniak R, Chodakowska-Zebrowska M, et al. Serum kynurenic acid positively correlates with cardiovascular disease risk factor, homocysteine: a study in stroke patients. Pharmacol Rep. 2006;58(4):507-11.
90. Valdiglesias V, Sanchez-Flores M, Maseda A, Lorenzo-Lopez L, Marcos-Perez D, Lopez-Corton A, et al. Immune biomarkers in older adults: Role of physical activity. Journal of Toxicology and Environmental Health - Part A: Current Issues. 2017;80(13-15):605-20.
91. van der Velpen V, Teav T, Gallart-Ayala H, Mehl F, Konz I, Clark C, et al. Systemic and central nervous system metabolic alterations in Alzheimer's disease. Alzheimers Res Ther. 2019;11(1):93.
92. Watkins SE, Thomas DE, Clifford M, Tidmarsh SF. Plasma amino acids in patients with senile dementia and in subjects with Down's syndrome at an age vulnerable to Alzheimer changes. Journal of Mental Deficiency Research. 1989;33(2):159-66.
93. Wennstrom M, Nielsen HM, Orhan F, Londos E, Minthon L, Erhardt S. Kynurenic Acid levels in cerebrospinal fluid from patients with Alzheimer's disease or dementia with lewy bodies. Int J Tryptophan Res. 2014;7:1-7.
94. Westbrook R, Chung T, Lovett J, Ward C, Joca H, Yang H, et al. Kynurenines link chronic inflammation to functional decline and physical frailty. JCI Insight. 2020;5(16).
95. Whiley L, Chappell KE, D'Hondt E, Lewis MR, Jiménez B, Snowden SG, et al. Metabolic phenotyping reveals a reduction in the bioavailability of serotonin and kynurenine pathway metabolites in both the urine and serum of individuals living with Alzheimer's disease. Alzheimers Res Ther. 2021;13(1):20.
96. Widner B, Leblhuber F, Walli J, Tilz GP, Demel U, Fuchs D. Degradation of tryptophan in neurodegenerative disorders. Adv Exp Med Biol. 1999;467:133-8.

97. Widner B, Leblhuber F, Walli J, Tilz GP, Demel U, Fuchs D. Tryptophan degradation and immune activation in Alzheimer's disease. Journal of Neural Transmission. 2000;107(3):343-53.
98. Willette AA, Pappas C, Hoth N, Wang Q, Klinedinst B, Willette SA, et al. Inflammation, Negative Affect, and Amyloid Burden in Alzheimer's Disease: Insights from the Kynurenine Pathway. Brain Behav Immun. 2021.
99. Wissmann P, Geisler S, Leblhuber F, Fuchs D. Immune activation in patients with Alzheimer's disease is associated with high serum phenylalanine concentrations. Journal of the Neurological Sciences. 2013;329(1-2):29-33.
100. Wu L, Han Y, Zheng Z, Peng G, Liu P, Yue S, et al. Altered Gut Microbial Metabolites in Amnestic Mild Cognitive Impairment and Alzheimer's Disease: Signals in Host-Microbe Interplay. Nutrients. 2021;13(1).
101. Xu J, Begley P, Church SJ, Patassini S, Hollywood KA, Jüllig M, et al. Graded perturbations of metabolism in multiple regions of human brain in Alzheimer's disease: Snapshot of a pervasive metabolic disorder. Biochim Biophys Acta. 2016;1862(6):1084-92.
102. Xyda SE, Vuckovic I, Petterson XM, Dasari S, Lalia AZ, Parvizi M, et al. Distinct Influence of Omega-3 Fatty Acids on the Plasma Metabolome of Healthy Older Adults. J Gerontol A Biol Sci Med Sci. 2020;75(5):875-84.
103. Yilmaz A, Ugur Z, Bisgin H, Akyol S, Bahado-Singh R, Wilson G, et al. Targeted Metabolic Profiling of Urine Highlights a Potential Biomarker Panel for the Diagnosis of Alzheimer's Disease and Mild Cognitive Impairment: A Pilot Study. Metabolites. 2020;10(9).

CHAPTER 3

Dysregulation of transcriptomic- and DNA (hydroxy)methylomic- profiles in the tryptophan catabolic pathway in patients with Alzheimer's disease

Kyonghwan Choe[1], Muhammad Ali[1, 2, 3], Roy Lardenoije[1, 4], Ehsan Pishva[1, 5], Markus Leber[6], Holger Wagner[7], Per Hoffmann[8, 9, 10], Tobias Luck[11], Steffi Riedel-Heller[11], Frank Jessen[12, 13], Wolfgang Maier[7, 12], Michael Wagner[7, 12], René Hurlemann[14], Diego Mastroeni[1, 15, 16], Elaine Delvaux[15, 16], Paul D. Coleman[15, 16], Alfredo Ramirez[6, 7], Inez H.G.B. Ramakers[1, 17], Frans R.J. Verhey[1, 17], Bart P.F. Rutten[1], Gunter Kenis[1], Daniël L.A. van den Hove[1, 18]

[1] *Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience (MHeNs) and European Graduate School of Neuroscience (EURON), Faculty of Health, Medicine and Life Sciences (FHML), Maastricht University, Maastricht, the Netherlands.*

[2] *Computational Biology Group, Luxembourg Centre for System Biomedicine (LCSB), University of Luxembourg, Belvaux, Luxembourg.*

[3] *Biomedical Data science Group, Luxembourg Centre for System Biomedicine (LCSB), University of Luxembourg, Belvaux, Luxembourg.*

[4] *Department of Psychiatry and Psychotherapy, University Medical Center Göttingen, 37075 Göttingen, Germany.*

[5] *University of Exeter Medical School, University of Exeter, Exeter, UK.*

[6] *Division of Neurogenetics and Molecular Psychiatry, Department of Psychiatry and Psychotherapy, University of Cologne, Medical Faculty, 50937 Cologne, Germany.*

[7] *Department of Neurodegeneration and Gerontopsychiatry, University of Bonn, 53127, Bonn, Germany.*

[8] *Institute of Human Genetics, University of Bonn, 53127 Bonn, Germany.*

[9] *Department of Genomics, Life & Brain Center, University of Bonn, 53127 Bonn, Germany.*

[10] *Division of Medical Genetics, University Hospital and Department of Biomedicine, University of Basel, CH-4058 Basel, Switzerland.*

[11] *Institute of Social Medicine, Occupational Health and Public Health, University of Leipzig, 04103 Leipzig, Germany.*

[12] *German Center for Neurodegenerative Diseases (DZNE), 53127 Bonn, Germany.*

[13] *Department of Psychiatry and Psychotherapy, University of Cologne, Medical Faculty, 50937 Cologne, Germany.*

[14] *Department of Psychiatry and Division of Medical Psychology, University of Bonn, 53105 Bonn, Germany.*
[15] *L.J. Roberts Center for Alzheimer's Research Banner Sun Health Research Institute, Sun City, AZ, USA.*
[16] *Biodesign Institute, Neurodegenerative Disease Research Center, Arizona State University, Tempe, AZ, USA.*
[17] *Department of Psychiatry and Neuropsychology, Alzheimer Center Limburg, Maastricht University, Maastricht, the Netherlands.*
[18] *Department of Psychiatry, Psychosomatic and Psychotherapy, University of Würzburg, Germany.*

Abstract

Neurodegenerative disorders such as Alzheimer's disease (AD) have been associated with alterations in tryptophan (TRP) catabolism, albeit that no studies investigate mRNA and epigenetic levels. The aim of the present study was to investigate transcriptomic and associated DNA (hydroxy)methylation changes within the genes of the TRP- and nicotinamide adenine dinucleotide (NAD)-pathways in AD and validate the findings in two independent cohorts. We used post-mortem middle temporal gyrus (MTG) tissue from AD patients (n = 45) and controls (n = 35), and blood from two independent cohorts, i.e., the German study on Ageing, Cognition, and Dementia in Primary Care Patients (AgeCoDe) cohort (n = 96), and the Dutch BioBank Alzheimer Center Limburg (BBACL) cohort (n = 262). Fifty-five TRP- and 52 NAD-associated genes were selected through the Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome, and WikiPathways databases. Gene expression, DNA (hydroxy) methylation profiling and associated bisulfite pyrosequencing data were analyzed. Gene regulatory network (GRN) and network perturbation analysis was performed using the Metacore database. Association analyses were done using linear regression, adjusting for several covariates. In our MTG analysis, 11 TRP- and 20 NAD-associated genes displayed differential expression in AD. Additionally, numerous loci displayed differential DNA (hydroxy)methylation, the levels of which correlated to the corresponding gene expressions on several occasions. Furthermore, GRN and network perturbation analysis also identified several genes that showed differential gene expression in the MTG. Based on these findings, three candidate genes, i.e., *IDO2*, *SLC7A5* and *PAPR14*, were assessed in blood samples of subjects of the AgeCoDe. One CpG site in *IDO2*, cg11251498, showed a significant difference in methylation level when comparing converters to AD dementia and non-converters, at a preclinical stage. In the BBACL cohort, whereas no significant difference in cg11251498 methylation between groups was observed, a significant negative association between cg11251498 methylation and age, representing the most important risk factor for AD, was found. Our preliminary data suggest numerous transcriptional and epigenetic differences in both TRP- and NAD-pathway associated genes in AD, and pinpoint the *IDO2* gene as a prime candidate gene. These genes and the encoded proteins may be targeted in the development of novel biomarkers and treatment strategies for AD.

Keywords: Tryptophan, Alzheimer's disease, Epigenetics, Brain, Blood

Introduction

Alzheimer's disease (AD), a heterogeneous neurodegenerative disorder characterized by gradual cognitive decline but also linked to multiple phenotypes and comorbidities, is the most common type of dementia, contributing to 50%-75% of cases [1]. For most people, the biggest risk factor for dementia is aging [2]. Currently, there is no treatment for AD and the exact cause and pathophysiology remain partly unclear. While the amyloid beta (Aβ) and tau hypotheses have been investigated for decades, recent studies have shown that other mechanisms could be involved in the development and course of AD as well. For example, recent work has shown that the tryptophan (TRP) catabolic pathway may contribute to neurodegenerative disorders such as AD [3]. TRP is an essential amino acid, supplied only through diet or supplements. TRP competes with other large neutral amino acids for the same large amino acid transporter (LAT) in order to cross the blood-brain barrier (BBB) [4]. Once TRP enters the brain, it acts as a precursor for many pathways centered around molecules such as kynurenine (KYN), serotonin, tryptamine, and protein synthesis [5].

Current studies on the TRP catabolic pathway largely focus on the kynurenine pathway (KP) as it is the dominant pathway, accounting for >90% of tryptophan metabolism. Tryptophan 2,3-dioxygenase 2 (TDO2), indoleamine 2,3-dioxygenase 1 (IDO1), and IDO2 are the first and rate-limiting enzymes that metabolize TRP and initiate the KP [6]. Although TDO2 is mainly expressed in the liver, it is also present in the brain. Astrocytes, microglia, microvascular endothelial cells, and macrophages are the main cell types expressing IDO [5]. Further downstream KP metabolites, also known as kynurenines, have shown to exert both neurotoxic (e.g., quinolinic acid [QA]) and neuroprotective (e.g., kynurenic acid [KA]) effects in the brain [4, 5]. In relation to the pathophysiology of AD, KA is considered neuroprotective because it is an antagonist for all ionotropic glutamate receptors as well as the α7 nicotinic acetylcholine (α7nACh) receptor [3]. On the contrary, QA is considered neurotoxic because it is an agonist of N-methyl-D-aspartate (NMDA) receptor with the potential to induce excitotoxicity [3]. In cross-sectional studies, plasma KA concentrations were shown to be decreased [7, 8] while QA concentrations were increased in AD patients compared to controls [7]. Additionally, in post-mortem brain studies, the highest QA expression was shown in the perimeter of

senile plaques in the hippocampus [9, 10]. Additionally, QA was co-localized with hyperphosphorylated tau within cortical neurons in AD brain and QA treatment increased tau phosphorylation in human primary neurons [11]. The KP also initiates the *de novo* synthesis of nicotinamide adenine dinucleotide (NAD) via the route through QA. NAD is a coenzyme central to metabolism and especially adenosine triphosphate (ATP) production. Studies have shown a decrease in NAD levels during normal aging, but also during neurodegeneration [12].

Studies systematically investigating the TRP catabolic pathway are lacking. Most studies have used concentrations of multiple metabolites or their ratio to deduce KP activity. Although these provide valuable information, investigating changes in mRNA expression and DNA methylation of genes linked to these pathways may be of great value in view of their potential role in the pathogenesis of AD and its molecular regulation. Therefore, the aim of the current study was to examine to which extent TRP- and NAD-associated genes were affected in AD at the transcriptional and DNA (hydroxy)methylation level. For this purpose, we first investigated TRP- and NAD- pathway associated genes through transcriptomic- and (hydroxy)methylation- profiling, gene regulatory network (GRN), and network perturbation analysis, making use of human post-mortem middle temporal gyrus (MTG) tissue from AD patients and controls. Subsequently, we aimed to validate the MTG findings through investigating blood based methylation profiling and pyrosequencing, making use of two independent longitudinal cohorts.

Materials and methods

Post-mortem MTG brain tissue

Detailed information about the MTG datasets can be found elsewhere [13]. Briefly, MTG DNA samples were obtained from AD patients (n = 45) and neurologically normal control (n = 35) from Brain and Body Donation Program (BBDP) donors and stored at the Brain and Tissue Bank of the Banner Sun Health Research Institute (BSHRI; Sun City, Arizona, USA) (Table 1). Detailed information about the BBDP has been reported elsewhere [14, 15]. The organization of the BBDP allows for fast tissue recovery after death and samples in this study had an average post-mortem interval of 2.8 hours. Additionally, Braak staging was carried out for assessing the degree of neurofibrillary

pathology. A consensus diagnosis of AD or non-demented control was reached by following National Institutes of Health (NIH) AD Center criteria [15]. Exclusion criteria were comorbidity with any other type of dementia, cerebrovascular disorders, mild cognitive impairment (MCI), and presence of non-microscopic infarcts. Informed consent was obtained from all human participants. This includes donors of the BSHRI-BBDP, who signed an Institutional Review Board-approved informed consent form, including specific consent to the use of donated tissue for future research [14, 15].

Table 1. MTG patient demographics

	AD patients	**Non-demented controls**
N	45	35
Gender (male/female)	22/23	17/18
Age of death	85.09 ± 6.24	84.46 ± 5.50
PMI	2.77 ± 0.69	2.87 ± 1.03
Plaque total	12.97 ± 2.25	4.65 ± 4.30
Tangle total	11.02 ± 4.16	3.96 ± 2.10
Braak Stage (range (median))	II – VI (V)	I – IV (III)

Overview of patient characteristics in post-mortem middle temporal gyrus (MTG) brains used in this study. Data are presented in n or mean ± standard deviation (SD). Patients were age- and gender- matched and diagnosed with either Alzheimer's disease (AD) or non-demented controls.

The AgeCoDe cohort

Detailed information about the Ageing, Cognition and Dementia in Primary Care Patients (AgeCoDe) datasets can be found elsewhere [13]. AgeCoDe, a prospective longitudinal study, aims to improve early detection of MCI and dementia in primary care and included 3327 non-demented individuals at baseline [16]. For this study, the dataset published by Lardenoije et al. (2019) was used [13]. Briefly, a subsample of 96 individuals (age >70 years) with whole blood DNA methylation data available, were selected. Of these, 41 individuals converted to AD during the course of the study, while 42 control subjects did not show cognitive impairment at baseline nor at follow-up 4.5 years later. The remaining 13 individuals did convert, but only after 4.5 years (Table 2). The German AgeCoDe study protocol was approved by the local ethics committees at the University of Bonn (Bonn, Germany), the University of Hamburg (Hamburg, Germany), the University of Düsseldorf (Düsseldorf, Germany), the University of Heidelberg/Mannheim (Mannheim, Germany), the University of

Leipzig (Leipzig, Germany), and the Technical University of Munich (Munich, Germany).

Table 2. AgeCoDe cohort patient demographics

	Controls	AD Converters	AD Converters (4.5 years)
Baseline (T1)			
N	42	54	41
Age at baseline	81.00 ± 3.11	82.31 ± 3.55	82.01 ± 3.51
Gender (male/female)	10/32	17/37	13/28
IDO2 (cg112551498) (%)	72.53 ± 5.36	75.59 ± 3.71	76.23 ± 3.73
Follow-up (T2)			
N	42	41	
Age at baseline	81.00 ± 3.11	82.01 ± 3.51	
Gender (male/female)	10/32	13/28	
IDO2 (cg112551498) (%)	73.07 ± 4.91	75.25 ± 4.96	

Overview of patient characteristics in the AgeCoDe cohort used in this study. Data are presented in n or mean ± standard deviation (SD). Patients were age- and gender-matched. Blood was collected at baseline and follow-up (average 4.5 years). All patients were controls at baseline and the patients were followed-up over time. Patients who converted to Alzheimer's disease (AD) after 4.5 years (n = 13) were included as converters at baseline. Converters within 4.5 years (n = 41) represent the baseline characteristics of patients who converted within 4.5 years.

The BBACL cohort

The Biobank Alzheimer Center Limburg (BBACL) study is an ongoing, prospective clinical cohort of patients referred to the Memory Clinic of the Maastricht University Medical Center + (MUMC+), the Netherlands, for the evaluation of their cognitive complaints. These patients were diagnosed either with subjective cognitive decline (SCD), mild cognitive impairment (MCI), or dementia. Inclusion criteria were a clinical dementia rating scale (CDR; Morris 1993) score from 0 to 1, and a Mini-Mental State Examination (MMSE; Folstein 1975) score ≥ 20, thereby including patients across the clinical spectrum of SCD, MCI and mild dementia. Exclusion criteria at baseline were non-degenerative neurological diseases, such as Normal Pressure Hydrocephalus, Huntington's disease, brain tumor, epilepsy, encephalitis, recent transient ischemic attack (TIA) or cerebrovascular accident (CVA) (< 2 years), or TIA/CVA with concurrent (within three months) cognitive decline; a history of psychiatric disorders, current major depressive disorder (within 12 months) (DSM IV), or alcohol abuse. All patients underwent a physical, cognitive and neuropsychiatric evaluation and biomaterials were collected. SCD and MCI patients were followed-up over time and a proportion developed dementia. For this study,

individuals were selected based on the availability of baseline DNA samples. As such, DNA (hydroxy)methylation levels were measured using pyrosequencing of DNA isolated from whole blood samples from 262 individuals: SCD (n = 39), MCI (n = 168), and dementia (n = 55). Amongst the 168 MCI patients, 80 patients developed dementia (MCI-D) while 88 individuals remained MCI (MCI-MCI) within 76 months after baseline (Table 3). The BBACL study protocol was approved by local ethics committees (METC 15-4-100) at the MUMC+ (Maastricht, the Netherlands). All participants gave their written informed consent.

Table 3. BBACL cohort patient demographics

	SCD	**MCI**	**Dementia**	**MCI-D**	**MCI-MCI**
Demographics variables					
N	39	168	55	80	88
Age at baseline	59.62 ± 9.50	72.65 ± 7.69	74.44 ± 7.89	74.94 ± 6.24	70.57 ± 8.30
Gender (male/female)	33/6	89/79	24/31	45/35	44/44
Education (low/middle/high)	13/16/10	64/66/38	27/20/8	26/31/23	38/35/15
Lifestyle variables					
BMI (kg/m^2)	25.95 ± 4.00 (n = 22)	26.66 ± 4.17 (n = 121)	24.67 ± 3.97 (n = 42)	26.28 ± 3.67 (n = 58)	27.01 ± 4.57 (n = 63)
Smoking status (never/< 6 months/ > 6 months/current)	17/0/13/8	74/1/61/22	30/0/17/4	38/0/32/7	36/1/29/15
Alcohol consumption (yes/no)	30/6	116/42	38/12	60/17	56/25
Cognitive test					
MMSE	28.31 ± 1.58	26.65 ± 2.30	24.07 ± 2.28	26.03 ± 2.42	27.23 ± 2.04
normMMSE	80.52 ± 14.04	68.41 ± 15.63	53.26 ± 11.03	64.38 ± 15.30	72.07 ± 15.10
Pyrosequencing (%)					
IDO2 (cg11251498)	64.96 ± 4.55	63.31 ± 5.12	63.91 ± 6.06	63.39 ± 5.93	63.23 ± 4.28

Overview of patient characteristics in the BBACL cohort used in this study. Data are presented in n or mean ± standard deviation (SD). Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; MCI-D, MCI to dementia converters; MCI-MCI, MCI to MCI non-converters; MMSE, mini mental state examination; normMMSE, normalized mini mental state examination.

Identification of tryptophan catabolic pathway-associated genes

The TRP catabolic pathway can be divided into two separate pathways, i.e., the TRP (metabolic) pathway and the NAD pathway. Our list of genes of the TRP- and NAD- pathways was generated through combining three databases: the Kyoto Encyclopedia of Genes and Genomes (KEGG), WikiPathways, and Reactome. In the KEGG database, the *Homo sapiens* "tryptophan metabolism pathway" (pathway: hsa00380) [17] and the "nicotinate and nicotinamide metabolism pathway" (pathway: hsa00760) [18] were selected. In WikiPathways, the "Tryptophan metabolism pathway (*Homo sapiens*)" from Lynn M. Ferrante et al. [19], and the "NAD biosynthetic pathways (*Homo sapiens*)" from Kristina Hanspers et al. [20], were selected. Finally, in Reactome, "Tryptophan catabolic" (Identifier: R-HSA-71240) [21], "Serotonin and melatonin biosynthesis" (Identifier: R-HSA-209931) [22], and "Nicotinate metabolism" (Identifier: R-HSA-196807) [23] were selected. This systematic search was validated and complemented by screening the available scientific literature on this matter. In the end, this procedure resulted in a gene set consisting of 59 TRP- and 57 NAD-associated genes (Supplementary Tables S1 and S2).

Transcriptomic- and DNA (hydroxy)methylomic- profiling

The brain tissue samples used for RNA extraction were identical to those used in the DNA methylation study. Both transcriptomic- and DNA (hydroxy) methylomic- profile data were obtained from a previous study by our group, as published by Lardinoije et al. [13]. The gene expression microarray data used in the present study were generated using Illumina HumanHT-12 v4 BeadChip arrays as described in more detail previously [24]. For differential DNA methylation analysis in the BSHRI-BBDP samples, bisulfite (BS) and oxidative BS (oxBS) conversion of genomic DNA (gDNA) derived from MTG tissue was performed using the TrueMethyl™ 24 Kit version 2.0 (Cambridge Epigenetix, Cambridge, UK). A total of 8µL from each BS/oxBS-treated DNA sample was amplified and hybridized on HM 450K arrays (Illumina, Inc., San Diego, CA, USA) for quantifying methylation status of different human '5-Cytosine-phosphate-Guanine-3' (CpG) sites. All procedures were performed according to the manufacturer's protocol. For the AgeCoDe samples, gDNA was isolated from whole blood and concentration was measured using the NanoDrop ND1000 spectrophotometer (Thermo Fisher scientific). Then, 200ng BS-treated DNA

was analyzed using HM 450K arrays according to the manufacturer's protocol. Imaging of the arrays were done using the Illumina iScan.

Gene-gene interaction network and network perturbation analysis

A detailed description of the protocol for the network analysis was published elsewhere [25]. Briefly, functional interactions between genes in the TRP- and NAD-associated pathways were identified through MetaCore (Clarivate Analytics). MetaCore is a collection of manually curated and experimentally validated direct gene-gene interactions. The analysis was restricted to "Functional interactions", "Binding interactions", and "Low trust interactions" to ensure a highly confident interaction network map and functional interaction among the selected genes. Additionally, a network perturbation analysis is a tool to identify common and phenotype-specific positive and negative elementary circuits. Studies have reported the significant role of these circuits in both maintaining network stability and having stable steady state [26, 27]. Network perturbation analysis was done through Java implementations as proposed by Zickenrott et al. [28], in which a network simulation analysis combined single genes and sets of up to four genes, in order to identify and rank genes and gene sets based on their ability to regulate the expression of downstream genes.

Pyrosequencing

DNA samples of 310 BBACL individuals were isolated from the buffy coat using the QIAsymphony DSP DNA Midi kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. Isolated DNA was aliquoted and stored at -80°C for later use. The DNA concentration was measured using the Qubit dsDNA BR Assay Kit (Q32853, Thermo Fisher Scientific, Waltham, MA, USA) and Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) analytical instrument, following the manufacturer's instructions. In the end, 262 DNA samples met the minimal requirement of 200ng to be used as input for BS treatment. For this purpose, DNA was BS-treated using the EZ-96 DNA Methylation-Gold kit (D5008, ZYMO RESEARCH, Irvine, CA, USA), following the manufacturer's instructions with one minor adjustment in which the BS-converted samples were eluted in 20µL of elution buffer. Then, 1µL of the resulting sample was used for polymerase chain reaction (PCR) amplification followed by BS pyrosequencing. All BS conversion assays included at least two negative controls.

IDO2 (cg11251498) PCR and pyrosequencing primers (reverse direction) were designed using the PyroMark Assay Design version 2.0.1.15 (QIAGEN, Hilden, Germany) (Supplementary Table S3) using Ensembl Genome Browser GRCh37 assemble database. All PCR reactions used FastStart™ Taq DNA Polymerase, dNTPack (Roche, Basel, Switzerland) following manufacturer's instructions. Briefly, each PCR reaction contained 2.5µL of PCR buffer (10x) with 20mM $MgCl_2$, 0.5µL of dNTPs, 1 µL of forward and reverse primers (5µM), 0.2µL of FastStart™ Taq DNA polymerase (5U/µL), and 1µL of BS treated gDNA in a total volume of 25µL. All PCR reactions had two positive and negative (BS negative and water each) controls. In addition, the PCR reaction was performed as follows: denaturation at 95°C for 5 minutes; 58 cycles of 95°C for 30 seconds, 57°C for 30 seconds, 72°C for 30 seconds; followed by final extension for 1 minute at 72°C. Each PCR product (150bp) was then size-fractionated on a 2% agarose gel in Tris-Acetate-EDTA (TAE) buffer.

Pyrosequencing was performed and quantified using the PyroMark Q48 Autoprep system and Pyro Q48 Autroprep 2.4.2 software (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The sensitivity of the assay was assessed using methylated and unmethylated DNA standards from the EpiTect PCR Control DNA set (QIAGEN, Hilden, Germany). All the pyrosequencing runs included multiple negative controls and only samples that passed the quality control were included.

Statistical analysis

A detailed description of the statistical analysis performed for the BSHRI-BBDP and AgeCoDe can be found elsewhere [13]. All computational and statistical analyses for these two cohorts were performed using R (version 3.3.2) and RStudio (version 1.0.136). Briefly, preprocessing and analysis of the raw data sets were conducted in R (version 3.4.4) [29]. Raw expression data was log-transformed and quantile-quantile normalized. For computing the cell composition, the Neun_pos cell percentage was calculated from the methylation data. The same regression model used for assessing methylation was applied to the expression data where the effects of age, gender and cell type composition were regressed out using limma with p-value less than 0.05 was considered as statistically significantly differentially expressed. Additionally, a false discovery rate (FDR) correction for multiple testing was applied for both transcriptomic- and DNA (hydroxy)methylomic- profiling in which a q-value less than 0.05 was

considered statistically significant. It is worth nothing that, in the DNA (hydroxy)methylomic profile analyses, the TRP- associated genes *ASMT* and *KYAT1* were not included in this analysis, as data on DNA methylation and hydroxymethylation were not available for these genes. In addition, *ALDH9A1* and *SLC36A4* were excluded from the 5hmC analysis due to missing data. Concerning the NAD pathway, the *NADK2, NMNAT1,* and *NMRK* genes were not included in the analysis, as data were not available for these genes. In addition, *PARP2* was excluded from the 5hmC analysis due to missing data.

All remaining computational and statistical analyses for BBACL and AgeCoDe (only for pyrosequencing and age association analyses) were performed using SPSS Statistics version 27. In BBACL, crude mini mental state exam (MMSE) score (range 0 – 30) was normalized (normMMSE; range 0 – 100) using the NormPsy (version 1.0.7) function. Various models were performed to adjust for the effect of covariates using general linear model univariate analysis, in which *model 1* adjusted for age, gender, education, and *model 2* (only for BBACL) additionally adjusted for lifestyle (smoking status, drinking status, and body mass index (BMI)). *Model 1* was considered the main model. All association studies reported the beta value (β), standard error (S.E.), p-value, and 95% confidential interval (95% CI). P-values less than 0.05 were considered as statistically significant.

Results

Altered gene expression profiles in the brain of patients with AD

Out of the 59 identified TRP pathway-associated genes, 55 genes (29 up-regulated and 26 down-regulated) were included in the microarray analysis. After FDR correction, 11 of these genes showed significant differential mRNA expression in patients with AD versus controls (Figure 1; Supplementary Table S4). In addition, microarray data were available for 52 genes of the 57 NAD pathway-associated genes. After FDR correction, mRNA levels of 20 genes showed statistically significant differences with 6 genes showing lower expressions and 14 genes showing a higher expressions (Figure 2; Supplementary Table S5).

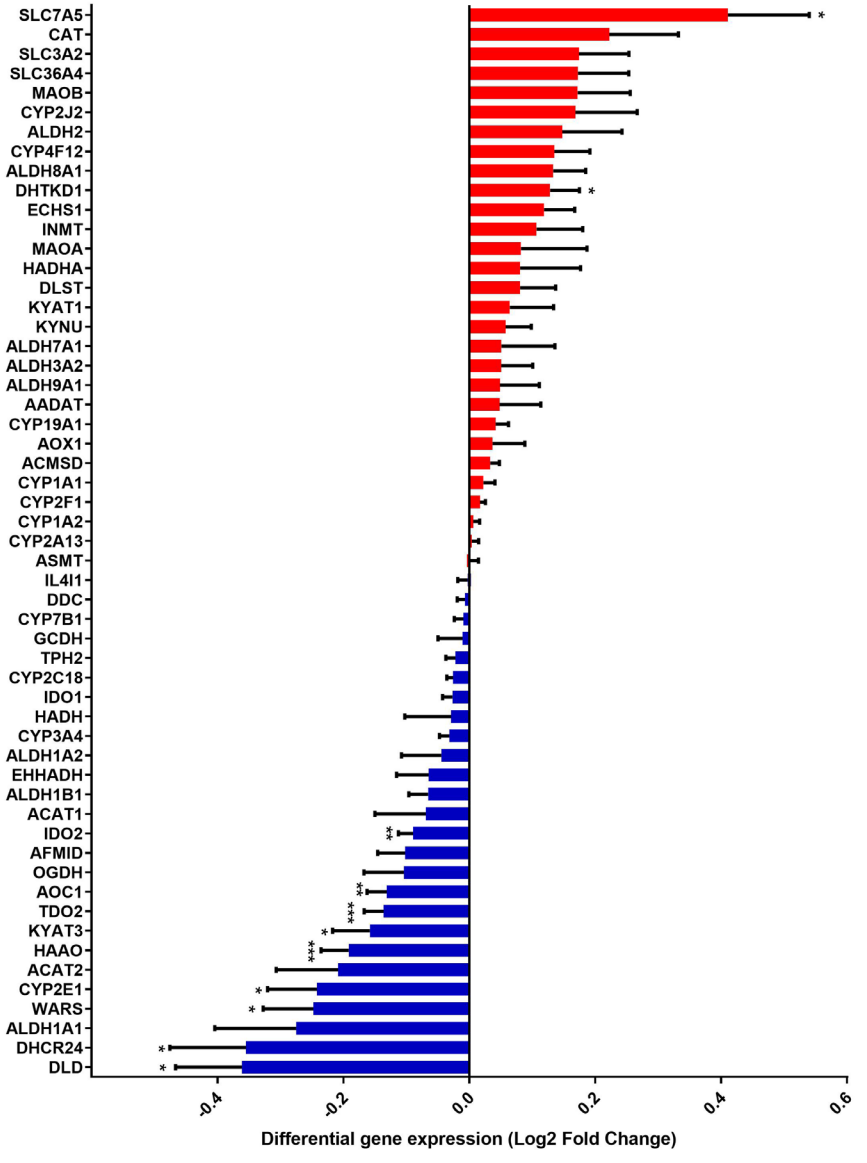


Figure 1. TRP pathway associated genes show significant differential gene expression. Shown are all the genes in the TRP pathway with up (red) or down (blue) regulation in the middle temporal gyrus of AD patients compared to age-matched controls. Differential expression is presented as log2 fold change. Differential regulation was assessed using false discovery rate (FDR) test and $q < 0.05$ was considered significant. * $q < 0.05$, ** $q < 0.01$ and *** $q < 0.001$. Error bars represent standard error of mean (SEM).

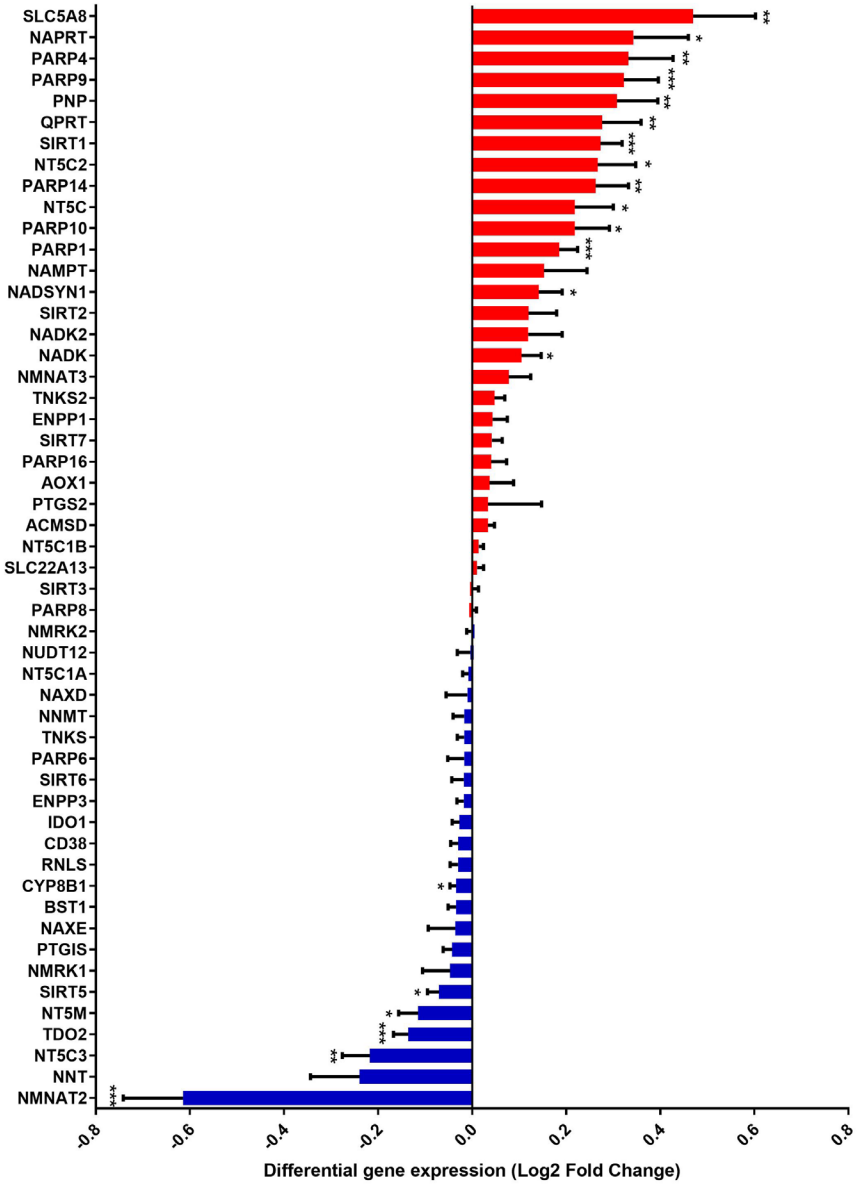


Figure 2. NAD pathway associated genes show significant differential gene expression. Shown are all the genes in the NAD metabolism pathway with up (red) or down (blue) regulation in the middle temporal gyrus of AD patients compared to age-matched controls. Differential expression is presented as log2 fold change. Differential regulation was assessed using false discovery rate (FDR) test and $q < 0.05$ was considered significant. * $q < 0.05$, ** $q < 0.01$ and *** $q < 0.001$. Error bars represent standard error of mean (SEM).

Transcriptomic enrichment analysis

In order to examine whether expression changes in TRP- and NAD-related genes are overrepresented in AD, an enrichment analysis was conducted based on the transcriptomic profiling within the MTG. It identified 31726 genes of which 11459 genes were identified to be significantly (limma p-value < 0.05) differentially expressed between AD patients and age-matched control individuals [13]. Once adjusting for multiple testing (limma FDR q-value < 0.05), 7776 genes were significantly differentially expressed at the mRNA level, resulting in an overall enrichment score of 24.5% as a baseline. The TRP pathway as a whole only displayed a 20% enrichment score (1-sided Fisher's exact test, p = 0.82, odds ratio [OR] = 0.77), indicating this gene set overall did not display a significant enrichment in view of differentially expressed genes. In contrast, the NAD pathway showed a 38.5% enrichment score (1-sided Fisher's exact test, p = 0.018, OR = 1.93), indicating a significant enrichment in differentially expressed genes within the NAD-associated gene set (Data not shown).

Alterations of DNA (hydroxy)methylation in the brain of patients with AD

As an exploratory approach, we investigated whether the aforementioned AD-specific TRP- and NAD-associated mRNA profiles were associated with DNA methylation differences at the level of 5-methylcytosine (5mC), 5-hydroxy methylcytosine (5hmC), or unmodified cytosoine (5uC). Within the TRP pathway, differences in 5mC, 5hmC, and 5uC levels showed nominal significance (p-value < 0.05) for 18/827 probes, 17/501 probes, and 30/827 probes, respectively (Figure 3; Supplementary Tables S6-S8). These differentially (hydroxy)methylated probes were linked to 12 significantly differentially expressed genes. Interestingly, six of these genes showed significant differences in different types of methylation modifications for the same CpG site (Supplementary Table S9). Moreover, two differentially hydroxymethylated CpG sites within the *SLC7A5* gene, i.e., cg10169763 and cg09409405, displayed a negative correlation between 5hmC levels and mRNA expressions (Figure 4A, B). For 5uC, both cg19571004 (*CYP2E1*) and cg01812894 (*ALDH1A1*) showed a positive correlation with mRNA expressions (Figure 4C, D).

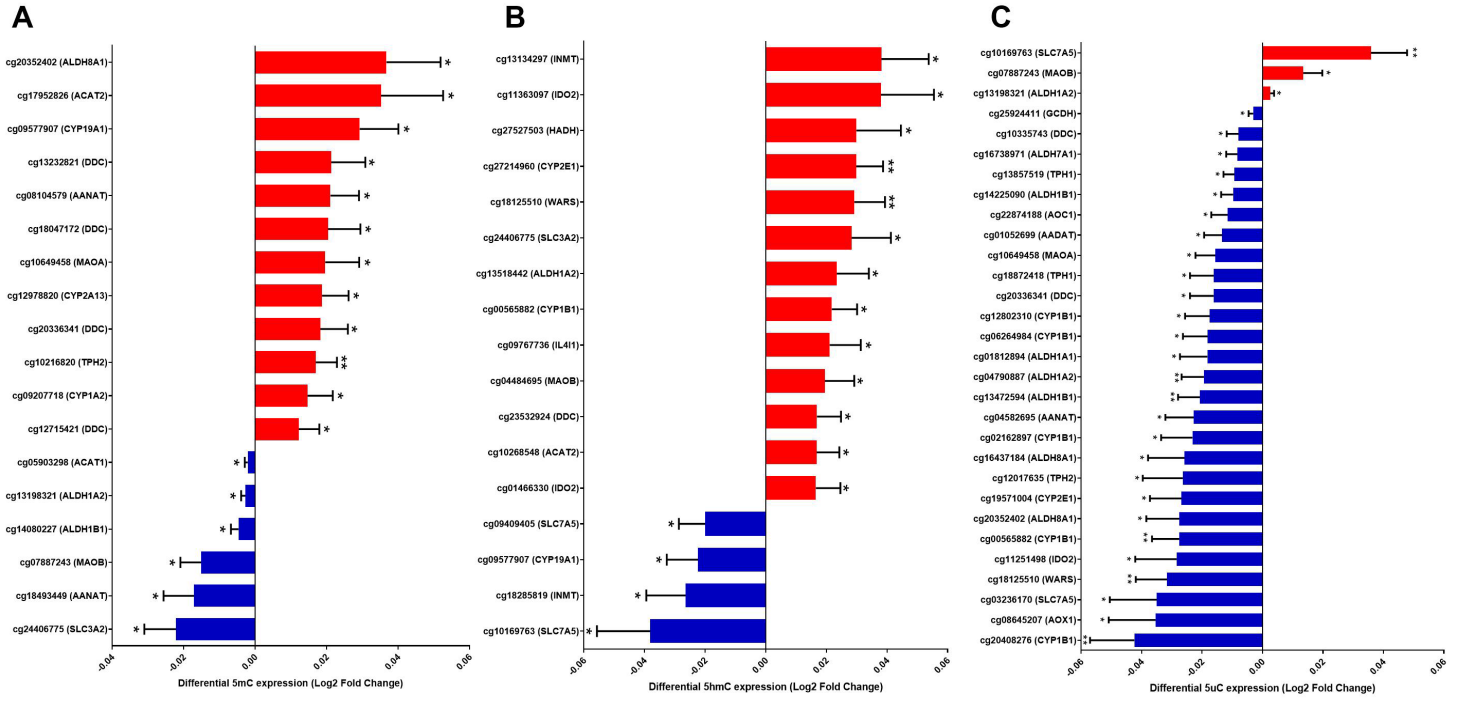


Figure 3. TRP pathway associated genes show significant differential (hydroxy)methylation levels. Shown are significant CpG sites with its corresponding gene name in the tryptophan metabolism pathway with up (red) or down (blue) regulation in the middle temporal gyrus of AD patients compared to age-matched controls. (**A**) Differential methylation (5mC) level. (**B**) Differential hydroxymethylation (5hmC) level. (**C**) Differential unmodified (5uC) level. Differential level is presented as log2 fold change. Differential regulation was assessed using limma differential expression analysis and $p < 0.05$ was considered significant. * $p < 0.05$ and ** $p < 0.01$. Error bars represent standard error of mean (SEM).

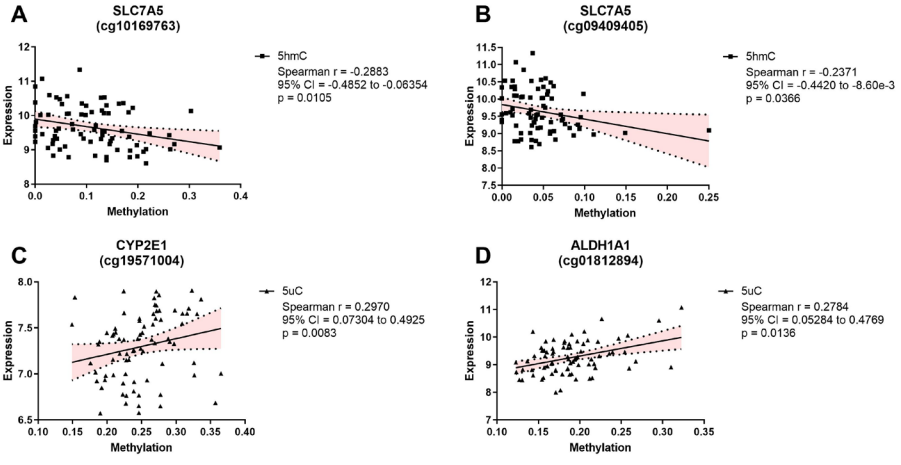


Figure 4. Spearman's correlation analysis of TRP pathway. Spearman's correlation analysis between methylation (■5hmC, ▲5uC) levels and mRNA expressions. (A-D) Gene name (probe ID): (A) *SLC7A5* (cg10168763), (B) *SLC7A5* (cg09409405), (C) *CYP2E1* (cg19571004), (D) *ALDH1A1* (cg01812894). Spearman's correlation analysis are presented with spearman r-value, 95% confidence interval, and p-value.

Within the NAD pathway, 5mC, 5hmC, and 5uC showed nominally significant differences for 21/1009 probes, 18/632 probes, and 36/1009 probes respectively (Figure 5; Supplementary Tables S10-S12). These differentially (hydroxy) methylated probes were linked to 18 significant differentially expressed genes. Five of these genes showed significant differences at different levels of methylation for the same CpG site (Supplementary Table S13). Eight CpG sites displayed significant correlations between DNA (hydroxy)methylation and mRNA expression (Figure 6-8). Concerning 5mC, three CpG sites (cg21580588 [*NADK*]; cg09185911 [*NADK*]; cg14750551 [*PARP14)*]) showed a positive correlation (Figure 6A, B and Figure 7A), while two other CpG sites (cg10582690 [*SIRT1*] and cg11229284 [*PARP14*]) showed a negative correlation (Figure 6C, D). At the 5hmC level, again, cg14750551 (*PARP14*) showed a significant negative correlation (Figure 7A), while cg24937136 (*PARP1*) was positively correlated (Figure 7B) to its mRNA expression profile. Finally, for 5uC, cg16373880 (*NMNAT2*) showed a positive correlation (Figure 8A), whereas two CpG sites (cg05215649 [*NADSYN1*] and cg15824543 [*NADK*]) showed a negative correlation (Figure 8B, C) with the corresponding mRNA levels.

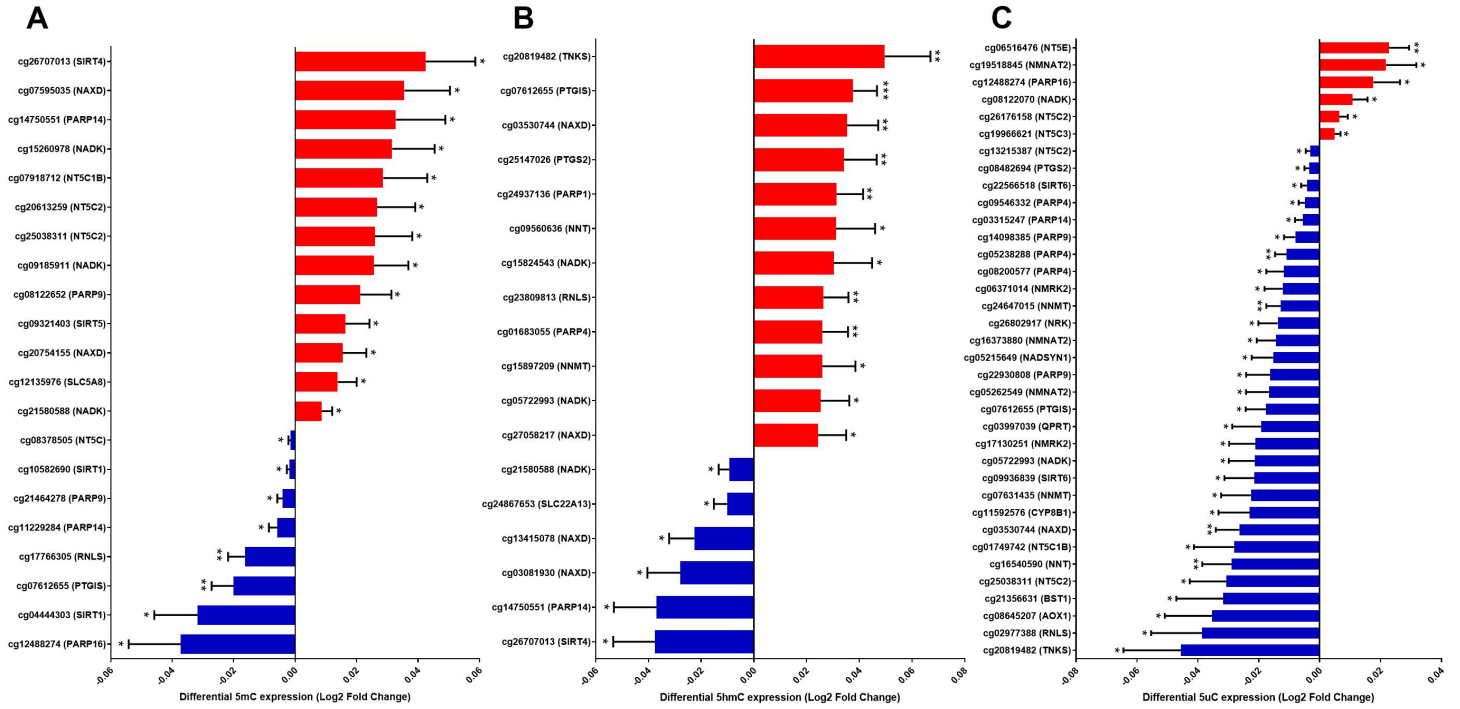


Figure 5. NAD pathway associated genes show significant differential (hydroxy)methylation levels. Shown are significant CpG sites with its corresponding gene name in the nicotinate and nicotinamide metabolism pathway with up (red) or down (blue) regulation in the middle temporal gyrus of AD patients compared to age-matched controls. (**A**) Differential methylation (5mC) level. (**B**) Differential hydroxymethylation (5hmC) level. (**C**) Differential unmodified (5uC) level. Differential level is presented as log2 fold change. Differential regulation was assessed using limma differential expression analysis and $p < 0.05$ was considered significant. * $p < 0.05$ and ** $p < 0.01$. Error bars represent standard error of mean (SEM).

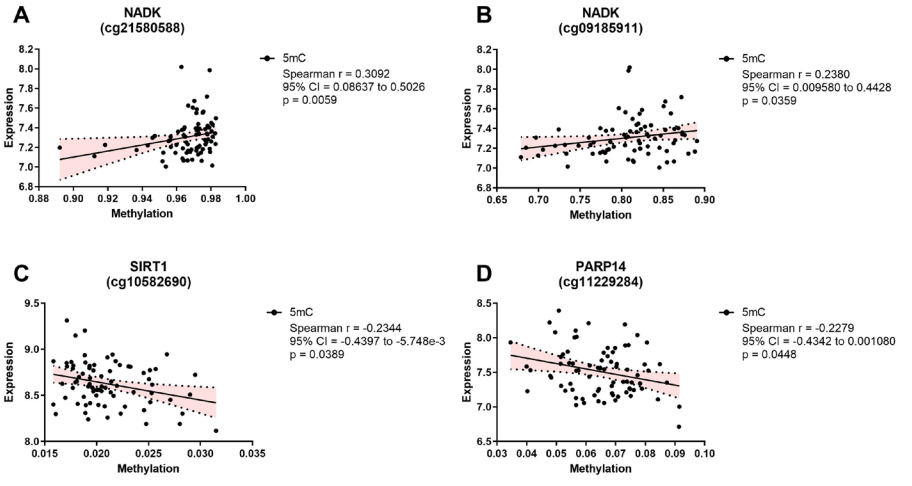


Figure 6. Spearman's correlation analysis of 5mC in NAD pathway. Spearman's correlation analysis between methylation (●5mC) levels and mRNA expressions. Gene name (probe ID): (A) *NADK* (cg21580588), (B) *NADK* (cg09185911), (C) *SIRT1* (cg10582690), and (D) *PARP14* (cg11229284). Spearman's correlation analysis are presented with spearman r-value, 95% confidence interval, and p-value.

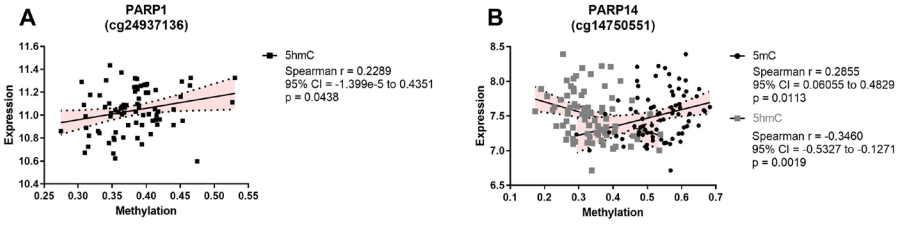


Figure 7. Spearman's correlation analysis of *PARP14* in NAD pathway. Spearman's correlation analysis between methylation (●5mC and ■5hmC) levels and mRNA expressions. Gene name (probe ID): (A) *PARP14* (cg14750551), (B) *PARP1* (cg24937136). Spearman's correlation analysis are presented with spearman r-value, 95% confidence interval, and p-value.

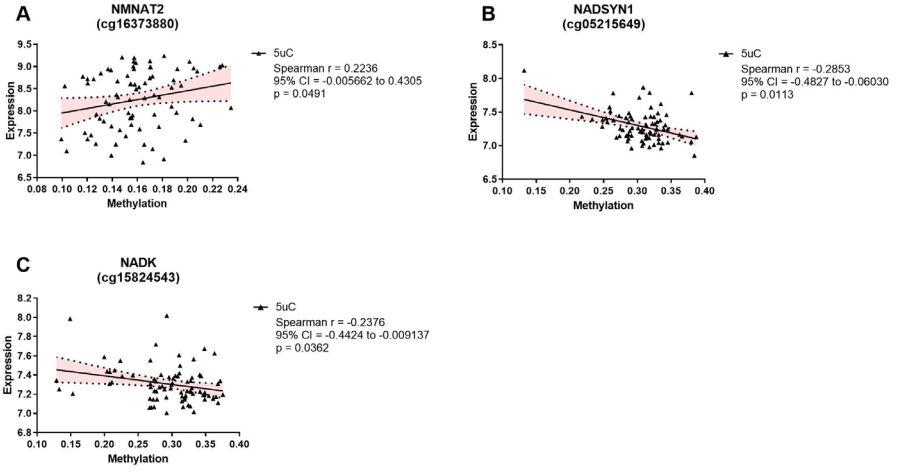


Figure 8. Spearman's correlation analysis of 5uC in NAD pathway. Spearman's correlation analysis between methylation (▲5uC) levels and mRNA expressions. Gene name (probe ID): (A) *NMNAT2* (cg16373880), (B) *NADSYN1* (cg05215649), (C) *NADK* (cg15824543). Spearman's correlation analysis are presented with spearman r-value, 95% confidence interval and, p-value.

Gene regulatory network and network perturbation analysis

GRN was generated through MetaCore (Clarivate Analytics), which builds gene networks by implementing direct functional interactions between genes acquired from experiments-based literature reports. In combination with identifying a GRN, we identified network perturbation candidates that have the potential to induce a positive phenotypic transition, i.e., moving from a diseased to a healthy GRN (Table 4). Concerning the TRP pathway, the reconstructed control network comprised 22 nodes and 30 interactions (Figure 9A), while the AD network comprised of 22 nodes and 25 interactions (Figure 9B). In the associated perturbation analysis, 9 genes were identified whose alteration holds the potential to revert the gene expression program from a diseased towards a healthy state. Two of these genes (*IDO2* and *CYP2E1*) showed significant changes in differential mRNA expression after FDR correction when comparing AD cases with control subjects. The highest perturbation score was obtained for a 3-gene perturbation combination, involving *CAT, IDO2,* and *CYP2E1* (Table 4).

In a similar manner, GRNs for the NAD pathway were constructed. The control NAD network comprised 13 nodes and 21 interactions (Figure 10A), while the AD network comprised 14 nodes and 24 interactions (Figure 10B). Furthermore,

in the perturbation analysis, 8 genes were identified, of which 2 genes (*SIRT1* and *PARP1*) showed significant differential mRNA expression after FDR correction. The highest perturbation score was obtained for a 2-gene perturbation combination, involving *SIRT1* and *PARP1* (Table 4).

Table 4. Perturbation scores of TRP- and NAD- pathway associated genes

TRP Pathway Genes		NAD Pathway Genes	
Perturbation Score	**Combination**	**Perturbation Score**	**Combination**
10	[CAT, IDO2]	8	[SIRT7, SIRT3]
10	[CAT, IDO1]	8	[IDO1, SIRT3]
9	[MAOA, CAT]	7	[SIRT7, SIRT1]
9	[IDO2, CYP3A4]	7	[SIRT7, PTGS2]
9	[CYP3A4, IDO1]	7	[SIRT1, IDO1]
9	[CYP2E1, IDO2]	7	[NAMPT, SIRT7]
9	[CYP2E1, IDO1]	6	[SIRT7, IDO1]
9	[CYP1A2, IDO2]	6	[PARP1, SIRT7]
9	[CYP1A2, IDO1]	6	[PARP1, SIRT3]
9	[CYP1A1, IDO2]	6	[PARP1, SIRT1]
9	[CYP1A1, IDO1]	6	[PARP1]
9	[CAT]	6	[NAMPT, IDO1]
8	[MAOB, CAT]	5	[SIRT3]
8	[MAOA, CYP3A4]	5	[PTGIS, SIRT3]
8	[MAOA, CYP2E1]	5	[PTGIS, PARP1]
8	[MAOA, CYP1A2]	5	[PARP1, NAMPT]
8	[MAOA, CYP1A1]	5	[PARP1, IDO1]
8	[CYP3A4]	4	[SIRT1, SIRT3]
8	[CYP2E1]	4	[SIRT1]
8	[CYP1A2]	4	[PTGS2, SIRT3]
8	[CYP1A1]	4	[PTGIS, SIRT1]
8	[CAT, CYP3A4]	4	[PTGIS, NAMPT]
8	[CAT, CYP2E1]	4	[PARP1, PTGS2]
8	[CAT, CYP1A2]	4	[NAMPT, SIRT3]
8	[CAT, CYP1A1]	4	[NAMPT]
7	[MAOB, CYP3A4]	3	[PTGS2, SIRT1]
7	[MAOB, CYP2E1]	3	[NAMPT, SIRT1]
7	[MAOB, CYP1A2]	3	[NAMPT, PTGS2]
7	[MAOB, CYP1A1]	2	[PTGIS, IDO1]
7	[CYP2E1, CYP3A4]	2	[PTGIS]
7	[CYP1A2, CYP3A4]	2	[IDO1]
7	[CYP1A2, CYP2E1]	1	[PTGS2, IDO1]
7	[CYP1A2, CYP1A1]	1	[PTGS2]
7	[CYP1A1, CYP3A4]	1	[PTGIS, PTGS2]
7	[CYP1A1, CYP2E1]		
6	[MAOA, IDO2]		
6	[MAOA, IDO1]		
5	[MAOB, IDO2]		
5	[MAOB, IDO1]		
5	[MAOA]		
5	[IDO2]		
5	[IDO1]		

Table 4. (Continue)

TRP Pathway Genes		NAD Pathway Genes	
Perturbation Score	**Combination**	**Perturbation Score**	**Combination**
4	[MAOB, MAOA]		
4	[MAOB]		
4	[IDO2, IDO1]		

Overview of network perturbation scores for TRP- and NAD-pathway associated genes. The list of the gene's full name can be found in the Supplementary Tables S1 and S2.

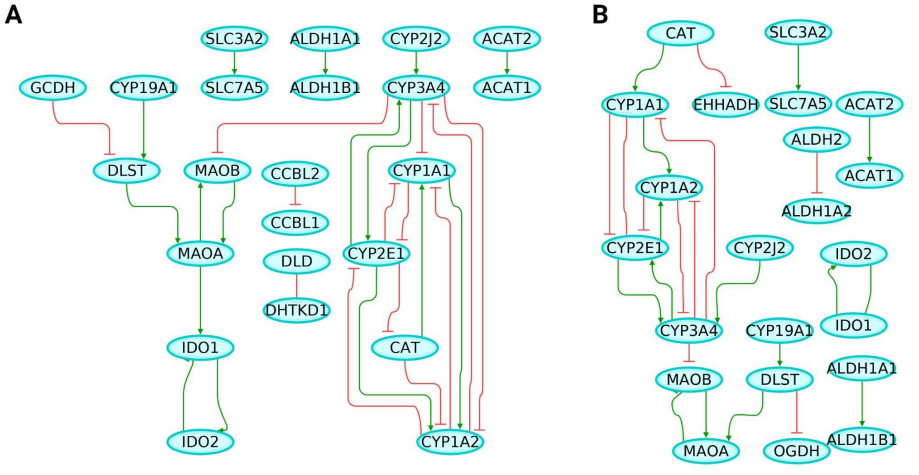


Figure 9. Gene regulatory network (GRN) of TRP pathway. (A) GRN representing the control phenotype and containing 22 nodes and 30 interactions; (B) GRN representing Alzheimer's disease (AD) phenotype and containing 22 nodes and 25 interactions. Green line indicates gene activation, while red line indicates gene inhibition.

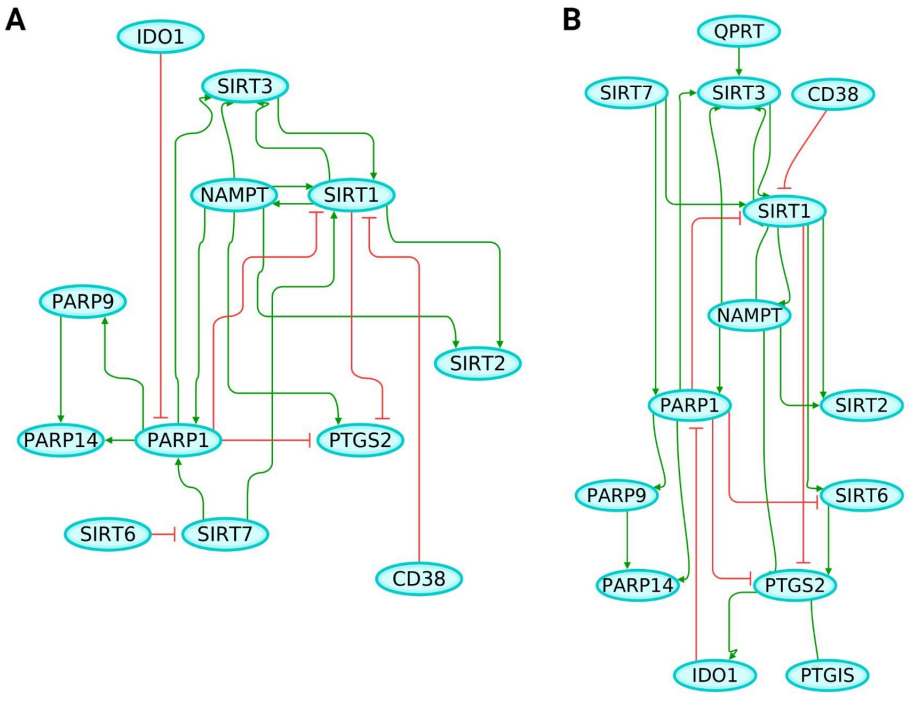


Figure 10. Gene regulatory network (GRN) of NAD pathway. (A) GRN representing the control phenotype and containing 13 nodes and 21 interactions; (B) GRN representing Alzheimer's disease (AD) phenotype and containing 14 nodes and 24 interactions. Green line indicates gene activation while red line indicates gene inhibition.

IDO2 methylation in AgeCoDe

Based on the MTG and *in silico*, such as GRN and gene perturbation, analyses, *IDO2*, *SLC7A5*, and *PARP14* were selected as potential candidate genes for further investigation. Therefore, an independent longitudinal cohort, i.e., AgeCoDe, with DNA derived from blood available, was used in an attempt to validate the MTG methylation findings. In the AgeCoDe cohort, across these genes, 8 out of 106 CpG sites showed nominal significant differential DNA methylation (data not shown), of which only cg11251498 (*IDO2*) was shown nominal significance for both MTG 5uC and AgeCoDe while the rest were not found in the MTG analysis. Upon further analysis, cg11251498 (*IDO2*) level was significantly higher in AD converters than controls at baseline ($p = 0.001$) and displayed a tendency towards higher methylation in AD patients after 4.5 years follow-up ($p = 0.051$), while controlling for age and gender for both time points (Table 2).

IDO2 pyrosequencing in BBACL

In order to validate cg11251498 (*IDO2*), we pyrosequenced this locus in DNA from blood samples of subjects from an independent longitudinal cohort, i.e., BBACL. At baseline, cg11251498 (*IDO2*) did not show a significant difference in DNA methylation when comparing SCD, MCI, and dementia patients (*model 1*, p = 0.58; *model 2*, p = 0.55). Similarly, no difference in *IDO2* DNA methylation was observed when comparing future converters and non-converters (MCI-D, MCI-MCI) at baseline (*model 1*, p = 0.38; *model 2*, p = 0.32) (Table 3). Despite no difference in *IDO2* DNA methylation, a strong negative association between *IDO2* DNA methylation and age was seen both when comparing SCD, MCI, and dementia patients (m*odel 1*: β = -0.11, S.E. = 0.04, p = 0.006, 95% CI = -0.19 to -0.031) and converters and non-converters (m*odel 1*: β = -0.14, S.E. = 0.053, p = 0.009, 95% CI = -0.24 to -0.035). Moreover, an age association was still significant for both comparisons in *model 2* (Table 5). Lastly, cg11251498 (*IDO2*) showed no significant association with cognition when comparing SCD, MCI, and dementia patients at baseline (*model 1*, p = 0.60; *model 2*, p = 0.15) and when comparing future converters and non-converters (*model 1*, p = 0.81; *model 2*, p = 0.30) (Table 5).

TABLE 5. *IDO2* methylation association with age and cognitive test in the BBACL cohort

		β	S.E.	t	p-value	95% CI	
Baseline	**Age**						
	Model 1	-0.110	0.040	-2.750	0.006	-0.189	-0.031
	Model 2	-0.126	0.049	-2.558	0.011	-0.223	-0.029
	normMMSE						
	Model 1	-0.012	0.023	-0.519	0.604	-0.058	0.034
	Model 2	-0.042	0.029	-1.464	0.145	-0.099	0.015
Converter	**Age**						
	Model 1	-0.139	0.053	-2.630	0.009	-0.244	-0.035
	Model 2	-0.163	0.067	-2.446	0.016	-0.296	-0.031
	normMMSE						
	Model 1	0.007	0.028	0.242	0.809	-0.048	0.061
	Model 2	-0.037	0.035	-1.042	0.300	-0.107	0.033

Association between *IDO2* percent methylation at position cg11251498 with age or normalized MMSE (normMMSE) for baseline diagnosis and in view of conversion within 76 months. *Model 1* adjusted for age, gender, education and diagnosis/converters, while *model 2* adjusted for *model 1* + lifestyle factors (tobacco use, alcohol use, and body mass index (BMI)). Abbreviations: normMMSE, normalized mini mental state exam; β, beta value; S.E., standard error; t, t statistic; 95% CI, 95% confidence interval.

Discussion

The aim of the present study was to investigate the TRP catabolic pathway and its potential dysregulation in the pathophysiology of AD. Making use of a selection of 59 TRP- and 57 NAD-associated genes, we conducted a hypothesis-driven pathway enrichment analysis, assessed gene-specific mRNA expression and DNA (hydroxy)methylation profiles, and performed a GRN and associated perturbation analysis. We then validated the findings by zooming in on blood *IDO2* methylation in two independent longitudinal cohorts, i.e., AgeCoDe and BBACL.

Transcriptional and epigenetic differences of TRP- and NAD-associated genes in the brain of patients with AD

For the TRP metabolic pathway, 11 genes showed significant differences in mRNA expression in the brain of patients with AD as compared to controls. These genes were associated with the KP (*TDO2, HAAO, IDO2, KYAT3*), the tryptamine pathway (*AOC1*), the acetyl-CoA pathway (*DLD, DHTKD1*), L-trytophanyl-tRNA synthesis (*WARS*), amino acid transport (*SLC7A5*), hydroxymelatonin (*CYP2E1*), and oxaloacetate (*DHCR24*). Of these, *AOC1*, *IDO2*, *SLC7A5,* and *WARS* showed nominal (but not after correction for multiple testing) significant differences in DNA (hydroxy)methylation levels.

TRP is an essential amino acid and transported from the periphery to the central nervous system (CNS) through amino acid transporter. Based on our results, the transporter gene solute carrier family 7 member 5 (*SLC7A5)*, also known as large amino acid transporter 1 (LAT1), showed a significant increase in mRNA expression, while exhibiting nominally significant differences at the level of 5hmC and 5uC in AD. The KP is active under inflammatory conditions, as such unbound free TRP competes with other large neutral amino acids (LNAAs) for the same LAT1 transporter. LAT1 is located in the capillaries of the blood-brain-barrier (BBB) and allows these amino acids to pass through the BBB, while LAT1 mRNA expression is (co)regulated by DNA methylation [30]. Taken together, these findings sketch a picture suggesting that alterations in LAT1 expression and function are regulated by DNA (hydroxy)methylation and may lead to an abnormal degree of delivery of TRP and/or other large amino acids to the CNS in AD, and future studies are warranted to test this hypothesis.

Furthermore, *IDO2* displayed decreased mRNA expression and increased 5hmC (two CpG sites) and 5uC (one CpG site, cg11251498) levels in AD. More importantly, both our GRN and network perturbation analysis pointed towards *IDO2* as a potentially critical player in the pathophysiology of AD. In fact, *IDO2* was part of the gene set with the highest perturbation score. In other words, *IDO2* is suggested to play a crucial role in the maintenance and stability of the phenotype under consideration and normalizing *IDO2* expression in AD has the potential to move the disease (AD) GRN towards that of healthy controls. *IDO2* is structurally linked and closely located to *IDO1* on chromosome 8, but its exact function is still unclear. Studies have reported that IDO1 mediates T cell suppressive effects, while IDO2 is a pro-inflammatory mediator of B cell responses and critical for IDO1-mediated T cell regulation [31, 32]. Studies have shown that under normal physiological or non-inflammatory conditions IDO mRNA expression is either undetectable or very low, but upon immune stimulation, it is up-regulated significantly [33]. Neuroinflammation seems an important contributor to the pathophysiology of AD and has been shown to be an early event in AD [34]. Interestingly, Guillemin et al. (2005) confirmed increased IDO activity in AD hippocampus via immunohistochemistry [10]. The same group has previously shown that both inflammatory cytokines and Aβ can lead to increased cellular expression of IDO [35-37].

***IDO2* in relation to age and conversion to AD**

Based on the MTG data, cg11251498 (*IDO2*) was selected as a potential candidate site to be further validated in the blood of two independent longitudinal cohorts, i.e., AgeCoDe and BBACL. In AgeCoDe, at baseline, cg11251498 displayed a significant increase in methylation in those subsequently converting to AD when compared to non-convertors. In BBACL, no significant difference in cg11251498 DNA methylation between SCD, MCI, and dementia patients was observed. However, a significant negative age association with cg11251498 methylation was seen in BBACL, an effect that was neither observed in AgeCoDe nor in the MTG data (Supplementary Table S14). One plausible explanation for this apparent discrepancy on the effect of age may be the different age ranges (BBACL: 43 years-90 years; MTG: 70-95; AgeCoDe: 75-89) of individuals assessed in the various studies. Moreover, while the MTG and AgeCoDe studies both represent age-matched studies, for BBACL, SCD individuals were about 10 years younger than MCI and dementia patients.

NAD pathway

TRP is also a precursor to the NAD pathway. In our transcriptomic analysis, 20 NAD pathway-associated genes showed significant differences in mRNA expression. These genes were associated with one of the three pathways which synthesize NAD, i.e., i) the *de novo* biosynthesis via the KP (*QPRT*), ii) the salvage pathway (*SIRT1, SIRT5, PARP1, PARP4, PARP9, PARP10, PARP14*), and iii) the Preiss-Handler pathway (*SLC5A8, NAPRT, NADSYN1*). Some genes were associated with both the salvage- and Preiss-Handler- pathways (*NMNAT2, NT5C3, PNP, NT5C2, NT5M, NT5C*).

Although the KP is often seen as the main driver for NAD production, recent studies suggest that the salvage pathway may be equally important in this respect [12, 38, 39]. Based on our MTG data, genes involved in the salvage pathways, especially poly (ADP-ribose) polymerases (PARPs), showed significant differences in gene expression. Studies have reported PARPs to be involved in DNA repair, cell proliferation, and cell death [40-42]. Amongst others, our data pointed towards *PARP14* both at the level of transcription and methylation, with cg14750551 displaying differential levels of 5mC and 5hmC, which correlated with *PARP14* mRNA expressions positively and negatively, respectively. Moreover, our GRN analysis showed that the *PARP14* node was linked to *PARP1* and *SIRT1*. Whereas no studies to date investigated the function of *PARP14* in AD, lipid studies have reported that *PAPR14* regulates low-density lipoproteins (LDL) receptors and apolipoproteins in macrophages, thus involved in hypercholesterolaemia and hyperlipidemia [43]. Furthermore, studies have reported that cholesterol affects amyloid, tau, and gliosis in AD, and elevated LDL is a risk factor for developing AD [44, 45]. Clearly, these results warrant further research into the role of these genes in AD.

Strengths and limitations

The strength of this study was our step-wise approach that used post-mortem tissues to find AD-related differences in expression and methylation of TRP- and NAD-associated genes, and subsequent validation of (hydroxy)methylation in two independent clinical cohorts. Additionally, we have used *in silico* modeling, including a GRN and network perturbation analysis, and various models to adjust for covariates. One limitation of the present study is that the BBACL cohort displayed significant age difference between the groups (which we have taken into account by adjusting for age in our analysis). Moreover, while representing

a multi-omics analysis, we have to take into consideration that these data are solely based on transcriptomic- and (hydroxy)methylation profiles. Gene expression does not represent protein expression, let alone protein activity, and according to Vogel et al., the correlation between mRNA and protein expression can be as little as 40% [46]. Lastly, since we used bulk post-mortem MTG tissues, factors such as cell type composition, the vulnerability of specific cell types in AD and accompanying differences in cell-type proportions between groups, may influence the acquired data.

Conclusion

Our integrative analyses making use of post-mortem brain tissue and blood samples from two independent longitudinal cohorts has provided new information on the (dys)regulation and expression of genes involved in TRP- and NAD pathway associated genes in AD. These findings provided first, preliminary evidence suggesting that *IDO2*, and more specifically cg11251498, could be a candidate biomarker for AD. Although further research is needed to reach a solid conclusion, the transcriptional and epigenetic data analysis combined with *in silico* modeling represents a powerful tool to gain more insight in the involvement of key biological pathways in the development and course of AD.

References

1. Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement. 2013;9(1):63-75.e2.
2. WHO. Global status report on the public health response to dementia: World Health Organization; 2021 [Available from: https://www.who.int/publications/i/item/9789240033245.
3. Maddison DC, Giorgini F. The kynurenine pathway and neurodegenerative disease. Semin Cell Dev Biol. 2015;40:134-41.
4. Jones SP, Guillemin GJ, Brew BJ. The kynurenine pathway in stem cell biology. Int J Tryptophan Res. 2013;6:57-66.
5. Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain: when physiology meets pathology. Nat Rev Neurosci. 2012;13(7):465-77.
6. Badawy AA. Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects. Int J Tryptophan Res. 2017;10:1178646917691938.
7. Gulaj E, Pawlak K, Bien B, Pawlak D. Kynurenine and its metabolites in Alzheimer's disease patients. Adv Med Sci. 2010;55(2):204-11.
8. Hartai Z, Juhász A, Rimanóczy A, Janáky T, Donkó T, Dux L, et al. Decreased serum and red blood cell kynurenic acid levels in Alzheimer's disease. Neurochem Int. 2007;50(2):308-13.
9. Guillemin GJ, Brew BJ, Noonan CE, Knight TG, Smythe GA, Cullen KM. Mass spectrometric detection of quinolinic acid in microdissected Alzheimer's disease plaques. International Congress Series. 2007;1304:404-8.
10. Guillemin GJ, Brew BJ, Noonan CE, Takikawa O, Cullen KM. Indoleamine 2,3 dioxygenase and quinolinic acid immunoreactivity in Alzheimer's disease hippocampus. Neuropathol Appl Neurobiol. 2005;31(4):395-404.
11. Rahman A, Ting K, Cullen KM, Braidy N, Brew BJ, Guillemin GJ. The excitotoxin quinolinic acid induces tau phosphorylation in human neurons. PLoS One. 2009;4(7):e6344-e.
12. Lautrup S, Sinclair DA, Mattson MP, Fang EF. NAD(+) in Brain Aging and Neurodegenerative Disorders. Cell Metab. 2019;30(4):630-55.
13. Lardenoije R, Roubroeks JAY, Pishva E, Leber M, Wagner H, Iatrou A, et al. Alzheimer's disease-associated (hydroxy)methylomic changes in the brain and blood. Clin Epigenetics. 2019;11(1):164.
14. Beach TG, Adler CH, Sue LI, Serrano G, Shill HA, Walker DG, et al. Arizona Study of Aging and Neurodegenerative Disorders and Brain and Body Donation Program. Neuropathology. 2015;35(4):354-89.
15. Beach TG, Sue LI, Walker DG, Roher AE, Lue L, Vedders L, et al. The Sun Health Research Institute Brain Donation Program: description and experience, 1987-2007. Cell Tissue Bank. 2008;9(3):229-45.
16. Luck T, Riedel-Heller SG, Kaduszkiewicz H, Bickel H, Jessen F, Pentzek M, et al. Mild Cognitive Impairment in General Practice: Age-Specific Prevalence and Correlate Results from the German Study on Ageing, Cognition and Dementia in Primary Care Patients (AgeCoDe). Dementia and Geriatric Cognitive Disorders. 2007;24(4):307-16.
17. Tryptophan metabolism - Homo sapiens (human) [Internet]. KEGG Pathway Database. 2019. Available from: https://www.genome.jp/kegg-bin/show_pathway?hsa00380.
18. Nicotinate and nicotinamide metabolism - Homo sapiens (human) [Internet]. KEGG Pathway database. 2019. Available from: https://www.genome.jp/kegg-bin/show_pathway?hsa00760.
19. Tryptophan metabolism (Homo sapiens) [Internet]. Wikipathways. 2019. Available from: https://www.wikipathways.org/index.php/Pathway:WP465.
20. NAD+ biosynthetic pathways (Homo sapiens) [Internet]. Wikipathways. 2019. Available from: https://www.wikipathways.org/index.php/Pathway:WP3645.
21. Tryptophan catabolism (R-HSA-71240) [Internet]. Reactome. 2005. Available from: https://reactome.org/content/detail/R-HSA-71240.

22. Serotonin and melatonin biosynthesis (R-HSA-209931) [Internet]. Reactome. 2008. Available from: https://reactome.org/content/detail/R-HSA-209931.
23. Nicotinate metabolism (R-HSA-196807) [Internet]. Reactome. 2007. Available from: https://reactome.org/content/detail/R-HSA-196807.
24. Piras IS, Krate J, Delvaux E, Nolz J, Mastroeni DF, Persico AM, et al. Transcriptome Changes in the Alzheimer's Disease Middle Temporal Gyrus: Importance of RNA Metabolism and Mitochondria-Associated Membrane Genes. J Alzheimers Dis. 2019;70(3):691-713.
25. Giovagnoni C, Ali M, Eijssen LMT, Maes R, Choe K, Mulder M, et al. Altered sphingolipid function in Alzheimer's disease; a gene regulatory network approach. Neurobiol Aging. 2021;102:178-87.
26. Gouzé J-L. Positive and Negative Circuits in Dynamical Systems. Journal of Biological Systems. 1998;06(01):11-5.
27. Thomas R, editor On the Relation Between the Logical Structure of Systems and Their Ability to Generate Multiple Steady States or Sustained Oscillations1981; Berlin, Heidelberg: Springer Berlin Heidelberg.
28. Zickenrott S, Angarica VE, Upadhyaya BB, del Sol A. Prediction of disease-gene-drug relationships following a differential network analysis. Cell Death Dis. 2016;7:e2040.
29. Team RC. R: A language and environment for statistical computing 2016 [Available from: https://www.r-project.org/.
30. Simner C, Novakovic B, Lillycrop KA, Bell CG, Harvey NC, Cooper C, et al. DNA methylation of amino acid transporter genes in the human placenta. Placenta. 2017;60:64-73.
31. Merlo LMF, DuHadaway JB, Montgomery JD, Peng W-D, Murray PJ, Prendergast GC, et al. Differential Roles of IDO1 and IDO2 in T and B Cell Inflammatory Immune Responses. Front Immunol. 2020;11.
32. Metz R, Smith C, DuHadaway JB, Chandler P, Baban B, Merlo LMF, et al. IDO2 is critical for IDO1-mediated T-cell regulation and exerts a non-redundant function in inflammation. International Immunology. 2014;26(7):357-67.
33. Yeung AW, Terentis AC, King NJ, Thomas SR. Role of indoleamine 2,3-dioxygenase in health and disease. Clin Sci (Lond). 2015;129(7):601-72.
34. Leng F, Edison P. Neuroinflammation and microglial activation in Alzheimer disease: where do we go from here? Nature Reviews Neurology. 2021;17(3):157-72.
35. Guillemin GJ, Brew BJ. Implications of the kynurenine pathway and quinolinic acid in Alzheimer's disease. Redox Rep. 2002;7(4):199-206.
36. Pemberton LA, Kerr SJ, Smythe G, Brew BJ. Quinolinic acid production by macrophages stimulated with IFN-gamma, TNF-alpha, and IFN-alpha. J Interferon Cytokine Res. 1997;17(10):589-95.
37. Guillemin GJ, Smythe GA, Veas LA, Takikawa O, Brew BJ. A beta 1-42 induces production of quinolinic acid by human macrophages and microglia. Neuroreport. 2003;14(18):2311-5.
38. Wang X, He H-J, Xiong X, Zhou S, Wang W-W, Feng L, et al. NAD+ in Alzheimer's Disease: Molecular Mechanisms and Systematic Therapeutic Evidence Obtained in vivo. Frontiers in Cell and Developmental Biology. 2021;9.
39. van der Velpen V, Rosenberg N, Maillard V, Teav T, Chatton J-Y, Gallart-Ayala H, et al. Sex-specific alterations in NAD+ metabolism in 3xTg Alzheimer's disease mouse brain assessed by quantitative targeted LC-MS. Journal of Neurochemistry. 2021;159(2):378-88.
40. Amé J-C, Spenlehauer C, de Murcia G. The PARP superfamily. BioEssays. 2004;26(8):882-93.
41. D'Amours D, Desnoyers S, D'Silva I, Poirier GG. Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. The Biochemical journal. 1999;342 (Pt 2)(Pt 2):249-68.
42. Morales J, Li L, Fattah FJ, Dong Y, Bey EA, Patel M, et al. Review of poly (ADP-ribose) polymerase (PARP) mechanisms of action and rationale for targeting in cancer and other diseases. Crit Rev Eukaryot Gene Expr. 2014;24(1):15-28.
43. Iwata H, Goettsch C, Sharma A, Ricchiuto P, Goh WWB, Halu A, et al. PARP9 and PARP14 cross-regulate macrophage activation via STAT1 ADP-ribosylation. Nature Communications. 2016;7(1):12849.

44. Dias HKI, Brown CLR, Polidori MC, Lip GYH, Griffiths HR. LDL-lipids from patients with hypercholesterolaemia and Alzheimer's disease are inflammatory to microvascular endothelial cells: mitigation by statin intervention. Clin Sci (Lond). 2015;129(12):1195-206.
45. Feringa FM, van der Kant R. Cholesterol and Alzheimer's Disease; From Risk Genes to Pathological Effects. Frontiers in Aging Neuroscience. 2021;13(333).
46. Vogel C, Marcotte EM. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat Rev Genet. 2012;13(4):227-32.

Supplemental Material

Table S1. List of TRP metabolic pathway associated genes

Tryptophan metabolism pathway gene	Abbreviation
Aminoadipate Aminotransferase	AADAT
Aralkylamine N-Acetyltransferase	AANAT
Acetyl-CoA Acetyltransferase 1	ACAT1
Acetyl-CoA Acetyltransferase 2	ACAT2
Aminocarboxymuconate Semialdehyde Decarboxylase	ACMSD
Arylformamidase	AFMID
Aldehyde Dehydrogenase 1 Family Member A1	ALDH1A1
Aldehyde Dehydrogenase 1 Family Member A2	ALDH1A2
Aldehyde Dehydrogenase 1 Family Member B1	ALDH1B1
Aldehyde Dehydrogenase 2 Family Member	ALDH2
Aldehyde Dehydrogenase 3 Family Member A2	ALDH3A2
Aldehyde Dehydrogenase 7 Family Member A1	ALDH7A1
Aldehyde Dehydrogenase 8 Family Member A1	ALDH8A1
Aldehyde Dehydrogenase 9 Family Member A1	ALDH9A1
Amine Oxidase, Copper Containing 1	AOC1
Aldehyde Oxidase 1	AOX1
Acetylserotonin O-Methyltransferase	ASMT
Catalase	CAT
Cytochrome P450 Family 19 Subfamily A Member 1	CYP19A1
Cytochrome P450 Family 1 Subfamily A Member 1	CYP1A1
Cytochrome P450 Family 1 Subfamily A Member 2	CYP1A2
Cytochrome P450 Family 1 Subfamily B Member 1	CYP1B1
Cytochrome P450 Family 2 Subfamily A Member 13	CYP2A13
Cytochrome P450 Family 2 Subfamily C Member 18	CYP2C18
Cytochrome P450 Family 2 Subfamily E Member 1	CYP2E1
Cytochrome P450 Family 2 Subfamily F Member 1	CYP2F1
Cytochrome P450 Family 2 Subfamily J Member 2	CYP2J2
Cytochrome P450 Family 3 Subfamily A Member 4	CYP3A4
Cytochrome P450 Family 4 Subfamily F Member 12	CYP4F12
Cytochrome P450 Family 7 Subfamily B Member 1	CYP7B1
Dopa Decarboxylase	DDC
24-Dehydrocholesterol Reductase	DHCR24
Dehydrogenase E1 And Transketolase Domain Containing 1	DHTKD1
Dihydrolipoamide Dehydrogenase	DLD
Dihydrolipoamide S-Succinyltransferase	DLST
Enoyl-CoA Hydratase, Short Chain 1	ECHS1
Enoyl-CoA Hydratase And 3-Hydroxyacyl CoA Dehydrogenase	EHHADH
Glutaryl-CoA Dehydrogenase	GCDH
3-Hydroxyanthranilate 3,4-Dioxygenase	HAAO
Hydroxyacyl-CoA Dehydrogenase	HADH
Hydroxyacyl-CoA Dehydrogenase Trifunctional Multienzyme Complex Subunit Alpha	HADHA
Indoleamine 2,3-Dioxygenase 1	IDO1
Indoleamine 2,3-Dioxygenase 2	IDO2
Interleukin 4 Induced 1	IL4I1
Indolethylamine N-Methyltransferase	INMT
Kynurenine 3-Monooxygenase	KMO

Table S1. *(Continue)*

Tryptophan metabolism pathway gene	Abbreviation
Kynurenine Aminotransferase 1	KYAT1
Kynurenine Aminotransferase 3	KYAT3
Kynureninase	KYNU
Monoamine Oxidase A	MAOA
Monoamine Oxidase B	MAOB
Oxoglutarate Dehydrogenase	OGDH
Solute Carrier Family 36 Member 4	SLC36A4
Solute Carrier Family 3 Member 2	SLC3A2
Solute Carrier Family 7 Member 5	SLC7A5
Tryptophan 2,3-Dioxygenase	TDO2
Tryptophan Hydroxylase 1	TPH1
Tryptophan Hydroxylase 2	TPH2
Tryptophanyl-TRNA Synthetase	WARS

Table S2. List of NAD pathway associated genes

Nicotinate and nicotinamide metabolism pathway gene	Abbreviation
Aminocarboxymuconate Semialdehyde Decarboxylase	ACMSD
Aldehyde Oxidase 1	AOX1
Bone Marrow Stromal Cell Antigen 1	BST1
CD38 Molecule	CD38
Cytochrome P450 Family 8 Subfamily B Member 1	CYP8B1
Ectonucleotide Pyrophosphatase/Phosphodiesterase 1	ENPP1
Ectonucleotide Pyrophosphatase/Phosphodiesterase 3	ENPP3
Indoleamine 2,3-Dioxygenase 1	IDO1
NAD Kinase	NADK
NAD Kinase 2, Mitochondrial	NADK2
NAD Synthetase 1	NADSYN1
Nicotinamide Phosphoribosyltransferase	NAMPT
Nicotinate Phosphoribosyltransferase	NAPRT
NAD(P)HX Dehydratase	NAXD
NAD(P)HX Epimerase	NAXE
Nicotinamide Nucleotide Adenylyltransferase 1	NMNAT1
Nicotinamide Nucleotide Adenylyltransferase 2	NMNAT2
Nicotinamide Nucleotide Adenylyltransferase 3	NMNAT3
Nicotinamide Riboside Kinase 1	NMRK1
Nicotinamide Riboside Kinase 2	NMRK2
Nicotinamide N-Methyltransferase	NNMT
Nicotinamide Nucleotide Transhydrogenase	NNT
Nik Related Kinase	NRK
5', 3'-Nucleotidase, Cytosolic	NT5C
5'-Nucleotidase, Cytosolic IA	NT5C1A
5'-Nucleotidase, Cytosolic IB	NT5C1B
5'-Nucleotidase, Cytosolic II	NT5C2
5'-Nucleotidase, Cytosolic IIIA	NT5C3A
5'-Nucleotidase Ecto	NT5E
5',3'-Nucleotidase, Mitochondrial	NT5M
Nudix Hydrolase 12	NUDT12
Poly(ADP-Ribose) Polymerase 1	PAPR1

Table S2. *(Continue)*

Nicotinate and nicotinamide metabolism pathway gene	Abbreviation
Poly(ADP-Ribose) Polymerase 2	PARP2
Poly(ADP-Ribose) Polymerase 4	PARP4
Poly(ADP-Ribose) Polymerase 6	PARP6
Poly(ADP-Ribose) Polymerase 8	PARP8
Poly(ADP-Ribose) Polymerase 9	PARP9
Poly(ADP-Ribose) Polymerase 10	PARP10
Poly(ADP-Ribose) Polymerase 14	PARP14
Poly(ADP-Ribose) Polymerase 16	PARP16
Purine Nucleoside Phosphorylase	PNP
Prostaglandin I2 Synthase	PTGIS
Prostaglandin-Endoperoxide Synthase 2	PTGS2
Quinolinate Phosphoribosyltransferase	QPRT
Renalase, FAD Dependent Amine Oxidase	RNLS
Sirtuin 1	SIRT1
Sirtuin 2	SIRT2
Sirtuin 3	SIRT3
Sirtuin 4	SIRT4
Sirtuin 5	SIRT5
Sirtuin 6	SIRT6
Sirtuin 7	SIRT7
Solute Carrier Family 22 Member 13	SLC22A13
Solute Carrier Family 5 Member 8	SLC5A8
Tryptophan 2,3-Dioxygenase	TDO2
Tankyrase	TNKS
Tankyrase 2	TNKS2

Table S3. *IDO2* PCR and sequencing primer overview

Gene	Forward Primer (5'-3')	Reverse primer (5'-3')	Target region (GRCh37)	Product size (bp)
IDO2	(Bio-)AGGAATTTTATA ATAGAGAATAGTGATTT	ACCACCACAAAA ATATTACATTTTCA	8:39792704: 39792853:1	150

Gene	Sequencing Primer	CpG	Target region (GRCh37)	PyroMark Orientation
IDO2	CTAATAACTTCTTTTCTAACCT	1	8:39792769: 39792774:1	Lower stand (5'-3')

The overview of the polymerase chain reaction (PCR) and pyrosequencing primers for *IDO2*. Abbreviation: Bio, biotinylation; GRCh37, Ensembl CRCh37 assembly; bp, base pair.

Table S4. TRP metabolic pathway gene expression

Gene Name	Fold Change	Ave Expr	t	S.E.	B	p-value	q-value (FDR)
TDO2	0.910	6.594	-4.349	0.031	1.865	0.000	0.001
HAAO	0.876	6.707	-4.336	0.044	1.821	0.000	0.001
AOC1	0.913	6.631	-4.180	0.031	1.300	0.000	0.001
IDO2	0.940	6.612	-3.826	0.023	0.153	0.000	0.004
DLD	0.778	8.701	-3.431	0.105	-1.045	0.001	0.011
SLC7A5	1.329	9.654	3.176	0.129	-1.766	0.002	0.020
WARS	0.842	8.749	-3.109	0.080	-1.949	0.003	0.020
CYP2E1	0.846	7.298	-3.081	0.079	-2.024	0.003	0.020
DHCR24	0.782	8.085	-2.930	0.121	-2.422	0.004	0.027
DHTKD1	1.093	7.489	2.762	0.046	-2.847	0.007	0.040
KYAT3	0.896	7.476	-2.679	0.059	-3.049	0.009	0.045
ALDH8A1	1.097	7.089	2.590	0.051	-3.261	0.012	0.053
CYP2C18	0.982	6.489	-2.491	0.010	-3.489	0.015	0.063
ECHS1	1.086	9.994	2.443	0.049	-3.598	0.017	0.067
CYP4F12	1.098	6.703	2.386	0.057	-3.723	0.020	0.072
AFMID	0.932	7.043	-2.348	0.043	-3.805	0.022	0.074
ACMSD	1.023	6.459	2.308	0.014	-3.892	0.024	0.077
SLC3A2	1.128	8.464	2.190	0.079	-4.136	0.032	0.094
ALDH1B1	0.956	6.855	-2.159	0.030	-4.199	0.034	0.094
SLC36A4	1.127	8.246	2.135	0.081	-4.247	0.036	0.094
ALDH1A1	0.827	9.257	-2.123	0.130	-4.271	0.037	0.094
ACAT2	0.866	7.815	-2.118	0.098	-4.279	0.037	0.094
CYP19A1	1.030	6.624	2.071	0.020	-4.371	0.042	0.098
MAOB	1.127	9.065	2.061	0.083	-4.391	0.043	0.098
CAT	1.167	8.504	2.029	0.110	-4.451	0.046	0.099
CYP3A4	0.978	6.490	-2.024	0.016	-4.462	0.047	0.099
CYP2F1	1.012	6.455	1.975	0.009	-4.553	0.052	0.106
CYP2J2	1.124	9.728	1.721	0.098	-4.995	0.089	0.176
IDO1	0.982	6.558	-1.695	0.016	-5.037	0.094	0.179
OGDH	0.931	7.210	-1.633	0.064	-5.136	0.107	0.196
ALDH2	1.108	10.897	1.559	0.095	-5.249	0.123	0.219
INMT	1.077	6.574	1.459	0.073	-5.393	0.149	0.256
DLST	1.057	7.608	1.426	0.057	-5.439	0.158	0.256
KYNU	1.041	6.746	1.412	0.041	-5.458	0.162	0.256
TPH2	0.985	6.577	-1.410	0.016	-5.461	0.163	0.256
EHHADH	0.956	7.432	-1.265	0.051	-5.647	0.210	0.321
CYP1A1	1.016	6.587	1.210	0.018	-5.714	0.230	0.342
ALDH3A2	1.036	8.366	1.012	0.050	-5.927	0.315	0.456
KYAT1	1.046	8.341	0.923	0.070	-6.010	0.359	0.506
ACAT1	0.953	9.451	-0.854	0.081	-6.071	0.396	0.540
HADHA	1.058	8.227	0.842	0.096	-6.081	0.403	0.540
MAOA	1.058	10.586	0.782	0.105	-6.128	0.436	0.562
ALDH9A1	1.034	11.593	0.777	0.063	-6.132	0.440	0.562
AADAT	1.034	7.409	0.735	0.065	-6.164	0.465	0.579
AOX1	1.026	6.556	0.720	0.051	-6.174	0.474	0.579
ALDH1A2	0.970	6.814	-0.702	0.063	-6.186	0.485	0.579
CYP7B1	0.994	6.556	-0.620	0.015	-6.240	0.537	0.620
CYP1A2	1.004	6.442	0.613	0.010	-6.244	0.542	0.620
ALDH7A1	1.036	9.729	0.597	0.085	-6.254	0.552	0.620

Table S4. *(Continue)*

Gene Name	Fold Change	Ave Expr	t	S.E.	B	p-value	q-value (FDR)
DDC	0.995	6.478	-0.578	0.012	-6.264	0.565	0.621
HADH	0.980	9.921	-0.392	0.074	-6.353	0.696	0.751
CYP2A13	1.003	6.538	0.352	0.011	-6.368	0.726	0.768
GCDH	0.993	7.301	-0.273	0.039	-6.392	0.785	0.815
IL4I1	0.998	6.478	-0.171	0.016	-6.415	0.865	0.881
ASMT	1.001	6.451	0.109	0.013	-6.423	0.914	0.914
AANAT	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CYP1B1	N/A	N/A	N/A	N/A	N/A	N/A	N/A
KMO	N/A	N/A	N/A	N/A	N/A	N/A	N/A
TPH1	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Gene expression of TRP pathway associated genes in the middle temporal gyrus (MTG) in a comparison of Alzheimer's disease (AD) patients and controls. Abbreviation: AveExpr, average expression; S.E., standard error; t, t-value; B, b value; FDR, false discovery rate.

Table S5. NAD pathway gene expression

Gene Name	Fold Change	Ave Expr	t	S.E.	B	p-value	q-value (FDR)
SIRT1	1.208	8.627	5.908	0.046	7.626	0.000	0.000
NMNAT2	0.653	8.255	-4.838	0.127	3.584	0.000	0.000
PARP1	1.137	11.041	4.702	0.039	3.096	0.000	0.000
PARP9	1.250	7.651	4.371	0.074	1.941	0.000	0.000
TDO2	0.910	6.594	-4.349	0.031	1.865	0.000	0.000
PARP14	1.200	7.511	3.746	0.070	-0.095	0.000	0.003
NT5C3	0.860	8.505	-3.692	0.059	-0.261	0.000	0.003
PNP	1.238	7.557	3.543	0.087	-0.713	0.001	0.004
SLC5A8	1.385	8.602	3.538	0.133	-0.729	0.001	0.004
PARP4	1.259	8.388	3.493	0.095	-0.862	0.001	0.004
QPRT	1.211	8.318	3.337	0.083	-1.313	0.001	0.006
NT5C2	1.203	10.366	3.297	0.081	-1.429	0.002	0.007
PARP10	1.163	7.305	2.970	0.074	-2.319	0.004	0.015
NAPRT	1.268	8.370	2.940	0.117	-2.397	0.004	0.015
NADSYN1	1.103	7.288	2.857	0.050	-2.611	0.006	0.018
SIRT5	0.953	7.547	-2.836	0.025	-2.663	0.006	0.018
NT5M	0.923	6.993	-2.784	0.041	-2.792	0.007	0.020
NT5C	1.164	8.532	2.676	0.082	-3.058	0.009	0.026
CYP8B1	0.977	6.498	-2.543	0.013	-3.370	0.013	0.035
NADK	1.075	7.307	2.489	0.042	-3.494	0.015	0.038
ACMSD	1.023	6.459	2.308	0.014	-3.892	0.024	0.057
NNT	0.847	8.713	-2.286	0.105	-3.937	0.025	0.058
PTGIS	0.971	6.551	-2.233	0.019	-4.047	0.029	0.063
TNKS2	1.034	6.599	2.205	0.022	-4.106	0.031	0.065
BST1	0.977	6.542	-2.025	0.017	-4.460	0.046	0.095
SIRT2	1.087	7.669	2.006	0.060	-4.494	0.048	0.095
SIRT7	1.030	6.746	1.904	0.022	-4.682	0.061	0.115
CD38	0.980	6.587	-1.872	0.016	-4.739	0.065	0.119

Table S5. *(Continue)*

Gene Name	Fold Change	Ave Expr	t	S.E.	B	p-value	q-value (FDR)
RNLS	0.980	6.973	-1.739	0.017	-4.965	0.086	0.152
IDO1	0.982	6.558	-1.695	0.016	-5.037	0.094	0.156
NMNAT3	1.056	7.480	1.694	0.046	-5.040	0.094	0.156
NADK2	1.086	8.149	1.670	0.072	-5.078	0.099	0.156
NAMPT	1.112	7.309	1.665	0.092	-5.085	0.100	0.156
NT5C1B	1.010	6.475	1.458	0.010	-5.395	0.149	0.226
ENPP1	1.031	6.764	1.396	0.031	-5.479	0.167	0.246
ENPP3	0.988	6.558	-1.253	0.014	-5.662	0.214	0.307
PARP16	1.028	6.948	1.217	0.033	-5.705	0.227	0.317
TNKS	0.988	6.585	-1.202	0.014	-5.723	0.233	0.317
NMRK1	0.968	7.716	-0.805	0.058	-6.110	0.423	0.561
SLC22A13	1.007	6.499	0.755	0.014	-6.148	0.452	0.581
AOX1	1.026	6.556	0.720	0.051	-6.174	0.474	0.581
NNMT	0.988	6.583	-0.716	0.024	-6.177	0.476	0.581
SIRT6	0.988	6.633	-0.706	0.025	-6.184	0.483	0.581
NAXE	0.975	8.960	-0.629	0.057	-6.234	0.531	0.626
NT5C1A	0.995	6.510	-0.583	0.013	-6.262	0.562	0.647
PARP6	0.988	7.513	-0.499	0.034	-6.306	0.619	0.698
PTGS2	1.024	8.070	0.296	0.114	-6.386	0.768	0.848
NMRK2	0.998	6.482	-0.243	0.009	-6.400	0.808	0.857
SIRT3	1.002	6.584	0.231	0.011	-6.403	0.818	0.857
NAXD	0.993	9.218	-0.222	0.045	-6.405	0.825	0.857
NUDT12	0.997	6.621	-0.133	0.028	-6.420	0.894	0.912
PARP8	1.000	6.479	0.068	0.008	-6.427	0.946	0.946
NMNAT1	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NRK	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NT5E	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PARP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SIRT4	N/A	N/A	N/A	N/A	N/A	N/A	N/A
SIRT7	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Gene expression of NAD pathway associated genes in the middle temporal gyrus (MTG) in a comparison of Alzheimer's disease (AD) patients and controls. Abbreviation: AveExpr, average expression; S.E., standard error; t, t-value; B, b value; FDR, false discovery rate.

Table S6. TRP pathway 5mC levels

Probe Name	Gene Name	Fold Change	Ave Expr	t	S.E.	p-value	B	Chromosome	SNP position	Region
cg10216820	TPH2	1.012	0.198	2.859	0.006	0.006	-4.376	12	72332539	TSS200
cg09577907	CYP19A1	1.021	0.704	2.704	0.011	0.008	-4.771	15	51535668	5'UTR
cg08104579	AANAT	1.015	0.718	2.643	0.008	0.010	-4.923	17	74467662	3'UTR;3'UTR
cg07887243	MAOB	0.990	0.066	-2.587	0.006	0.012	-5.058	X	43741530	1stExon
cg12978820	CYP2A13	1.013	0.342	2.528	0.007	0.014	-5.198	19	41593202	TSS1500
cg24406775	SLC3A2	0.985	0.508	-2.488	0.009	0.015	-5.292	11	62655842	Body
cg20352402	ALDH8A1	1.026	0.531	2.434	0.015	0.017	-5.417	6	135271333	TSS200
cg05903298	ACAT1	0.999	0.020	-2.419	0.001	0.018	-5.450	11	107992155	TSS200
cg20336341	DDC	1.013	0.279	2.379	0.008	0.020	-5.539	7	50628841	TSS200;5'UTR
cg13198321	ALDH1A2	0.998	0.019	-2.275	0.001	0.026	-5.768	15	58357891	5'UTR;1stExon; 1stExon;5'UTR
cg18047172	DDC	1.014	0.552	2.240	0.009	0.028	-5.844	7	50670059	Body
cg13232821	DDC	1.015	0.334	2.221	0.010	0.029	-5.883	7	50628718	5'UTR;5'UTR;1stExon
cg14080227	ALDH1B1	0.997	0.052	-2.176	0.002	0.033	-5.979	9	38363157	
cg12715421	DDC	1.009	0.422	2.132	0.006	0.036	-6.068	7	50629987	5'UTR;TSS1500
cg09207718	CYP1A2	1.010	0.690	2.093	0.007	0.040	-6.146	15	75041386	5'UTR
cg10649458	MAOA	1.014	0.819	2.072	0.009	0.042	-6.188	X	43512216	
cg17952826	ACAT2	1.025	0.278	2.024	0.017	0.047	-6.283	6	160182184	TSS1500
cg18493449	AANAT	0.988	0.869	-1.998	0.009	0.049	-6.333	17	74467972	Body

List of nominal significant differentially methylated (5mC) probes in the TRP pathway associated gene in the middle temporal gyrus (MTG) in a comparison of Alzheimer's disease (AD) patients and controls. Abbreviation: AveExpr, average expression; S.E., standard error; t, t-value; B, b value; TSS, transcription start site; 5'UTR, 5'untranslated region; 3'UTR, 3'untranslated region.

Table S7. TRP pathway 5hmC levels

Probe Name	Gene Name	Fold Change	Ave Expr	t	S.E.	p-value	B	Chromosome	SNP position	Region
cg27214960	CYP2E1	1.021	0.042	3.392	0.009	0.001	-1.590	10	135343280	Body

cg18125510	WARS	1.020	0.213	2.893	0.010	0.005	-2.981	14	100841768	1stExon;TSS1500;5'UTR;5'UTR;TSS1500;1stExon;5'UTR;5'UTR
cg00565882	CYP1B1	1.015	0.100	2.623	0.008	0.010	-3.661	2	38300707	Body
cg13134297	INMT	1.027	0.124	2.449	0.016	0.017	-4.070	7	30737556	
cg09409405	SLC7A5	0.986	0.045	-2.310	0.009	0.024	-4.379	16	87911198	
cg10268548	ACAT2	1.012	0.159	2.243	0.007	0.028	-4.524	6	160184041	Body
cg13518442	ALDH1A2	1.016	0.234	2.214	0.011	0.030	-4.584	15	58358879	TSS1500;TSS1500
cg24406775	SLC3A2	1.020	0.152	2.188	0.013	0.032	-4.639	11	62655842	Body
cg11363097	IDO2	1.027	0.107	2.173	0.017	0.033	-4.669	8	39792086	TSS1500
cg10169763	SLC7A5	0.974	0.110	-2.164	0.018	0.034	-4.687	16	87873389	Body
cg09577907	CYP19A1	0.985	0.144	-2.163	0.010	0.034	-4.689	15	51535668	5'UTR;5'UTR
cg23532924	DDC	1.012	0.065	2.129	0.008	0.036	-4.758	7	50633445	TSS1500
cg09767736	IL4I1	1.015	0.156	2.059	0.010	0.043	-4.898	19	50400119	Body;1stExon;5'UTR
cg18285819	INMT	0.982	0.158	-2.039	0.013	0.045	-4.936	7	30736630	
cg01466330	IDO2	1.012	0.056	2.039	0.008	0.045	-4.936	8	39835697	Body
cg27527503	HADH	1.021	0.231	2.033	0.015	0.045	-4.948	4	108909664	TSS1500
cg04484695	MAOB	1.014	0.181	2.008	0.010	0.048	-4.997	X	43742501	TSS1500

List of nominal significant differentially hydroxymethylated (5hmC) probes in the TRP pathway associated gene in the middle temporal gyrus (MTG) in a comparison of Alzheimer's disease (AD) patients and controls. Abbreviation: AveExpr, average expression; S.E., standard error; t, t-value; B, b value; TSS, transcription start site; 5'UTR, 5'untranslated region.

Table S8. TRP pathway 5uC levels

Probe Name	Gene Name	Fold Change	Ave Expr	t	S.E.	p-value	B	Chromosome	SNP position	Region
cg00565882	CYP1B1	0.981	0.778	-3.067	0.009	0.003	-3.750	2	38300707	Body
cg18125510	WARS	0.978	0.682	-3.034	0.010	0.003	-3.838	14	100841768	1stExon;TSS1500;5'UTR;5'UTR;TSS1500;1stExon;5'UTR;5'UTR
cg10169763	SLC7A5	1.025	0.242	2.992	0.012	0.004	-3.953	16	87873389	Body
cg20408276	CYP1B1	0.971	0.328	-2.888	0.015	0.005	-4.230	2	38300586	Body

Table S8. (Continue)

Probe Name	Gene Name	Fold Change	Ave Expr	t	S.E.	p-value	B	Chromosome	SNP position	Region
cg13472594	ALDH1B1	0.986	0.474	-2.871	0.007	0.005	-4.275	9	38346777	
cg04790887	ALDH1A2	0.987	0.160	-2.626	0.007	0.010	-4.894	15	58515544	
cg19571004	CYP2E1	0.982	0.250	-2.566	0.010	0.012	-5.038	10	135340850	TSS200
cg13857519	TPH1	0.994	0.088	-2.556	0.004	0.013	-5.062	11	18062735	TSS1500
cg20352402	ALDH8A1	0.981	0.213	-2.505	0.011	0.014	-5.182	6	135271333	TSS200
cg14225090	ALDH1B1	0.993	0.091	-2.441	0.004	0.017	-5.330	9	38237796	
cg04582695	AANAT	0.984	0.282	-2.435	0.009	0.017	-5.344	17	74449757	5'UTR;1stExon;TSS1500
cg10649458	MAOA	0.989	0.124	-2.360	0.007	0.021	-5.514	X	43512216	
cg08645207	AOX1	0.976	0.296	-2.285	0.015	0.025	-5.678	2	201489698	Body
cg03236170	SLC7A5	0.976	0.570	-2.247	0.016	0.028	-5.758	16	87915396	
cg16738971	ALDH7A1	0.994	0.923	-2.238	0.004	0.028	-5.779	5	125931166	TSS200
cg01052699	AADAT	0.991	0.668	-2.235	0.006	0.028	-5.785	4	171030995	
cg02162897	CYP1B1	0.984	0.211	-2.233	0.010	0.029	-5.789	2	38300537	Body
cg06264984	CYP1B1	0.988	0.763	-2.208	0.008	0.030	-5.841	2	38300885	Body
cg22874188	AOC1	0.992	0.125	-2.141	0.005	0.036	-5.980	7	150555302	Body
cg16437184	ALDH8A1	0.982	0.314	-2.136	0.012	0.036	-5.990	6	135157459	
cg12802310	CYP1B1	0.988	0.594	-2.122	0.008	0.037	-6.019	2	38304720	TSS1500
cg07887243	MAOB	1.009	0.929	2.119	0.006	0.037	-6.026	X	43741530	1stExon
cg11251498	IDO2	0.981	0.304	-2.077	0.014	0.041	-6.109	8	39792769	Body
cg20336341	DDC	0.989	0.641	-2.065	0.008	0.042	-6.133	7	50628841	TSS200;5'UTR
cg18872418	TPH1	0.989	0.149	-2.062	0.008	0.043	-6.140	11	18068355	
cg13198321	ALDH1A2	1.002	0.977	2.037	0.001	0.045	-6.188	15	58357891	5'UTR;1stExon;1stExon;5'UTR
cg01812894	ALDH1A1	0.987	0.189	-2.017	0.009	0.047	-6.227	9	75568506	TSS1500
cg25924411	GCDH	0.998	0.955	-2.009	0.002	0.048	-6.243	19	13001836	TSS200
cg10335743	DDC	0.995	0.091	-2.002	0.004	0.049	-6.256	7	50634611	TSS1500

List of nominal significant differentially unmodified (5uC) probes in the TRP pathway associated gene in the middle temporal gyrus (MTG) in a comparison of Alzheimer's disease (AD) patients and controls. Abbreviation: AveExpr, average expression; S.E., standard error; t, t-value; B, b value; TSS, transcription start site; 5'UTR, 5'untranslated region.

Table S9. Summary of TRP pathway genes with both differential gene expression and methylation levels in the middle temporal gyrus of controls and AD patients

	5mC (Probe ID)	**5hmC** (Probe ID)	**5uC** (Probe ID)
ACAT2	(cg17952826)	(cg10268548)	
ALDH1A1			(cg01812894)
ALDH1B1	(cg14080227)		(cg13472594) (cg14225090)
ALDH8A1	**(cg20352402)**		**(cg20352402)** (cg16437184)
AOC1*			(cg22874188)
CYP19A1	**(cg09577907)**	**(cg09577907)**	
CYP2E1*		(cg27214960)	(cg19571004)
IDO2*		(cg11363097) (cg01466330)	(cg11251498)
MAOB	**(cg07887243)**	(cg04484695)	**(cg07887243)**
SLC3A2	**(cg24406775)**	**(cg24406775)**	
SLC7A5*		(cg09409405) **(cg10169763)**	**(cg10169763)** (cg03236170)
WARS*		**(cg18125510)**	**(cg18125510)**

Bold probe IDs are nominal significant probes in multiple regions of TRP pathway. Abbreviation: 5mC, methylcytosine; 5hmC, hydroxymethylcytosine; 5uC, unmodified cytosine; FDR, false discovery rate; * FDR significant gene expression.

Table S10. NAD pathway 5mC levels

Probe Name	Gene Name	Fold Change	Ave Expr	t	S.E.	p-value	B	Chromosome	SNP position	Region
cg17766305	RNLS	0.989	0.111	-2.896	0.006	0.005	-4.280	10	90147030	Body
cg07612655	PTGIS	0.986	0.288	-2.821	0.007	0.006	-4.476	20	48185517	TSS1500
cg26707013	SIRT4	1.030	0.516	2.635	0.016	0.010	-4.943	12	120752270	
cg21580588	NADK	1.006	0.967	2.492	0.003	0.015	-5.281	1	1685723	Body
cg07595035	NAXD	1.025	0.595	2.389	0.015	0.019	-5.518	13	111291582	3'UTR
cg21464278	PARP9	0.997	0.045	-2.358	0.002	0.021	-5.586	3	122283593	TSS1500;TSS1500;TSS200; TSS1500;Body;TSS200; TSS1500
cg08378505	NT5C	0.999	0.022	-2.319	0.001	0.023	-5.673	17	73127297	Body
cg09185911	NADK	1.018	0.808	2.284	0.011	0.025	-5.749	1	1688883	Body
cg15260978	NADK	1.022	0.612	2.281	0.014	0.025	-5.755	1	1685832	Body
cg12135976	SLC5A8	1.010	0.116	2.255	0.006	0.027	-5.811	12	101604147	TSS200
cg04444303	SIRT1	0.978	0.779	-2.252	0.014	0.027	-5.818	10	69634106	
cg10582690	SIRT1	0.999	0.021	-2.229	0.001	0.029	-5.866	10	69644422	TSS1500;TSS200
cg12488274	PARP16	0.975	0.580	-2.189	0.017	0.032	-5.950	15	65594642	
cg20613259	NT5C2	1.019	0.411	2.149	0.012	0.035	-6.034	10	104868306	Body
cg25038311	NT5C2	1.018	0.499	2.125	0.012	0.037	-6.082	10	104964751	
cg11229284	PARP14	0.996	0.065	-2.113	0.003	0.038	-6.106	3	122399506	TSS200
cg08122652	PARP9	1.015	0.472	2.081	0.010	0.041	-6.170	3	122281939	5'UTR;5'UTR;TSS1500; 5'UTR;5'UTR;5'UTR; 5'UTR
cg09321403	SIRT5	1.011	0.112	2.077	0.008	0.041	-6.179	6	13555624	
cg14750551	PARP14	1.023	0.534	2.029	0.016	0.046	-6.273	3	122401343	Body
cg20754155	NAXD	1.011	0.809	2.013	0.008	0.048	-6.304	13	111256586	

List of nominal significant differentially methylated (5mC) probes in the NAD pathway associated gene in the middle temporal gyrus (MTG) in a comparison of Alzheimer's disease (AD) patients and controls. Abbreviation: AveExpr, average expression; S.E., standard error; t, t-value; B, b value; TSS, transcription start site; 5'UTR, 5'untranslated region; 3'UTR, 3'untranslated region.

Table S11. NAD pathway 5hmC levels

Probe Name	Gene Name	Fold Change	Ave Expr	t	S.E.	p-value	B	Chromosome	SNP position	Region
cg07612655	PTGIS	1.026	0.218	4.164	0.009	0.000	0.874	20	48185517	TSS1500
cg24937136	PARP1	1.022	0.382	3.137	0.010	0.002	-2.323	1	226593346	Body
cg03530744	NAXD	1.025	0.332	2.965	0.012	0.004	-2.793	13	111270991	Body
cg20819482	TNKS	1.035	0.303	2.867	0.017	0.005	-3.049	8	9472057	Body
cg23809813	RNLS	1.018	0.352	2.793	0.009	0.007	-3.240	10	90217672	Body
cg25147026	PTGS2	1.024	0.273	2.725	0.013	0.008	-3.412	1	186650441	TSS1500
cg01683055	PARP4	1.018	0.041	2.689	0.010	0.009	-3.501	13	25026835	Body
cg26707013	SIRT4	0.974	0.347	-2.365	0.016	0.021	-4.259	12	120752270	
cg13415078	NAXD	0.984	0.045	-2.363	0.010	0.021	-4.264	13	111287117	Body
cg21580588	NADK	0.994	0.017	-2.349	0.004	0.021	-4.295	1	1685723	Body
cg05722993	NADK	1.018	0.331	2.329	0.011	0.023	-4.339	1	1727796	Body
cg14750551	PARP14	0.975	0.335	-2.294	0.016	0.025	-4.414	3	122401343	Body
cg27058217	NAXD	1.017	0.121	2.252	0.011	0.027	-4.504	13	111289041	Body
cg03081930	NAXD	0.981	0.173	-2.214	0.013	0.030	-4.584	13	111288583	Body
cg09560636	NNT	1.022	0.156	2.096	0.015	0.039	-4.824	5	43631579	Body
cg15897209	NNMT	1.018	0.267	2.074	0.013	0.041	-4.867	11	114151397	
cg15824543	NADK	1.021	0.199	2.074	0.015	0.041	-4.868	1	1695391	Body
cg24867653	SLC22A13	0.993	0.054	-2.024	0.005	0.046	-4.966	3	38306594	TSS1500

List of nominal significant differentially hydroxymethylated (5hmC) probes in the NAD pathway associated gene in the middle temporal gyrus (MTG) in a comparison of Alzheimer's disease (AD) patients and controls. Abbreviation: AveExpr, average expression; S.E., standard error; t, t-value; B, b value; TSS, transcription start site.

Table S12. NAD pathway 5uC levels

Probe Name	Gene Name	Fold Change	Ave Expr	t	S.E.	p-value	B	Chromosome	SNP position	Region
cg03530744	NAXD	0.982	0.184	-3.410	0.008	0.001	-2.762	13	111270991	Body
cg06516476	NT5E	1.016	0.343	3.406	0.007	0.001	-2.775	6	86174584	Body
cg16540590	NNT	0.980	0.193	-2.963	0.010	0.004	-4.032	5	43802496	
cg05238288	PARP4	0.992	0.933	-2.869	0.004	0.005	-4.279	13	25087299	TSS1500
cg24647015	NNMT	0.991	0.131	-2.645	0.005	0.010	-4.848	11	114191367	
cg07612655	PTGIS	0.988	0.493	-2.634	0.007	0.010	-4.875	20	48185517	TSS1500
cg25038311	NT5C2	0.979	0.340	-2.532	0.012	0.013	-5.120	10	104964751	
cg19966621	NT5C3A	1.003	0.970	2.513	0.002	0.014	-5.163	7	33102440	TSS200
cg05722993	NADK	0.985	0.230	-2.468	0.009	0.016	-5.267	1	1727796	Body
cg17130251	NMRK2	0.985	0.342	-2.459	0.009	0.016	-5.289	19	3945936	
cg20819482	TNKS	0.969	0.382	-2.408	0.019	0.019	-5.406	8	9472057	Body
cg09546332	PARP4	0.997	0.941	-2.388	0.002	0.019	-5.451	13	25086777	5'UTR
cg02977388	RNLS	0.974	0.599	-2.319	0.017	0.023	-5.605	10	90253615	Body
cg07631435	NNMT	0.984	0.723	-2.315	0.010	0.023	-5.613	11	114043903	Body
cg26176158	NT5C2	1.004	0.052	2.289	0.003	0.025	-5.670	10	104936358	5'UTR
cg08645207	AOX1	0.976	0.296	-2.285	0.015	0.025	-5.678	2	201489698	Body
cg13215387	NT5C2	0.998	0.966	-2.273	0.001	0.026	-5.704	10	104953220	TSS200
cg16373880	NMNAT2	0.990	0.160	-2.270	0.006	0.026	-5.710	1	183274638	Body;TSS1500
cg11592576	CYP8B1	0.984	0.273	-2.261	0.010	0.027	-5.730	3	42915467	3'UTR;1stExon
cg05262549	NMNAT2	0.989	0.775	-2.206	0.008	0.030	-5.846	1	183256588	Body
cg05215649	NADSYN1	0.989	0.306	-2.191	0.007	0.032	-5.878	11	71196591	Body
cg19518845	NMNAT2	1.015	0.206	2.190	0.010	0.032	-5.880	1	183338346	Body
cg09936839	SIRT6	0.985	0.466	-2.168	0.010	0.033	-5.925	19	4181854	Body;TSS1500
cg14098385	PARP9	0.994	0.107	-2.166	0.004	0.034	-5.929	3	122285010	TSS1500;TSS1500;Body
cg08122070	NADK	1.007	0.096	2.153	0.005	0.035	-5.956	1	1689610	Body
cg26802917	NRK	0.991	0.126	-2.148	0.006	0.035	-5.965	X	105064008	
cg01749742	NT5C1B	0.981	0.462	-2.131	0.013	0.036	-6.000	2	18944047	
cg08482694	PTGS2	0.998	0.960	-2.106	0.002	0.039	-6.052	1	186649530	5'UTR;1stExon
cg03315247	PARP14	0.996	0.937	-2.093	0.003	0.040	-6.078	3	122399498	TSS200
cg22566518	SIRT6	0.997	0.951	-2.074	0.002	0.042	-6.115	19	4182796	TSS1500

cg22930808	PARP9	0.989	0.173	-2.059	0.008	0.043	-6.145	3	122281881	5'UTR;5'UTR;TSS1500; 5'UTR;5'UTR;5'UTR; 5'UTR
cg21356631	BST1	0.978	0.380	-2.043	0.015	0.045	-6.177	4	15702461	
cg06371014	NMRK2	0.992	0.176	-2.034	0.006	0.046	-6.194	19	3924433	Body
cg08200577	PARP4	0.992	0.891	-2.017	0.006	0.047	-6.228	13	25086393	5'UTR
cg12488274	PARP16	1.012	0.324	2.009	0.009	0.048	-6.242	15	65594642	

List of nominal significant differentially unmodified (5uC) probes in the NAD pathway associated gene in the middle temporal gyrus (MTG) in a comparison of Alzheimer's disease (AD) patients and controls. Abbreviation: AveExpr, average expression; S.E., standard error; t, t-value; B, b value; TSS, transcription start site; 5'UTR, 5'untranslated region; 3'UTR, 3'untranslated region.

Table S13. Summary of NAD pathway genes with both differential gene expression and methylation levels in the middle temporal gyrus of controls and AD patients

	5mC (Probe ID)	**5hmC** (Probe ID)	**5uC** (Probe ID)
BST1			(cg21356631)
CYP8B1*			(cg11592576)
NADK*	**(cg21580588)** (cg09185911) (cg15260978)	**(cg21580588)** ***(cg05722993)*** (cg15824543)	***(cg05722993)*** (cg08122070)
NADSYN1*			(cg05215649)
NMNAT2*			(cg16373880) (cg05262549) (cg19518845)
NNT		(cg09560636)	(cg16540590)
NT5C*	(cg08378505)		
NT5C2*	(cg20613259) **(cg25038311)**		**(cg25038311)** (cg26176158) (cg13215387)
NT5C3*			(cg19966621)
PARP1*		(cg24937136)	
PARP14*	(cg11229284) **(cg14750551)**	**(cg14750551)**	(cg03315247)
PARP4*		(cg01683055)	(cg05238288) (cg09546332) (cg08200577)
PARP9*	(cg21464278)		(cg14098385)
PTGIS	**(cg07612655)**	**(cg07612655)**	**(cg07612655)**
QPRT*			(cg03997039)
SIRT1*	(cg04444303) (cg10582690)		
SIRT5*	(cg09321403)		
SLC5A8*	(cg12135976)		

Bold probe IDs are nominal significant probes in multiple regions of NAD pathway. Abbreviation: 5mC, methylcytosine; 5hmC, hydroxymethylcytosine; 5uC, unmodified cytosine; FDR, false discovery rate; * FDR significant gene expression.

Table S14. AgeCoDe and MTG *IDO2* methylation association with age

	β	S.E.	t	p-value	95% CI	
AgeCoDe						
Baseline	-0.149	0.139	-1.075	0.285	-0.424	0.126
Follow-ups	-0.137	0.166	-0.825	0.412	-0.467	0.193
MTG						
mRNA	-0.001	0.002	-0.285	0.777	-0.004	0.003
5mC	0.001	0.001	0.892	0.375	-0.001	0.002
5hmC	7.097E-5	0.001	0.064	0.949	-0.002	0.002
5uC	-0.001	0.001	-1.026	0.308	-0.003	0.001

Association between *IDO2* percent methylation at position cg11251498 with age in AgeCoDe baseline and follow-up, and the middle temporal gyrus (MTG). Both analyses adjusted gender and diagnosis stage. Abbreviations: MTG, middle temporal gyrus; β, beta value; S.E., standard error; t, t statistic; 95% CI, 95% confidence interval.

CHAPTER 4

Associations between plasma metabolites of the kynurenine pathway and affective symptomatology in subjects with or at risk for dementia

Kyonghwan Choe[1], Lieke Bakker[1, 2], Sebastian Köhler[1, 2], Inez H.G.B. Ramakers[1, 2], Simone J.P.M. Eussen[3], Myeong Ok Kim[4, 5], Per M. Ueland[6, 7], Øivind Midttun[8], Frans R.J. Verhey[1, 2], Daniël L.A. van den Hove[1, 9], Gunter Kenis[1], Bart P.F. Rutten[1]

[1] *Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience (MHeNs) and the European Graduate School of Neuroscience (EURON), Maastricht University, Maastricht, the Netherlands.*

[2] *Department of Psychiatry and Neuropsychology, Alzheimer Center Limburg, Maastricht University, Maastricht, the Netherlands.*

[3] *Department of Epidemiology, Maastricht University, Maastricht, the Netherlands.*

[4] *Division of Life Science and Applied Life Science (BK21 FOUR), College of Natural Sciences, Gyeongsang National University, Jinju, Republic of Korea.*

[5] *Alz-Dementia Korea Co., Jinju 52828, Republic of Korea.*

[6] *University of Bergen, Bergen, Norway.*

[7] *Haukeland University Hospital, Bergen, Norway.*

[8] *Bevital AS, Bergen, Norway.*

[9] *Laboratory of Translational Neuroscience, Department of psychiatry, psychosomatics and psychotherapy, University of Würzburg, Würzburg, Germany.*

Abstract

Affective symptomatology such as depression and anxiety are more commonly observed in patients with dementia or in individuals at risk of developing dementia compared to individuals without cognitive complaints. One pathway that has been associated with neurodegenerative disease, cognitive function and affective symptoms is the kynurenine pathway (KP). However, studies investigate affective symptoms and dementia independently. Therefore, the aim of the present study was to investigate associations between plasma KP metabolites and affective symptoms in patients with and without cognitive impairments from the memory clinic setting. Plasma KP metabolites were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in patients with subjective cognitive decline (SCD; n = 296), mild cognitive impairment (MCI; n = 308), and dementia (n = 164) who participated in the Biobank Alzheimer Center Limburg (BBACL) study. Symptoms of depression and anxiety were measured using the neuropsychiatric inventory (NPI) and the 15-item geriatric depression scale (GDS15). Cross-sectional associations between metabolites and affective symptoms were analyzed using negative binomial and binary logistic regression analyses, adjusted for several covariates. Higher plasma levels of xanthurenic acid (XA; incidence rate ratio [IRR] 0.91 [95% confidence interval 0.86, 0.97]), picolinic acid (PIC; 0.93 [0.88, 0.99], kynurenic acid/quinolinic acid ratio (KA/QA; 0.92 [0.86, 0.97]), kynurenic acid/kynurenine ratio (KA/KYN; 0.94 [0.89, 0.99]), and XA/QA ratio (0.90 [0.85, 0.96]) levels were associated with lower IRR of depressive symptoms on the self-rated GDS15. Furthermore, XA, KA/QA, KA/KYN, and XA/QA associations were robust and independent from various other measured factors. Additionally, symptoms of depression and anxiety were more commonly reported by care partners in patients with dementia than in patients with SCD. Lastly, higher plasma levels of KA (0.81 [0.66, 1.00]) was associated with lower IRR of anxiety-like symptoms reported by informants. Although the exact mechanisms through which the KP plays a role in both cognitive and affective symptoms remains unclear, our findings provide evidence that higher plasma KP metabolite concentrations were associated with lower IRR of affective symptoms in patients with or at risk of developing dementia.

Keywords: Kynurenine pathway, dementia, affective symptomatology, cognitive impairment

Introduction

Dementia is a general term to describe loss of memory, language, problem-solving, and social abilities severely enough to interfere with daily life. Mild cognitive impairment (MCI) is similar to dementia with the difference being that impairment is less pronounced and day-to-day functioning is still intact [1]. Recently, research in patients with subjective cognitive decline (SCD) has been growing. SCD refers to individuals who notice a decline in memory or other cognitive abilities, while clinical diagnosis shows no proof of such decline [2]. A meta-analysis reported that the annual conversion rate of people with SCD to dementia was 2.33% and to MCI was 6.67% [3]. In general, cognitive and affective symptomatology are two symptom dimensions which are considered to being qualitatively distinct from each other, yet depressive- and anxiety-like symptoms were commonly observed in patients with dementia or at risk of developing dementia, suggesting that they are linked to each other. For example, two independent meta-analyses reported that the overall pooled prevalence of depression in MCI patient was 32% [4] and the prevalence rates of depression and anxiety across dementia stages, mild, moderate, and severe, were 38%, 41%, and 37%, respectively, for both symptoms [5].

Recent studies suggest that dysregulation of certain metabolic pathways could be involved in the development of both neurodegenerative and neuropsychiatric disorders. Amongst others, studies point towards the tryptophan-kynurenine metabolic pathway [6-12] as an important mechanism in this respect. Tryptophan (TRP) is an essential amino acid, which can only be obtained through diet or supplements and is a precursor to multiple biochemical pathways such as kynurenine (KYN), serotonin, tryptamine, and protein synthesis [6]. The kynurenine pathway (KP) is the dominant pathway and accounts for more than 90% of tryptophan metabolism [13]. Multiple studies in individuals with AD have shown diverse and even opposite effects of KP metabolites, also known as kynurenines, with some exhibiting neurotoxic and others neuroprotective properties. For example, quinolinic acid (QA) is a well-known kynurenines that displays neurotoxic properties in AD, most likely due to its role as an agonist of the N-methyl-D-aspartate (NMDA) receptor [14]. Additionally, QA has been shown to induce the generation of free radicals, mitochondrial dysfunction and inflammation [15]. Contrary to QA, kynurenic acid (KA) is a competitive antagonist of NMDA receptors as well as noncompetitive α7 nicotinic

acetylcholine receptor inhibitor, thus considered as a neuroprotective factor in AD [16]. Besides focused analyses of individual KP metabolite, ratios between certain KP metabolites, such as KYN/TRP ratio (KTR; reflecting changes in indoleamine 2,3-dioxygenase [IDO] activity) and KA/QA ratio (reflecting the balance between neuroprotection and neurotoxicity) are also well investigated [17]. Additionally, a meta-analysis has reported other ratios such as KA/KYN and KA/HK to be lower in patients with major depressive disorder (MDD) compared to controls [18]. However, to date, no studies have addressed the hypothesis that affective symptomatology in people with cognitive complaints, impairments or with dementia is associated with alterations in KP metabolites (and their ratios). Based on this, this cross-sectional study measured plasma TRP and KP metabolites and addressed associations of these with expression of affective symptomatology in people with SCD, MCI and in people with dementia, and analyzed associations across these diagnostic groups, taking into account various covariates.

Materials and Methods

Study participants

Cross-sectional data was used from individuals who participated in the Biobank Alzheimer Center Limburg (BBACL) study and for whom plasma kynurenines were determined. The BBACL study is an ongoing, prospective clinical cohort of patients referred to the Memory Clinic of the Maastricht University Medical Center+ (MUMC+, Maastricht, the Netherlands), for the evaluation of their cognitive complaints. Patients were classified with either SCD (n = 296), MCI (n = 308), or dementia (n = 164). Inclusion criteria were a clinical dementia rating scale (CDR; Morris 1993) score from 0 to 1, and a Mini-Mental State Examination (MMSE; Folstein 1975) score ≥ 20, thereby including patients across the clinical spectrum of SCD, MCI and mild dementia. Exclusion criteria at baseline were non-degenerative neurological disorders, such as Normal Pressure Hydrocephalus, Morbus Huntington, brain tumor, epilepsy, encephalitis, recent transient ischemic attack (TIA) or cerebrovascular accident (CVA) (< 2 years), or TIA/CVA with concurrent (within three months) cognitive decline; a history of psychiatric disorders, current major depressive disorder (within 12 months) (DSM IV), or alcohol abuse. All patients underwent a physical, cognitive and neuropsychiatric evaluation. Additionally, a structural magnetic resonance imaging (MRI) cerebrum was made, and biomaterials were

collected. The BBACL study protocol was approved by the local ethics committee (METC 15-4-100) at the MUMC+ (Maastricht, the Netherlands). All participants gave their written informed consent.

Neuropsychiatric assessment

In addition to extensive cognitive assessments, the present study used the 15-item geriatric depression scale (GDS15) and the domains Depression and Anxiety of the Neuropsychiatric Inventory (NPI) for measuring expression of affective symptomatology.

The GDS15, a short version of the 30-item based GDS (GDS30), is a self-administered questionnaire to estimate the presence or absence of depressive symptoms and, if present, its severity [19]. In general, scores from 0-4 are considered normal (depending on age, education, and complaints); 5-8 reflect mild depression; 9-11 moderate; and 12-15 severe depression. The GDS15 cut-off scale was set at 4/5 (0-4: not depressed; 5-15: depressed) as multiple studies reported this cut-off displaying the highest accuracy, sensitivity, and specificity [20-22].

The NPI-, an informant-based questionnaire, is a validated clinical instrument for evaluating neuropsychiatric symptoms [23]. Briefly, the informant indicates the presence or absence of 12 NPI symptoms: delusions, hallucinations, agitation/aggression, depression/dysphoria, anxiety, elation/euphoria, apathy/ indifference, disinhibition, irritability/lability, aberrant motor behavior, night-time restlessness, and eating disorder. For this study, only symptoms of depression (NPID) and anxiety (NPIA) were included. If an informant indicated the presence of symptoms of depression or anxiety, the frequency (1 = rarely, less than once per week; 2 = sometimes, about once per week; 3 = often, several times per week; and 4 = very often, once or more per day) and severity (1 = mild; 2 = moderate; 3 = severe) were rated. If an informant indicated absence of symptoms, they were rated 0 for frequency and severity. A domain score (0 to 12 points) per symptom (NPID-DS, depression; NPIA-DS, anxiety) was calculated by multiplying its frequency and severity.

Blood collection and biochemical analysis

Venous non-fasting EDTA blood samples were collected at baseline, centrifuged at 4°C at 2000g for 10 minutes, aliquoted into 0.5 ml polypropylene vials, and

stored at -80°C until analysis. Plasma concentrations of TRP (μM) and KP metabolites (KYN (μM), KA (nM), hydroxykynurenine (3-HK [nM]), anthranilic acid (AA [nM]), xanthurenic acid (XA [nM]), 3-hydroxyanthranilic acid (3-HAA [nM]), picolinic acid (PIC [nM]), and QA (nM)) of the whole cohort were analyzed at once in 2019 by liquid chromatograph-tandem mass spectrometry (LC-MS/MS) by Bevital (Bergen, Norway, https://bevital.no). In addition, KTR (KYN/TRP * 1000), KA/QA, KA/KYN (KYN unit converted to nM), KA/HK, and XA/QA ratios were calculated.

Potential covariates

The following relevant characteristics and covariates were measured. Estimated glomerular filtration rate (eGFR), which is calculated using the chronic kidney disease epidemiology collaboration (CKD-EPI) formula [24], was used to measure general kidney function. Additionally, data were collected on body mass index (BMI), smoking status (never, ex-smoker [more than 6 months ago], and current smoker), and alcohol consumption (none, low [≤ 7 glasses per week for women, ≤14 glasses per week for men], high [> 7 for women, >14 for men]), given their known links to cognition, depression, and anxiety [25-27]. In addition, data on blood levels of vitamin B2 (riboflavin [RIBO]) and B6 (pyridoxal 5'-phosphate [PLP] i.e., coenzymes in the KP) were collected, given their known links with cognition [28, 29]. General cognition was scored using the mini mental state examination (MMSE), given its links is associated with affective symptoms [30, 31], and presence of cardiovascular disease, cerebrovascular disease, and hypertension were recorded, given their known links with cognitive and affective symptomatology [32-37].

Statistical analysis

All statistical analyses were done using IBM SPSS Statistics version 27. Based on the Shapiro-Wilk normality test, patient characteristics were statistically analyzed using Kruskal-Wallis (with bonferroni corrections) or Pearson chi-square tests. Furthermore, all metabolite levels were log transformed and standardized prior to statistical analysis for comparative purposes. To investigate associations of plasma metabolites with affective symptomatology, binary logistic- and negative binomial- regression analyses were done while controlling for covariates. All assumptions were tested and met for all statistical models and results were presented in incidence rate ratio (IRR) and 95% confidence interval (95% CI).

First, interaction analyses between age and baseline diagnosis were done for group comparisons as age is the main risk factor for developing dementia. Additionally, as cognitive impairment may influence metabolite levels, interaction analyses between baseline diagnosis and metabolite concentrations were done. All factors and covariates were analyzed as main effects, unless otherwise stated.

Different models were done to adjust for the effect of covariates on the association between TRP, KP metabolites, and its ratios with affective symptomatology tests. The models were adjusted as follows:

- *Model 1*: demographics and kidney function;
- *Model 2* (main model): model 1 with baseline cognitive diagnosis status;
- *Model 3*: model 2 with lifestyle factors;
- *Sensitivity analysis (SA)*: model 3 with vitamin B, cognitive function, and comorbidities;

Model 2 with SA conditions and *Model 2 with model 3 conditions* analyses were only performed to examine if the change in sample size influenced the outcome. Demographics consists of age, gender, and educational level (low, middle, high). Lifestyle includes BMI, smoking status, and alcohol consumption. Finally, vitamin B includes PLP and RIBO, and comorbidities include cardiovascular disease, cerebrovascular disease, and hypertension. P-value less than 0.05 was considered as nominal significant and Bonferroni multiple testing (q-value) less than 0.05 were considered as statistically significant. For analyses investigating associations with metabolite levels, a p-value lower than 0.004 (0.05/14 variables) was considered statistically significant after Bonferroni correction.

Results

Characteristics of study population

In this study population, patients with dementia were older compared to patients with SCD and MCI, and patients with MCI were also older compared to SCD patients (Table 1; Supplementary Tables S1 and S8). On the contrary, for BMI, eGFR, and MMSE, an opposite trend was shown in which patients with SCD showed higher BMI than patients with dementia and higher eGFR levels than patients with MCI and dementia. Additionally, patients with MCI had higher eGFR levels than patients with dementia. As expected, MMSE score was highest

in patients with SCD and lowest in patients with dementia. Moreover, there was a significant difference in the history of cardiovascular diagnoses between the groups.

Table 1. Demographics

	SCD (n = 296)	MCI (n = 308)	Dementia (n = 164)	p-value
Demographics				
Age (year)	61.8 ± 10.8	71.2 ± 9.7[a****]	75.5 ± 7.7[b****, c***]	0.000
Gender				
Male/Female	184/112	173/135	86/78	0.102
Education				
Low/Mid/High	95/122/79	110/122/76	75/56/33	0.069
BMI (kg/m^2)	27.0 ± 5.2 (n = 229)	26.7 ± 4.5 (n = 240)	25.8 ± 4.0[b*] (n = 129)	0.027
eGFR	84.0 ± 13.9	76.1 ± 15.6[a****]	74.0 ± 14.6[b****]	0.000
Tobacco usage				
Never/Former/Current	133/105/47	125/121/45	80/59/18	0.388
Alcohol consumption				
None/Low/High	70/172/35	76/166/40	36/92/18	0.915
Cardiovascular disease				
Absent/Present	153/140	129/174	61/97	0.009
Hypertension				
Absent/Present	57/85	72/103	40/58	0.984
Cerebrovascular disease				
Absent/Present	267/26	255/41	136/22	0.120
Cognition				
MMSE	28.7 ± 1.4	26.9 ± 2.4[a****]	24.9 ± 2.5[b****, c****]	0.000

Data are presented as n or mean ± standard deviation (SD). The sample size of each measurements are equal to the group sample size, unless otherwise stated. Kruskal-Wallis with Bonferroni correction or Pearson chi-square tests were used to analyze the difference between subjective cognitive decline (SCD), mild cognitive impairment (MCI), and dementia. Significance after Bonferroni correction: [a]SCD vs MCI; [b]SCD vs dementia; [c]MCI vs dementia; [*]$q < 0.05$; [**]$q < 0.01$; [***]$q < 0.001$; [****]$q < 0.0001$. Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; MMSE, mini-mental state exam.

With regards to the affective symptomatology, NPIA differed between groups with dementia had higher NPIA-DS than MCI patients. Patients with SCD had a higher GDS15 score than MCI and dementia and significant difference in GDS cut-off scale was shown (Table 2). On the NPI comparisons between the groups, once adjusted for age, gender, educational level and bonferroni correction, informants of patients with dementia showed an increased IRR of reporting a presence of depression symptoms (NPID) than informants of people with SCD patients. Additionally, dementia patient informants had a higher IRR of reporting anxiety symptoms (NPIA) than informants of people with MCI and SCD (Table

3). Lastly, NPID-DS (p = 0.76), NPIA-DS, (p = 0.19), and GDS15 (p = 0.34) and GDS15 cut-off scale (p = 0.81) showed no difference between the groups (data not shown). Lastly, many downstream KP metabolites and its ratios such as KYN, 3-HK, AA, PIC, QA, and KTR were lower in patients with SCD than in patients with MCI and/or dementia, while others such as TRP, XA, KA/QA, KA/HK, and XA/QA were higher in patients with SCD than in patients with MCI and/or dementia (Table 4).

Table 2. Scores on neuropsychiatric tests

	SCD (n = 296)	MCI (n = 308)	Dementia (n = 164)	p-value
Neuropsychiatric symptoms				
NPID				
Absent/Present	157/135	170/136	83/78	0.660
NPID-DS	2.0 ± 2.9 (n = 292)	1.9 ± 2.9 (n = 306)	1.8 ± 2.6 (n = 160)	0.775
NPIA				
Absent/Present	215/77	229/77	102/59	0.023
NPIA-DS	1.2 ± 2.4 (n = 292)	1.1 ± 2.2 (n = 305)	1.6 ± 2.7[c]* (n = 161)	0.023
GDS15	4.0 ± 3.1 (n = 286)	3.2 ± 2.8[a]** (n = 294)	2.8 ± 2.3[b]*** (n = 157)	0.00016
GDS15 cut-off scale (4/5)				
No depression/Depression	216/70	248/46	139/18	0.015

Data are presented as n or mean ± standard deviation (SD). The sample size of each measurements are equal to the group sample size, unless otherwise stated. Kruskal-Wallis with Bonferroni correction or Pearson chi-square tests were used to analyze the difference between subjective cognitive decline (SCD), mild cognitive impairment (MCI), and dementia. Significance after Bonferroni correction: [a]SCD vs MCI; [b]SCD vs dementia; [c]MCI vs dementia; *q < 0.05; **q < 0.01; ***q < 0.001; ****q < 0.0001. Abbreviations: NPID, neuropsychiatric inventory depression; NPID-DS, NPID-domain score; NPIA, NPI anxiety; NPIA-DS, NPIA-domain score; GDS15, 15-item geriatric depression scale.

Table 3. Presence of depression and anxiety across the groups

	NPID		NPIA	
	IRR (95% CI)	**q-value**	**IRR (95% CI)**	**q-value**
SCD[a] vs MCI	1.329 (0.925, 1.909)	0.366	1.092 (0.730, 1.633)	1.00
SCD[a] vs Dementia	1.805 (1.151, 2.829)	0.028	2.026 (1.252, 3.281)	0.014
MCI[a] vs Dementia	1.358 (0.915, 2.016)	0.382	1.855 (1.215, 2.833)	0.016

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Binary logistic regression with Bonferroni correction (q-value) and adjusted for age, gender, and educational level was used to analyze the association between the groups. [a]Reference group. NPID (n = 759) and NPIA (n = 759). Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; NPID, neuropsychiatric inventory depression; NPIA, NPI anxiety.

Table 4. Plasma levels of metabolites of the kynurenine pathway, its ratios, and vitamin B

	SCD (n = 296)	MCI (n = 308)	Dementia (n = 164)	p-value
TRP and KP metabolites				
TRP (μM)	58.5 ± 10.5	55.8 ± 11.6[a**]	55.9 ± 12.1[b*]	0.001
KYN (μM)	1.7 ± 0.4	1.8 ± 0.5	1.8 ± 0.5[b*]	0.012
3-HK (nM)	48.5 ± 17.3 (n = 290)	58.0 ± 39.7 (n = 306)	60.8 ± 46.3[b**] (n = 161)	0.003
KA (nM)	51.7 ± 18.0	55.6 ± 41.9	52.2 ± 23.1	0.705
XA (nM)	15.9 ± 6.8	15.0 ± 9.6[a**]	14.3 ± 6.9[b*]	0.003
AA (nM)	14.7 ± 5.2 (n = 290)	16.1 ± 6.5[a*] (n = 306)	16.9 ± 7.6[b***] (n = 161)	0.0002
3-HAA (nM)	42.4 ± 14.7 (n = 290)	43.2 ± 17.5 (n = 306)	42.8 ± 18.7 (n = 161)	0.858
PIC (nM)	47.0 ± 18.4	50.0 ± 22.7	53.0 ± 28.4[b*]	0.032
QA (nM)	448.2 ± 201.7	521.0 ± 356.6[a*]	542.7 ± 442.6[b**]	0.002
KP metabolite ratios				
KTR	29.3 ± 8.1	32.8 ± 10.9[a****]	32.8 ± 10.7[b***]	5.0E-06
KA/QA	0.12 ± 0.04	0.12 ± 0.04[a*]	0.11 ± 0.04[b***]	0.001
KA/KYN	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.067
KA/HK	1.1 ± 0.3 (n = 290)	1.1 ± 0.4 (n = 308)	1.0 ± 0.3[b***] (n = 164)	0.001
XA/QA	0.04 ± 0.02	0.03 ± 0.02[a****]	0.03 ± 0.02[b****]	1.1E-07
Vitamin B				
PLP (nM)	63.8 ± 66.3	54.0 ± 52.5[a***]	59.3 ± 74.1[b**]	6.0E-05
RIBO (nM)	20.4 ± 29.6	21.4 ± 39.5	25.0 ± 54.6	0.904

Data are presented as mean ± standard deviation (SD). Median and IQR can be found in Supplementary Table S1. The sample size of each measurements are equal to the group sample size, unless otherwise stated. Kruskal-Wallis with Bonferroni correction or Pearson chi-square tests were used to analyze the difference between subjective cognitive decline (SCD), mild cognitive impairment (MCI), and dementia. Significance after Bonferroni correction: [a]SCD vs MCI; [b]SCD vs dementia; [c]MCI vs dementia; [*]$q < 0.05$; [**]$q < 0.01$; [***]$q < 0.001$; [****]$q < 0.0001$. Abbreviations: TRP, tryptophan; KYN, kynurenine; 3-HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; AA, anthranilic acid; 3-HAA, 3-hydroxyanthranilic acid; PIC, picolinic acid; QA: quinolinic acid; KTR, kynurenine/tryptophan ratio; PLP, pyridoxal 5'-phophate; RIBO, riboflavin.

Associations between KP metabolites and self-reported depressive symptoms

Although self-reported depressive symptoms (GDS15) did not differ between the groups, GDS15 was significantly associated with several metabolites and its ratios (Table 5). In *model 2*, higher levels of XA, PIC, KA/QA, KA/KYN, and XA/QA ratios were associated with reduced IRR in GDS15, where KA/QA and XA/QA ratios remained significant after multiple test correction (Table 5). Additionally, after various covariate adjustment analyses, XA, KA/QA, KA/KYN, and XA/QA were still associated with reduced IRR in GDS15,

indicating that these associations were robust and independent of several factors (Supplementary Table S2). Furthermore, KA levels showed no association in *model 2*, but higher KA levels were associated with a reduced IRR in *model 3* and in *model 2 with model 3 conditions*, suggesting that lifestyle factors play a role in the association (Supplementary Table S2). Lastly, BMI mediated the association with PIC based on *model* 3 analysis (Data not shown).

In the GDS15 cut-off scale analysis, on the contrary to the score analysis, only higher XA/QA ratio level showed lower IRR of depressive status in *model 2* and was significant throughout the entire models, but did not survive correction for multiple testing (Table 5; Supplementary Table S3). Additionally, higher XA levels were associated with lower IRR in *model 3*, *SA*, *model 2 with SA and model 3 conditions*, but was non-significant in *model 1* and *model 2* (Table 5; Supplementary Table S3).

Table 5. Association between TRP, KP metabolite and its ratios with GDS15

	GDS15	GDS15 cut-off
TRP		
Model 1	0.987 (0.932, 1.045)	0.925 (0.777, 1.102)
Model 2	0.984 (0.930, 1.042)	0.921 (0.773, 1.097)
KYN		
Model 1	1.021 (0.954, 1.092)	0.978 (0.793, 1.204)
Model 2	1.018 (0.951, 1.089)	0.973 (0.789, 1.200)
3-HK		
Model 1	0.998 (0.932, 1.069)	1.005 (0.815, 1.238)
Model 2	0.999 (0.932, 1.070)	1.005 (0.815, 1.240)
KA		
Model 1	0.943 (0.878, 1.012)	0.893 (0.717, 1.113)
Model 2	0.937 (0.872, 1.006)	0.884 (0.708, 1.104)
XA		
Model 1	0.921 (0.865, 0.980)**	0.854 (0.705, 1.035)
Model 2	0.914 (0.859, 0.974)**	0.845 (0.697, 1.026)
AA		
Model 1	1.056 (0.993, 1.124)	1.169 (0.964, 1.418)
Model 2	1.058 (0.994, 1.126)	1.170 (0.965, 1.419)
3-HAA		
Model 1	0.989 (0.931, 1.051)	0.952 (0.791, 1.145)
Model 2	0.988 (0.929, 1.049)	0.948 (0.787, 1.141)
PIC		
Model 1	0.930 (0.877, 0.986)*	0.888 (0.739, 1.067)
Model 2	0.931 (0.878, 0.987)*	0.889 (0.740, 1.068)
QA		
Model 1	1.065 (0.993, 1.142)	1.139 (0.919, 1.413)
Model 2	1.062 (0.990, 1.139)	1.134 (0.914, 1.408)

Table 5. (Continue)

	GDS15	GDS15 cut-off
KTR		
Model 1	1.037 (0.966, 1.114)	1.070 (0.863, 1.328)
Model 2	1.037 (0.966, 1.114)	1.071 (0.863, 1.329)
KA/QA		
Model 1	0.916 (0.866, 0.970)[#]	0.841 (0.704, 1.003)
Model 2	0.915 (0.864, 0.969)[#]	0.839 (0.703, 1.002)
KA/KYN		
Model 1	0.939 (0.886, 0.994)[*]	0.922 (0.769, 1.104)
Model 2	0.939 (0.886, 0.994)[*]	0.921 (0.769, 1.104)
KA/HK		
Model 1	0.961 (0.906, 1.020)	0.924 (0.774, 1.103)
Model 2	0.957 (0.902, 1.016)	0.918 (0.768, 1.097)
XA/QA		
Model 1	0.902 (0.850, 0.958)[#]	0.814 (0.676, 0.980)
Model 2	0.899 (0.847, 0.955)[#]	0.810 (0.673, 0.976)

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Negative binominal regression (GDS15) or binary logistic regression (cut-off scale) was used to analyze the association. Model 1: adjusted for age, gender, educational level, and eGFR; Model 2: adjusted for model 1 and diagnosis status (SCD, MCI, and dementia). Further covariate adjusted models can be found in the Supplementary Tables S2 and S3. *$p < 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$. #Significance after Bonferroni correction ($p < 0.004$). Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; eGFR, estimated glomerular filtration rate; TRP, tryptophan; KYN, kynurenine; 3-HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; AA, anthranilic acid; 3-HAA, 3-hydroxyanthranilic acid; PIC, picolinic acid; QA: quinolinic acid; KTR, kynurenine/tryptophan ratio; GDS15, 15-item geriatric depression scale.

Associations between KP metabolites and informant reported depressive- and anxiety- symptoms

In the NPID analyses, there were no significant associations between TRP, KP metabolites and its ratios with NPID and NPID-DS in *model 2* (Table 6; Supplementary Tables S4 and S5). NPIA was investigated in the same manner as NPID. Higher levels of KA showed a nominal significant association with lower IRR in NPIA for *model 1* and *model 2*, but was not significant in *model 3*. Furthermore, 3-HK showed no association in *model 2*, but higher levels of 3-HK were associated with lower IRR with NPIA in *model 3* (Table 6; Supplementary Table S6). Finally, NPIA-DS analysis showed no association between TRP, KP metabolite and its ratios in all models (Table 6; Supplementary Table S7).

Table 6. Association between TRP and KP metabolites and ratio in NPI

	NPID	NPID-DS	NPIA	NPIA-DS
TRP				
Model 1	1.087 (0.938, 1.260)	1.069 (0.940, 1.215)	1.004 (0.854, 1.180)	1.051 (0.869, 1.272)
Model 2	1.099 (0.947, 1.276)	1.072 (0.941, 1.220)	1.007 (0.856, 1.185)	1.051 (0.866, 1.275)
KYN				
Model 1	0.923 (0.772, 1.103)	0.938 (0.794, 1.108)	0.869 (0.714, 1.057)	0.810 (0.625, 1.048)
Model 2	0.938 (0.784, 1.122)	0.940 (0.795, 1.110)	0.882 (0.724, 1.074)	0.826 (0.639, 1.067)
3-HK				
Model 1	0.918 (0.772, 1.092)	0.919 (0.778, 1.085)	0.827 (0.678, 1.010)	0.893 (0.726, 1.100)
Model 2	0.921 (0.775, 1.096)	0.920 (0.779, 1.086)	0.827 (0.678, 1.010)	0.915 (0.743, 1.126)
KA				
Model 1	0.879 (0.729, 1.060)	0.858 (0.722, 1.019)	0.788 (0.641, 0.969)*	0.825 (0.652, 1.042)
Model 2	0.904 (0.749, 1.092)	0.862 (0.725, 1.025)	0.809 (0.657, 0.998)*	0.836 (0.661, 1.057)
XA				
Model 1	1.023 (0.873, 1.199)	0.994 (0.861, 1.147)	0.911 (0.766, 1.083)	0.955 (0.779, 1.170)
Model 2	1.048 (0.892, 1.230)	0.999 (0.864, 1.155)	0.925 (0.777, 1.102)	0.962 (0.785, 1.180)
AA				
Model 1	1.064 (0.901, 1.255)	0.999 (0.869, 1.149)	0.871 (0.723, 1.048)	0.888 (0.700, 1.125)
Model 2	1.068 (0.904, 1.262)	0.997 (0.866, 1.147)	0.869 (0.721, 1.048)	0.896 (0.709, 1.133)
3-HAA				
Model 1	1.079 (0.924, 1.261)	1.049 (0.912, 1.207)	0.901 (0.759, 1.069)	0.912 (0.736, 1.128)
Model 2	1.096 (0.937, 1.281)	1.051 (0.914, 1.210)	0.913 (0.769, 1.085)	0.931 (0.751, 1.154)
PIC				
Model 1	1.023 (0.878, 1.193)	0.976 (0.855, 1.115)	0.959 (0.811, 1.135)	0.947 (0.777, 1.156)
Model 2	1.023 (0.876, 1.193)	0.977 (0.855, 1.116)	0.953 (0.805, 1.128)	0.947 (0.776, 1.155)
QA				
Model 1	0.970 (0.802, 1.172)	0.914 (0.770, 1.087)	0.873 (0.706, 1.080)	0.846 (0.663, 1.081)
Model 2	0.989 (0.818, 1.196)	0.917 (0.771, 1.091)	0.888 (0.718, 1.099)	0.858 (0.671, 1.098)

KTR				
Model 1	0.837 (0.695, 1.008)	0.856 (0.720, 1.017)	0.867 (0.707, 1.064)	0.801 (0.634, 1.013)
Model 2	0.839 (0.697, 1.011)	0.855 (0.719, 1.017)	0.876 (0.713, 1.076)	0.815 (0.645, 1.029)
KA/QA				
Model 1	0.937 (0.804, 1.091)	0.963 (0.837, 1.108)	0.939 (0.795, 1.109)	0.979 (0.805, 1.191)
Model 2	0.942 (0.809, 1.098)	0.965 (0.839, 1.110)	0.946 (0.801, 1.119)	0.979 (0.804, 1.191)
KA/KYN				
Model 1	0.962 (0.825, 1.122)	0.925 (0.807, 1.061)	0.909 (0.768, 1.077)	0.956 (0.791, 1.156)
Model 2	0.964 (0.826, 1.124)	0.926 (0.808, 1.062)	0.908 (0.767, 1.076)	0.951 (0.786, 1.150)
KA/HK				
Model 1	0.984 (0.846, 1.145)	0.955 (0.822, 1.108)	0.991 (0.840, 1.169)	0.961 (0.784, 1.177)
Model 2	1.001 (0.860, 1.165)	0.959 (0.825, 1.113)	1.010 (0.855, 1.193)	0.952 (0.779, 1.164)
XA/QA				
Model 1	1.037 (0.886, 1.214)	1.041 (0.904, 1.200)	0.989 (0.832, 1.175)	1.045 (0.852, 1.281)
Model 2	1.047 (0.894, 1.227)	1.045 (0.906, 1.204)	0.994 (0.836, 1.182)	1.044 (0.852, 1.279)

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Binary logistic regression (NPID and NPIA) and negative binominal regression (NPID-DS and NPIA-DS) were performed. *Model 1*: adjusted for age, gender, educational level, and eGFR; *Model 2*: adjusted for *model 1* and diagnosis status (SCD, MCI, and dementia). Further covariate adjusted models can be found in the Supplementary Tables S4-S7. $^{*}p < 0.05$. Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; eGFR, estimated glomerular filtration rate; TRP, tryptophan; KYN, kynurenine; 3-HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; AA, anthranilic acid; 3-HAA, 3-hydroxyanthranilic acid; PIC, picolinic acid; QA: quinolinic acid; KTR, kynurenine/tryptophan ratio; NPID, neuropsychiatric inventory depression; NPID-DS, NPID-domain score; NPIA, NPI anxiety; NPIA-DS, NPIA-domain score.

Discussion

The main aim of this large cohort study was to investigate the cross-sectional associations between plasma TRP, KP metabolites, and its ratios with affective symptomatology across a wide cognitive spectrum ranging from SCD, MCI, and dementia. Overall, our study showed that higher plasma concentrations of XA, PIC, KA/QA, KA/KYN, and XA/QA levels were associated with lower IRR of self-reported depressive symptoms. In addition, caretakers of dementia patients had higher IRR in reporting depressive symptoms than SCD patients, and also in anxiety symptoms than SCD and MCI patients. Furthermore, higher KA levels were associated with lower IRR of informants reporting anxiety-like symptoms. Therefore, the overall findings indicate a negative association between KP metabolites with affective symptoms in patients with or at risk of developing dementia.

Although the screening tools used in this study are clinically validated screening tools, each screening tool has its strengths and weaknesses because affective symptomatology are heterogeneous symptoms and vary between individuals. As demonstrated in this study, the findings in the NPID and GDS15 were not consistent and plausible explanations could be the purpose and design of the tests itself. First, the informants rate NPI and the patients themselves rate GDS15. Moreover, a review by Lai (2014) concluded that NPI tests were valid and reliable, even across different ethnic groups, but the major issue is the scoring system (no multiples of 5, 7, and 11), thus parametric tests may not be appropriate [38]. While GDS15 is an effective, reliable and accurate screening test for depression, in populations that contain large numbers of patients with AD dementia, it lost its validity [39]. However, more recent study concluded that GDS15 was able to assess depressive symptoms among very old people with an MMSE score of 10 or more, but not for older people with MMSE scores lower than 10 [40]. Our dementia patients had an average MMSE score of 25 and our *models 1 and 2* analyses showed that patient diagnosis had little-to-no influence in the association outcome. Therefore, as demonstrated in this study, it is important to assess affective symptoms using multiple screening tools.

KP metabolites and its ratios are associated with depressive-like symptoms

This study has shown that multiple metabolites and its ratios were indeed associated with depression- and anxiety-related symptoms, and our results,

comparing *model 1* with *model 2*, also showed that diagnosis status had little-to-no influence on these associations. This important notion suggests that changes in circulating KP metabolites may be related to depressive- and anxiety-like symptoms, independent from cognitive status or the presence of dementia. Moreover, this study is the first study to investigate the association between KP-associated metabolites and affective symptoms in patients with cognitive issue. Therefore, the findings could only be compared with studies investigating the associations between the KP and patients with clinically diagnosed major depression since it has been extensively examined before and two independent systematic reviews and meta-analyses were recently published [18, 41]. In accordance to our findings, the reviews reported no differences in QA [18, 41], 3-HK [18, 41], and KTR [18] levels between those with depression and matched controls, which our findings also showed no associations with self-reported depressive symptoms and informant reported depressive symptom. Lastly, a recent study reported, after correcting for multiple testing, no difference in TRP, but lower levels of PIC and XA in patients suffering from major depressive episodes compared to healthy controls [42], which was similar to our data on TRP, and, to a lesser extent, our nominal significant associations for PIC and XA. KP metabolites are part of multiple biochemical processes, and may have neuromodulating properties though diverse mechanisms. For example, although the exact mechanism of XA is still unclear and not widely studied, studies have reported XA inhibits metal ion-induced lipid peroxidation and has anti- and sometimes pro-oxidative properties [43-45]. In addition, the endogenous functions of PIC is also unclear, but studies have suggested it may be neuroprotective due to its metal chelation property [46]. Having said that, PIC demonstrated mildly inhibit the effects of QA neurotoxicity [47].

Although most studies examine individual metabolite, investigating different KP metabolite ratios are as important. KA/QA ratio is a widely studied ratio since it determines the balance between neuroprotective and neurotoxic effects of the KP. Both reviews and our data have shown, respectively, lower and negative association of KA/QA ratio in patients with depression and GDS15 [18, 41]. We have further shown that the KA/QA ratio association was robust, independent from various factors, and remained significant after multiple testing. It is worth mentioning that KA/QA ratio in the GDS15 cut-off scale also showed tendency towards negative association, but did not reach statistical significance ($p = 0.053$). Furthermore, KYN has shown neurodegenerative and excitotoxic

properties [48], as such, KA/KYN showed lower levels in patients with depression [18], which was in line with our association with GDS15. To the best of our knowledge, we are the first to report the association between XA/QA ratio and depressive symptoms in patients with cognitive impairment or dementia. Despite individual metabolite measurements, such as KA and QA, were not statistically significant in our GDS15 score association, both KA/QA ratio and XA/QA ratio were statistically significant across different *models* and even after multiple testing correction.

KP metabolites and its ratios across the cognitive spectrum

Even though our study investigated affective symptomatology, our study population were patients with cognitive issues. Studies have reported differences in plasma KP metabolite levels in patients with cognitive impairment or dementia, such as AD, compared to neurologically healthy controls. For example, studies have reported lower levels of XA [9, 49], TRP [7, 49-55], and KA/KYN [52], an increased levels of KTR [52] in AD patients compared to controls, and no difference in KA levels [7, 8, 49, 50, 56]. In addition, neuroinflammation has a role in the pathogenesis of AD [57], thus lower TRP and higher KTR concentration may indicate inflammation since KTR is an inflammatory marker for IDO activity. IDO is the first and rate-limiting enzyme that is activated upon inflammatory stimulation such as interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα) which converts TRP to KYN and initiates the KP [48, 58]. In addition to differential levels in cognitive disorders, KP metabolites and its ratios have also shown to be associated with cognitive function. TRP [9, 59], XA [60] and KA/KYN ratio [52] has shown positive association with MMSE score, while KTR [61, 62] was negatively associated with MMSE score. Additionally, other disease have shown similar results. Our group recently reported that individuals with type-2 diabetes showed plasma levels of XA to be associated with lower odds of cognitive impairment and better executive functioning and attention [63]. Although further research is needed, the findings indicating that some KP metabolites and its ratios are involved in both cognitive and affective symptoms while others were specifically to one specific disorder.

Strengths and limitations

The strengths of this study are that, to our knowledge, this is the first large cross-sectional study to report the associations between plasma TRP, KP metabolites

(and its ratios) with affective symptoms in patients with cognitive complaints, cognitive impairment, and dementia. Also, the study investigated affective symptoms using different clinically validated tests while adjusting for various covariates. Nevertheless, there were some limitations in this study, such as the absence of neurologically healthy controls, the use of non-fasted blood samples and the significant age difference between the diagnosis groups. On average, SCD patients were 10 years younger when compared to MCI or dementia patients, a notion that was taken into account by correcting for age in our analysis. Lastly, despite the uncertainty whether the KP metabolites act independent from each other, we still corrected for multiple testing, which may be associated with and increased chance for type II errors. As such, it is also important to interpret our data based on the pattern of associations (e.g., strength, consistency, or directional expectation).

Conclusion

While it has been suggested that the KP is associated with cognitive function and affective symptoms, the exact nature of this relationship is still unknown. Here we show that several KP metabolites such as XA and PIC and its ratios such as KA/QA, KA/KYN, and XA/QA were associated with affective symptoms across a spectrum of cognitive performance, including neurodegeneration. Since neuroinflammation plays a key role in affective symptoms and in the KP, future prospective studies should investigate associations between inflammatory markers and their interaction with KP metabolites.

References

1. Petersen RC. Mild cognitive impairment as a diagnostic entity. J Intern Med. 2004;256(3):183-94.
2. Jessen F, Amariglio RE, Van Boxtel M, Breteler M, Ceccaldi M, Chételat G, et al. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. Alzheimer's & dementia. 2014;10(6):844-52.
3. Mitchell A, Beaumont H, Ferguson D, Yadegarfar M, Stubbs B. Risk of dementia and mild cognitive impairment in older people with subjective memory complaints: meta-analysis. Acta Psychiatrica Scandinavica. 2014;130(6):439-51.
4. Ismail Z, Elbayoumi H, Fischer CE, Hogan DB, Millikin CP, Schweizer T, et al. Prevalence of depression in patients with mild cognitive impairment: a systematic review and meta-analysis. JAMA psychiatry. 2017;74(1):58-67.
5. Leung DKY, Chan WC, Spector A, Wong GHY. Prevalence of depression, anxiety, and apathy symptoms across dementia stages: A systematic review and meta-analysis. Int J Geriatr Psychiatry. 2021;36(9):1330-44.
6. Maddison DC, Giorgini F. The kynurenine pathway and neurodegenerative disease. Seminars in cell & developmental biology. 2015;40:134-41.
7. González-Sánchez M, Jiménez J, Narváez A, Antequera D, Llamas-Velasco S, Martín AH, et al. Kynurenic Acid Levels are Increased in the CSF of Alzheimer's Disease Patients. Biomolecules. 2020;10(4).
8. Jacobs KR, Lim CK, Blennow K, Zetterberg H, Chatterjee P, Martins RN, et al. Correlation between plasma and CSF concentrations of kynurenine pathway metabolites in Alzheimer's disease and relationship to amyloid-β and tau. Neurobiology of Aging. 2019;80:11-20.
9. Sorgdrager FJH, Vermeiren Y, Van Faassen M, van der Ley C, Nollen EAA, Kema IP, et al. Age- and disease-specific changes of the kynurenine pathway in Parkinson's and Alzheimer's disease. Journal of Neurochemistry. 2019.
10. Shao Y, Ouyang Y, Li T, Liu X, Xu X, Li S, et al. Alteration of Metabolic Profile and Potential Biomarkers in the Plasma of Alzheimer's Disease. Aging Dis. 2020;11(6):1459-70.
11. Wu L, Han Y, Zheng Z, Peng G, Liu P, Yue S, et al. Altered Gut Microbial Metabolites in Amnestic Mild Cognitive Impairment and Alzheimer's Disease: Signals in Host-Microbe Interplay. Nutrients. 2021;13(1).
12. Yilmaz A, Ugur Z, Bisgin H, Akyol S, Bahado-Singh R, Wilson G, et al. Targeted Metabolic Profiling of Urine Highlights a Potential Biomarker Panel for the Diagnosis of Alzheimer's Disease and Mild Cognitive Impairment: A Pilot Study. Metabolites. 2020;10(9).
13. Badawy AA. Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects. Int J Tryptophan Res. 2017;10:1178646917691938.
14. Stone TW, Perkins MN. Quinolinic acid: a potent endogenous excitant at amino acid receptors in CNS. Eur J Pharmacol. 1981;72(4):411-2.
15. Zádori D, Veres G, Szalárdy L, Klivényi P, Vécsei L. Alzheimer's Disease: Recent Concepts on the Relation of Mitochondrial Disturbances, Excitotoxicity, Neuroinflammation, and Kynurenines. J Alzheimers Dis. 2018;62(2):523-47.
16. Platten M, Nollen EAA, Röhrig UF, Fallarino F, Opitz CA. Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond. Nature Reviews Drug Discovery. 2019;18(5):379-401.
17. Savitz J, Drevets WC, Smith CM, Victor TA, Wurfel BE, Bellgowan PS, et al. Putative neuroprotective and neurotoxic kynurenine pathway metabolites are associated with hippocampal and amygdalar volumes in subjects with major depressive disorder. Neuropsychopharmacology. 2015;40(2):463-71.
18. Marx W, McGuinness AJ, Rocks T, Ruusunen A, Cleminson J, Walker AJ, et al. The kynurenine pathway in major depressive disorder, bipolar disorder, and schizophrenia: a meta-analysis of 101 studies. Molecular Psychiatry. 2021;26(8):4158-78.

19. Yesavage JA, Sheikh JI. 9/Geriatric Depression Scale (GDS). Clinical Gerontologist. 1986;5(1-2):165-73.
20. Dias F, Teixeira AL, Guimarães HC, Barbosa MT, Resende EPF, Beato RG, et al. Accuracy of the 15-item Geriatric Depression Scale (GDS-15) in a community-dwelling oldest-old sample: the Pietà Study. Trends Psychiatry Psychother. 2017;39(4):276-9.
21. Marc LG, Raue PJ, Bruce ML. Screening performance of the 15-item geriatric depression scale in a diverse elderly home care population. Am J Geriatr Psychiatry. 2008;16(11):914-21.
22. Weintraub D, Oehlberg KA, Katz IR, Stern MB. Test characteristics of the 15-item geriatric depression scale and Hamilton depression rating scale in Parkinson disease. Am J Geriatr Psychiatry. 2006;14(2):169-75.
23. Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J. The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. Neurology. 1994;44(12):2308-14.
24. Valente MAE, Hillege HL, Navis G, Voors AA, Dunselman PHJM, van Veldhuisen DJ, et al. The Chronic Kidney Disease Epidemiology Collaboration equation outperforms the Modification of Diet in Renal Disease equation for estimating glomerular filtration rate in chronic systolic heart failure. European Journal of Heart Failure. 2014;16(1):86-94.
25. Fluharty M, Taylor AE, Grabski M, Munafò MR. The Association of Cigarette Smoking With Depression and Anxiety: A Systematic Review. Nicotine Tob Res. 2017;19(1):3-13.
26. Luppino FS, de Wit LM, Bouvy PF, Stijnen T, Cuijpers P, Penninx BW, et al. Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. Arch Gen Psychiatry. 2010;67(3):220-9.
27. Cournot M, Marquié JC, Ansiau D, Martinaud C, Fonds H, Ferrières J, et al. Relation between body mass index and cognitive function in healthy middle-aged men and women. Neurology. 2006;67(7):1208-14.
28. Midttun Ø, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom. 2009;23(9):1371-9.
29. Kennedy DO. B Vitamins and the Brain: Mechanisms, Dose and Efficacy--A Review. Nutrients. 2016;8(2):68.
30. Gotlib IH, Joormann J. Cognition and depression: current status and future directions. Annu Rev Clin Psychol. 2010;6:285-312.
31. Ma L. Depression, Anxiety, and Apathy in Mild Cognitive Impairment: Current Perspectives. Frontiers in Aging Neuroscience. 2020;12(9).
32. Alfaddagh A, Martin SS, Leucker TM, Michos ED, Blaha MJ, Lowenstein CJ, et al. Inflammation and cardiovascular disease: From mechanisms to therapeutics. American Journal of Preventive Cardiology. 2020;4:100130.
33. Hare DL, Toukhsati SR, Johansson P, Jaarsma T. Depression and cardiovascular disease: a clinical review. Eur Heart J. 2014;35(21):1365-72.
34. Patrick DM, Van Beusecum JP, Kirabo A. The role of inflammation in hypertension: novel concepts. Current Opinion in Physiology. 2021;19:92-8.
35. Seidel GA, Giovannetti T, Libon DJ. Cerebrovascular disease and cognition in older adults. Curr Top Behav Neurosci. 2012;10:213-41.
36. Stuart MJ, Baune BT. Depression and type 2 diabetes: inflammatory mechanisms of a psychoneuroendocrine co-morbidity. Neurosci Biobehav Rev. 2012;36(1):658-76.
37. Wang K, Li F, Cui Y, Cui C, Cao Z, Xu K, et al. The Association between Depression and Type 1 Diabetes Mellitus: Inflammatory Cytokines as Ferrymen in between? Mediators of Inflammation. 2019;2019:2987901.
38. Lai CKY. The merits and problems of Neuropsychiatric Inventory as an assessment tool in people with dementia and other neurological disorders. Clin Interv Aging. 2014;9:1051-61.
39. Burke WJ, Houston MJ, Boust SJ, Roccaforte WH. Use of the Geriatric Depression Scale in dementia of the Alzheimer type. J Am Geriatr Soc. 1989;37(9):856-60.

40. Conradsson M, Rosendahl E, Littbrand H, Gustafson Y, Olofsson B, Lövheim H. Usefulness of the Geriatric Depression Scale 15-item version among very old people with and without cognitive impairment. Aging Ment Health. 2013;17(5):638-45.
41. Ogyu K, Kubo K, Noda Y, Iwata Y, Tsugawa S, Omura Y, et al. Kynurenine pathway in depression: A systematic review and meta-analysis. Neuroscience & Biobehavioral Reviews. 2018;90:16-25.
42. Colle R, Masson P, Verstuyft C, Fève B, Werner E, Boursier-Neyret C, et al. Peripheral tryptophan, serotonin, kynurenine, and their metabolites in major depression: A case-control study. Psychiatry Clin Neurosci. 2020;74(2):112-7.
43. Murakami K, Ito M, Yoshino M. Xanthurenic acid inhibits metal ion-induced lipid peroxidation and protects NADP-isocitrate dehydrogenase from oxidative inactivation. J Nutr Sci Vitaminol (Tokyo). 2001;47(4):306-10.
44. Murakami K, Haneda M, Yoshino M. Prooxidant action of xanthurenic acid and quinoline compounds: role of transition metals in the generation of reactive oxygen species and enhanced formation of 8-hydroxy-2'-deoxyguanosine in DNA. Biometals. 2006;19(4):429-35.
45. Hirai T, Fukushima K, Kumamoto K, Iwahashi H. Effects of some naturally occurring iron ion chelators on in vitro superoxide radical formation. Biol Trace Elem Res. 2005;108(1-3):77-85.
46. Suzuki K, Yasuda M, Yamasaki K. Stability Constants of Picolinic and Quinaldic Acid Chelates of Bivalent Metals. The Journal of Physical Chemistry. 1957;61(2):229-31.
47. Cockhill J, Jhamandas K, Boegman RJ, Beninger RJ. Action of picolinic acid and structurally related pyridine carboxylic acids on quinolinic acid-induced cortical cholinergic damage. Brain Res. 1992;599(1):57-63.
48. Baumgartner R, Forteza MJ, Ketelhuth DFJ. The interplay between cytokines and the Kynurenine pathway in inflammation and atherosclerosis. Cytokine. 2019;122:154148.
49. Giil LM, Midttun O, Refsum H, Ulvik A, Advani R, Smith AD, et al. Kynurenine Pathway Metabolites in Alzheimer's Disease. Journal of Alzheimer's Disease. 2017;60(2):495-504.
50. van der Velpen V, Teav T, Gallart-Ayala H, Mehl F, Konz I, Clark C, et al. Systemic and central nervous system metabolic alterations in Alzheimer's disease. Alzheimers Res Ther. 2019;11(1):93.
51. Trushina E, Dutta T, Persson XM, Mielke MM, Petersen RC. Identification of altered metabolic pathways in plasma and CSF in mild cognitive impairment and Alzheimer's disease using metabolomics. PLoS One. 2013;8(5):e63644.
52. Gulaj E, Pawlak K, Bien B, Pawlak D. Kynurenine and its metabolites in Alzheimer's disease patients. Adv Med Sci. 2010;55(2):204-11.
53. Li NJ, Liu WT, Li W, Li SQ, Chen XH, Bi KS, et al. Plasma metabolic profiling of Alzheimer's disease by liquid chromatography/mass spectrometry. Clinical Biochemistry. 2010;43(12):992-7.
54. Fekkes D, van der Cammen TJ, van Loon CP, Verschoor C, van Harskamp F, de Koning I, et al. Abnormal amino acid metabolism in patients with early stage Alzheimer dementia. J Neural Transm (Vienna). 1998;105(2-3):287-94.
55. Watkins SE, Thomas DE, Clifford M, Tidmarsh SF. Plasma amino acids in patients with senile dementia and in subjects with Down's syndrome at an age vulnerable to Alzheimer changes. Journal of Mental Deficiency Research. 1989;33(2):159-66.
56. Schwarz MJ, Guillemin GJ, Teipel SJ, Buerger K, Hampel H. Increased 3-Hydroxykynurenine serum concentrations differentiate Alzheimer's disease patients from controls. European Archives of Psychiatry and Clinical Neuroscience. 2013;263(4):345-52.
57. Leng F, Edison P. Neuroinflammation and microglial activation in Alzheimer disease: where do we go from here? Nature Reviews Neurology. 2021;17(3):157-72.
58. Spalletta G, Bossù P, Ciaramella A, Bria P, Caltagirone C, Robinson RG. The etiology of poststroke depression: a review of the literature and a new hypothesis involving inflammatory cytokines. Molecular Psychiatry. 2006;11(11):984-91.
59. Widner B, Leblhuber F, Walli J, Tilz GP, Demel U, Fuchs D. Degradation of tryptophan in neurodegenerative disorders. Adv Exp Med Biol. 1999;467:133-8.

60. Whiley L, Chappell KE, D'Hondt E, Lewis MR, Jiménez B, Snowden SG, et al. Metabolic phenotyping reveals a reduction in the bioavailability of serotonin and kynurenine pathway metabolites in both the urine and serum of individuals living with Alzheimer's disease. Alzheimers Res Ther. 2021;13(1):20.
61. Wissmann P, Geisler S, Leblhuber F, Fuchs D. Immune activation in patients with Alzheimer's disease is associated with high serum phenylalanine concentrations. Journal of the Neurological Sciences. 2013;329(1-2):29-33.
62. Widner B, Leblhuber F, Walli J, Tilz GP, Demel U, Fuchs D. Tryptophan degradation and immune activation in Alzheimer's disease. Journal of Neural Transmission. 2000;107(3):343-53.
63. Bakker L, Ramakers I, van Boxtel MPJ, Schram MT, Stehouwer CDA, van der Kallen CJH, et al. Associations between plasma kynurenines and cognitive function in individuals with normal glucose metabolism, prediabetes and type 2 diabetes: the Maastricht Study. Diabetologia. 2021;64(11):2445-57.

Supplementary Material

Table S1. BBACL characteristics in median (IQR)

	SCD (n = 296)	MCI (n = 308)	Dementia (n = 164)
TRP and KP metabolites			
TRP (μM)	58.35 (51.60, 64.05)	55.65 (47.90, 62.30)	54.55 (48.75, 63.00)
KYN (μM)	1.62 (1.44, 1.85)	1.69 (1.44, 1.99)	1.76 (1.47, 2.00)
3-HK (nM)	45.15 (37.70, 54.50)	47.65 (38.50, 62.80)	49.50 (40.80, 66.60)
KA (nM)	47.90 (40.30, 60.70)	48.95 (37.10, 63.60)	46.20 (38.05, 62.10)
XA (nM)	14.45 (11.10, 19.30)	12.75 (9.75, 18.65)	12.65 (9.13, 18.65)
AA (nM)	13.75 (11.40, 16.80)	14.75 (11.60, 19.10)	15.40 (12.60, 19.50)
3-HAA (nM)	39.95 (31.40, 51.00)	38.50 (31.00, 52.00)	39.70 (29.70, 51.00)
PIC (nM)	42.25 (33.65, 55.85)	46.00 (35.70, 59.65)	46.55 (37.75, 62.25)
QA (nM)	399.50 (325.50, 521.00)	450.50 (335.50, 581.00)	450.00 (346.00, 600.00)
KP metabolite ratios			
KTR	27.71 (24.31, 32.09)	0.11 (0.09, 0.14)	0.11 (0.08, 0.13)
KA/QA	0.12 (0.10, 0.15)	0.03 (0.02, 0.04)	0.03 (0.02, 0.03)
KA/KYN	0.03 (0.03, 0.04)	1.01 (0.79, 1.25)	0.95 (0.74, 1.17)
KA/HK	1.06 (0.91, 1.28)	0.03 (0.02, 0.04)	0.03 (0.02, 0.04)
XA/QA	0.04 (0.03, 0.05)	0.11 (0.09, 0.14)	0.11 (0.08, 0.13)
Vitamin B			
PLP (nM)	47.85 (34.25, 66.65)	39.15 (25.85, 56.10)	37.90 (28.05, 58.35)
Riboflavin (nM)	12.50 (7.76, 20.75)	12.40 (7.93, 20.55)	12.00 (7.24, 19.30)

Data are presented as median and interquartile range (IQR). The sample size of each measurements are equal to the group sample size, unless otherwise stated in Table 3. Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; TRP, tryptophan; KYN, kynurenine; 3-HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; AA, anthranilic acid; 3-HAA, 3-hydroxyanthranilic acid; PIC, picolinic acid; QA: quinolinic acid; KTR, kynurenine/tryptophan ratio; PLP, pyridoxal 5'-phophate; RIBO, riboflavin.

Table S2. GDS15

	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)
	Model 1		**Model 2**		**Model 3**	
TRP	0.651	0.987 (0.932, 1.045)	0.584	0.984 (0.930, 1.042)	0.326	0.967 (0.905, 1.034)
KYN	0.552	1.021 (0.954, 1.092)	0.613	1.018 (0.951, 1.089)	0.891	0.994 (0.913, 1.082)
3-HK	0.960	0.998 (0.932, 1.069)	0.972	0.999 (0.932, 1.070)	0.241	0.951 (0.874, 1.034)
KA	0.102	0.943 (0.878, 1.012)	0.071	0.937 (0.872, 1.006)	**0.017**	0.898 (0.821, 0.981)
XA	**0.010**	0.921 (0.865, 0.980)	**0.005**	0.914 (0.859, 0.974)	**0.001**	0.881 (0.815, 0.951)
AA	0.084	1.056 (0.993, 1.124)	0.074	1.058 (0.994, 1.126)	0.314	1.039 (0.964, 1.120)
3-HAA	0.730	0.989 (0.931, 1.051)	0.688	0.988 (0.929, 1.049)	0.493	0.974 (0.903, 1.050)
PIC	**0.015**	0.930 (0.877, 0.986)	**0.017**	0.931 (0.878, 0.987)	0.172	0.953 (0.888, 1.021)
QA	0.077	1.065 (0.993, 1.142)	0.091	1.062 (0.990, 1.139)	0.864	1.008 (0.922, 1.102)
KTR	0.317	1.037 (0.966, 1.114)	0.315	1.037 (0.966, 1.114)	0.384	1.040 (0.952, 1.137)
KA/QA	**0.003**	0.916 (0.866, 0.970)	**0.002**	0.915 (0.864, 0.969)	**0.044**	0.932 (0.870, 0.998)
KA/KYN	**0.032**	0.939 (0.886, 0.994)	**0.032**	0.939 (0.886, 0.994)	**0.028**	0.924 (0.862, 0.991)
KA/HK	0.190	0.961 (0.906, 1.020)	0.149	0.957 (0.902, 1.016)	0.446	0.973 (0.907, 1.044)
XA/QA	**0.001**	0.902 (0.850, 0.958)	**0.0005**	0.899 (0.847, 0.955)	**0.005**	0.902 (0.839, 0.969)
	Sensitivity analysis (SA)		**Model 2 with SA conditions**		**Model 2 with model 3 conditions**	
TRP	0.191	0.937 (0.850 ,1.033)	0.930	0.930 (0.844, 1.024)	0.301	0.965 (0.902, 1.032)
KYN	0.678	1.028 (0.904, 1.168)	0.395	1.056 (0.931, 1.199)	0.558	1.025 (0.943, 1.115)
3-HK	0.646	0.973 (0.865, 1.094)	0.779	1.016 (0.911, 1.132)	0.884	0.994 (0.915, 1.079)
KA	**0.001**	0.813 (0.719, 0.920)	**0.020**	0.869 (0.772, 0.978)	**0.046**	0.916 (0.841, 0.998)
XA	**0.00023**	0.829 (0.750, 0.916)	**0.002**	0.855 (0.775, 0.943)	**0.001**	0.882 (0.817, 0.952)
AA	0.828	1.012 (0.906, 1.131)	0.547	1.034 (0.927, 1.153)	0.228	1.047 (0.972, 1.129)
3-HAA	0.133	0.925 (0.835, 1.024)	0.416	0.960 (0.871, 1.059)	0.983	1.001 (0.929, 1.078)
PIC	**0.036**	0.898 (0.812, 0.993)	**0.019**	0.887 (0.803, 0.981)	0.097	0.942 (0.878, 1.011)
QA	0.717	1.024 (0.902, 1.161)	0.312	1.064 (0.944, 1.199)	0.290	1.048 (0.961, 1.143)
KTR	0.102	1.109 (0.980, 1.255)	**0.033**	1.139 (1.010, 1.283)	0.111	1.074 (0.984, 1.173)
KA/QA	**0.005**	0.874 (0.796, 0.959)	**0.006**	0.880 (0.803, 0.964)	**0.010**	0.914 (0.853, 0.979)
KA/KYN	**0.000355**	0.836 (0.757, 0.922)	**0.003**	0.864 (0.785, 0.952)	**0.017**	0.920 (0.859, 0.985)

Table S2. (Continue)

	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)
	Sensitivity analysis (SA)		**Model 2 with SA conditions**		**Model 2 with model 3 conditions**	
KA/HK	**0.035**	0.895 (0.808, 0.992)	**0.045**	0.908 (0.826, 0.998)	0.154	0.951 (0.887, 1.019)
XA/QA	**0.001**	0.849 (0.772, 0.935)	**0.001**	0.849 (0.771, 0.933)	**0.001**	0.882 (0.821, 0.948)

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Negative binominal regression was used to analyze the association. P-value less than 0.05 (in bold) was considered nominal significant and p-value less than 0.004 was considered statically significant after Bonferroni correction. *Model 1*: adjusted for age, gender, educational level, and eGFR; *Model 2*: adjusted for *model 1* and diagnosis status (SCD, MCI, and dementia); *Model 3*: adjusted for *model 2* and lifestyle (BMI, smoking status, and alcohol consumption); *Sensitivity analysis (SA)*: adjusted for *model 3* and PLP, RIBO, MMSE, cardiovascular disease, cerebrovascular disease, and hypertension; *Model 2 with SA condition*: only included patients with all factors; *Model 2 with model 3 conditions*: only included patients with all factors up to lifestyle. Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; eGFR, estimated glomerular filtration rate; TRP, tryptophan; KYN, kynurenine; 3-HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; AA, anthranilic acid; 3-HAA, 3-hydroxyanthranilic acid; PIC, picolinic acid; QA: quinolinic acid; KTR, kynurenine/tryptophan ratio; BMI, body mass index; PLP, pyridoxal 5'-phosphate; RIBO, riboflavin; GDS15, 15-item geriatric depression scale.

Table S3. GDS15 cut-off (4/5) scale

	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)
	Model 1		**Model 2**		**Model 3**	
TRP	0.383	0.925 (0.777, 1.102)	0.356	0.921 (0.773, 1.097)	0.101	0.842 (0.686, 1.034)
KYN	0.831	0.978 (0.793, 1.204)	0.797	0.973 (0.789, 1.200)	0.651	0.942 (0.726, 1.222)
3-HK	0.964	1.005 (0.815, 1.238)	0.963	1.005 (0.815, 1.240)	0.620	0.936 (0.719, 1.217)
KA	0.313	0.893 (0.717, 1.113)	0.278	0.884 (0.708, 1.104)	0.058	0.764 (0.578, 1.009)
XA	0.107	0.854 (0.705, 1.035)	0.089	0.845 (0.697, 1.026)	**0.009**	0.722 (0.566, 0.921)
AA	0.111	1.169 (0.964, 1.418)	0.110	1.170 (0.965, 1.419)	0.469	1.090 (0.863, 1.378)
3-HAA	0.600	0.952 (0.791, 1.145)	0.571	0.948 (0.787, 1.141)	0.306	0.885 (0.701, 1.118)
PIC	0.206	0.888 (0.739, 1.067)	0.209	0.889 (0.740, 1.068)	0.258	0.880 (0.705, 1.098)

QA	0.236	1.139 (0.919, 1.413)	0.254	1.134 (0.914, 1.408)	0.852	1.027 (0.780, 1.352)
KTR	0.537	1.070 (0.863, 1.328)	0.534	1.071 (0.863, 1.329)	0.227	1.179 (0.903, 1.539)
KA/QA	0.054	0.841 (0.704, 1.003)	0.053	0.839 (0.703, 1.002)	0.096	0.835 (0.675, 1.033)
KA/KYN	0.376	0.922 (0.769, 1.104)	0.374	0.921 (0.769, 1.104)	0.121	0.838 (0.670, 1.048)
KA/HK	0.382	0.924 (0.774, 1.103)	0.347	0.918 (0.768, 1.097)	0.315	0.898 (0.727, 1.108)
XA/QA	**0.029**	0.814 (0.676, 0.980)	**0.027**	0.810 (0.673, 0.976)	**0.019**	0.762 (0.606, 0.957)
	Sensitivity analysis (SA)		**Model 2 with SA conditions**		**Model 2 with model 3 conditions**	
TRP	0.066	0.734 (0.528, 1.020)	0.073	0.754 (0.554, 1.026)	0.093	0.839 (0.685, 1.029)
KYN	0.806	1.052 (0.703, 1.575)	0.533	1.131 (0.767, 1.668)	0.938	1.010 (0.787, 1.296)
3-HK	0.861	0.966 (0.658, 1.420)	0.729	1.064 (0.750, 1.509)	0.806	1.031 (0.806, 1.320)
KA	**0.013**	0.571 (0.367, 0.888)	0.065	0.688 (0.462, 1.024)	0.132	0.817 (0.628, 1.063)
XA	**0.003**	0.571 (0.397, 0.821)	**0.008**	0.636 (0.455, 0.889)	**0.011**	0.735 (0.581, 0.932)
AA	0.674	1.080 (0.755, 1.545)	0.519	1.119 (0.795, 1.576)	0.313	1.125 (0.895, 1.413)
3-HAA	0.173	0.784 (0.552, 1.113)	0.345	0.858 (0.623, 1.180)	0.688	0.955 (0.764, 1.195)
PIC	0.132	0.767 (0.544, 1.083)	0.093	0.755 (0.545, 1.048)	0.181	0.861 (0.691, 1.072)
QA	0.507	1.145 (0.767, 1.709)	0.321	1.204 (0.835, 1.736)	0.358	1.128 (0.873, 1.458)
KTR	**0.043**	1.532 (1.013, 2.318)	**0.023**	1.553 (1.061, 2.272)	0.092	1.248 (0.965, 1.614)
KA/QA	**0.017**	0.691 (0.511, 0.936)	**0.022**	0.712 (0.532, 0.953)	**0.039**	0.802 (0.650, 0.989)
KA/KYN	**0.010**	0.636 (0.452, 0.896)	**0.022**	0.685 (0.495, 0.947)	0.843	0.680 (1.044, 0.843)
KA/HK	0.083	0.747 (0.538, 1.039)	0.087	0.771 (0.572, 1.038)	0.153	0.862 (0.702, 1.057)
XA/QA	**0.003**	0.612 (0.441, 0.849)	**0.004**	0.632 (0.463, 0.864)	**0.006**	0.728 (0.582, 0.912)

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Binary logistic regression was used to analyze the association. P-value less than 0.05 (in bold) was considered nominal significant and p-value less than 0.004 was considered statically significant after Bonferroni correction. *Model 1*: adjusted for age, gender, educational level, and eGFR; *Model 2*: adjusted for *model 1* and diagnosis status (SCD, MCI, and dementia); *Model 3*: adjusted for *model 2* and lifestyle (BMI, smoking status, and alcohol consumption); *Sensitivity analysis (SA)*: adjusted for *model 3* and PLP, RIBO, MMSE, cardiovascular disease, cerebrovascular disease, and hypertension; *Model 2 with SA condition*: only included patients with all factors; *Model 2 with model 3 conditions*: only included patients with all factors up to lifestyle. Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; eGFR, estimated glomerular filtration rate; TRP, tryptophan; KYN, kynurenine; 3-HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; AA, anthranilic acid; 3-HAA, 3-hydroxyanthranilic acid; PIC, picolinic acid; QA: quinolinic acid; KTR, kynurenine/tryptophan ratio; BMI, body mass index; PLP, pyridoxal 5'-phosphate; RIBO, riboflavin; GDS15, 15-item geriatric depression scale.

Table S4. NPID

	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)
		Model 1		**Model 2**		**Model 3**
TRP	0.269	1.087 (0.938, 1.260)	0.212	1.099 (0.947, 1.276)	0.175	1.130 (0.947, 1.350)
KYN	0.378	0.923 (0.772, 1.103)	0.483	0.938 (0.784, 1.122)	0.780	1.032 (0.828, 1.285)
3-HK	0.335	0.918 (0.772, 1.092)	0.354	0.921 (0.775, 1.096)	0.439	0.919 (0.742, 1.138)
KA	0.176	0.879 (0.729, 1.060)	0.296	0.904 (0.749, 1.092)	0.132	0.833 (0.656, 1.057)
XA	0.780	1.023 (0.873, 1.199)	0.571	1.048 (0.892, 1.230)	0.982	1.002 (0.817, 1.230)
AA	0.466	1.064 (0.901, 1.255)	0.438	1.068 (0.904, 1.262)	0.211	1.138 (0.929, 1.394)
3-HAA	0.336	1.079 (0.924, 1.261)	0.253	1.096 (0.937, 1.281)	0.079	1.194 (0.980, 1.454)
PIC	0.769	1.023 (0.878, 1.193)	0.777	1.023 (0.876, 1.193)	0.778	1.028 (0.849, 1.244)
QA	0.749	0.970 (0.802, 1.172)	0.909	0.989 (0.818, 1.196)	0.822	1.027 (0.812, 1.300)
KTR	0.060	0.837 (0.695, 1.008)	0.066	0.839 (0.697, 1.011)	0.277	0.879 (0.697, 1.109)
KA/QA	0.401	0.937 (0.804, 1.091)	0.446	0.942 (0.809, 1.098)	0.167	0.879 (0.732, 1.056)
KA/KYN	0.624	0.962 (0.825, 1.122)	0.636	0.964 (0.826, 1.124)	0.103	0.854 (0.707, 1.032)
KA/HK	0.835	0.984 (0.846, 1.145)	0.991	1.001 (0.860, 1.165)	0.710	0.966 (0.806, 1.158)
XA/QA	0.650	1.037 (0.886, 1.214)	0.568	1.047 (0.894, 1.227)	0.901	0.988 (0.812, 1.201)
		Sensitivity analysis (SA)		**Model 2 with SA conditions**		
TRP	0.115	1.250 (0.947, 1.650)	0.239	1.168 (0.902, 1.514)		
KYN	0.370	0.852 (0.600, 1.210)	0.409	0.872 (0.629, 1.208)		
3-HK	0.143	0.774 (0.550, 1.090)	0.300	0.854 (0.633, 1.152)		
KA	**0.004**	0.562 (0.381, 0.831)	**0.013**	0.647 (0.458, 0.912)		
XA	0.576	0.918 (0.679, 1.240)	0.882	0.979 (0.742, 1.292)		
AA	0.225	1.212 (0.889, 1.652)	0.345	1.149 (0.861, 1.532)		
3-HAA	0.632	1.073 (0.803, 1.435)	0.648	1.063 (0.818, 1.382)		
PIC	0.707	0.945 (0.706, 1.266)	0.578	0.925 (0.703, 1.217)		
QA	0.956	0.990 (0.698, 1.405)	0.810	1.039 (0.760, 1.420)		

KTR	**0.018**	0.639 (0.442, 0.925)	0.060	0.726 (0.519, 1.014)	
KA/QA	**0.035**	0.749 (0.573, 0.980)	**0.035**	0.765 (0.596, 0.982)	
KA/KYN	**0.020**	0.704 (0.524, 0.947)	**0.043**	0.755 (0.575, 0.992)	
KA/HK	0.389	0.878 (0.653, 1.181)	0.356	0.885 (0.683, 1.147)	
XA/QA	0.666	0.940 (0.711, 1.244)	0.779	0.963 (0.741, 1.252)	

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Binary logistic regression was used to analyze the association. P-value less than 0.05 (in bold) was considered nominal significant and p-value less than 0.004 was considered statically significant after Bonferroni correction. *Model 1*: adjusted for age, gender, educational level, and eGFR; *Model 2*: adjusted for *model 1* and diagnosis status (SCD, MCI, and dementia); *Model 3*: adjusted for *model 2* and lifestyle (BMI, smoking status, and alcohol consumption); *Sensitivity analysis (SA)*: adjusted for *model 3* and PLP, RIBO, MMSE, cardiovascular disease, cerebrovascular disease, and hypertension; *Model 2 with SA condition*: only included patients with all factors; *Model 2 with model 3 conditions*: only included patients with all factors up to lifestyle. Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; eGFR, estimated glomerular filtration rate; TRP, tryptophan; KYN, kynurenine; 3-HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; AA, anthranilic acid; 3-HAA, 3-hydroxyanthranilic acid; PIC, picolinic acid; QA: quinolinic acid; KTR, kynurenine/tryptophan ratio; BMI, body mass index; PLP, pyridoxal 5'-phosphate; RIBO, riboflavin; NPID, neuropsychiatric inventory depression.

Table S5. NPID-DS

	p-value	**IRR (95% CI)**	**p-value**	**IRR (95% CI)**	**p-value**	**IRR (95% CI)**
	Model 1		**Model 2**		**Model 3**	
TRP	0.310	1.069 (0.940, 1.215)	0.297	1.072 (0.941, 1.220)	0.341	1.080 (0.922, 1.265)
KYN	0.451	0.938 (0.794, 1.108)	0.466	0.940 (0.795, 1.110)	0.511	0.935 (0.765, 1.143)
3-HK	0.317	0.919 (0.778, 1.085)	0.324	0.920 (0.779, 1.086)	0.248	0.880 (0.708, 1.093)
KA	0.080	0.858 (0.722, 1.019)	0.092	0.862 (0.725, 1.025)	0.109	0.835 (0.670, 1.041)
XA	0.933	0.994 (0.861, 1.147)	0.992	0.999 (0.864, 1.155)	0.799	1.024 (0.854, 1.227)
AA	0.993	0.999 (0.869, 1.149)	0.965	0.997 (0.866, 1.147)	0.736	1.029 (0.872, 1.214)
3-HAA	0.505	1.049 (0.912, 1.207)	0.484	1.051 (0.914, 1.210)	0.125	1.152 (0.961, 1.380)
PIC	0.724	0.976 (0.855, 1.115)	0.733	0.977 (0.855, 1.116)	0.609	0.959 (0.817, 1.126)

Table S5. (Continue)

	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)
	Model 1		**Model 2**		**Model 3**	
QA	0.309	0.914 (0.770, 1.087)	0.328	0.917 (0.771, 1.091)	0.468	0.923 (0.744, 1.146)
KTR	0.077	0.856 (0.720, 1.017)	0.077	0.855 (0.719, 1.017)	0.091	0.827 (0.664, 1.031)
KA/QA	0.597	0.963 (0.837, 1.108)	0.615	0.965 (0.839, 1.110)	0.549	0.950 (0.804, 1.123)
KA/KYN	0.267	0.925 (0.807, 1.061)	0.271	0.926 (0.808, 1.062)	0.303	0.916 (0.776, 1.082)
KA/HK	0.542	0.955 (0.822, 1.108)	0.580	0.959 (0.825, 1.113)	0.791	0.976 (0.813, 1.170)
XA/QA	0.575	1.041 (0.904, 1.200)	0.548	1.045 (0.906, 1.204)	0.512	1.059 (0.892, 1.257)
	Sensitivity analysis (SA)		**Model 2 with SA conditions**			
TRP	0.690	1.058 (0.802, 1.395)	0.478	1.096 (0.851, 1.411)		
KYN	0.090	0.740 (0.523, 1.048)	0.315	0.848 (0.615, 1.170)		
3-HK	0.162	0.790 (0.567, 1.099)	0.445	0.893 (0.667, 1.195)		
KA	0.196	0.797 (0.565, 1.124)	0.228	0.826 (0.605, 1.127)		
XA	0.996	1.001 (0.759, 1.320)	0.999	1.000 (0.774, 1.292)		
AA	0.682	1.062 (0.798, 1.413)	0.940	1.010 (0.776, 1.315)		
3-HAA	0.649	1.064 (0.814, 1.392)	0.617	1.063 (0.837, 1.348)		
PIC	0.284	0.868 (0.670, 1.125)	0.204	0.859 (0.680, 1.086)		
QA	0.818	0.960 (0.675, 1.364)	0.947	1.011 (0.737, 1.386)		
KTR	0.055	0.720 (0.516, 1.007)	0.110	0.777 (0.570, 1.059)		
KA/QA	0.445	0.906 (0.704, 1.167)	0.316	0.882 (0.691, 1.127)		
KA/KYN	0.807	0.967 (0.737, 1.268)	0.527	0.921 (0.713, 1.190)		
KA/HK	0.858	1.027 (0.767, 1.376)	0.770	0.962 (0.742, 1.247)		
XA/QA	0.891	1.019 (0.783, 1.326)	0.968	0.995 (0.774, 1.278)		

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Negative binomial regression was used to analyze the association. P-value less than 0.05 (in bold) was considered nominal significant and p-value less than 0.004 was considered statically significant after Bonferroni correction. *Model 1*: adjusted for age, gender, educational level, and eGFR; *Model 2*: adjusted for *model 1* and diagnosis status (SCD, MCI, and dementia); *Model 3*: adjusted for *model 2* and lifestyle (BMI,

smoking status, and alcohol consumption); *Sensitivity analysis (SA)*: adjusted for *model 3* and PLP, RIBO, MMSE, cardiovascular disease, cerebrovascular disease, and hypertension; *Model 2 with SA condition*: only included patients with all factors. Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; eGFR, estimated glomerular filtration rate; TRP, tryptophan; KYN, kynurenine; 3-HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; AA, anthranilic acid; 3-HAA, 3-hydroxyanthranilic acid; PIC, picolinic acid; QA: quinolinic acid; KTR, kynurenine/tryptophan ratio; BMI, body mass index; PLP, pyridoxal 5'-phosphate; RIBO, riboflavin; NPID-DS, neuropsychiatric inventory depression-domain score.

Table S6. NPIA

	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)
	Model 1		**Model 2**		**Model 3**	
TRP	0.964	1.004 (0.854, 1.180)	0.930	1.007 (0.856, 1.185)	0.760	0.970 (0.799, 1.178)
KYN	0.161	0.869 (0.714, 1.057)	0.212	0.882 (0.724, 1.074)	0.227	0.860 (0.674, 1.098)
3-HK	0.063	0.827 (0.678, 1.010)	0.063	0.827 (0.678, 1.010)	**0.045**	0.768 (0.594, 0.994)
KA	**0.024**	0.788 (0.641, 0.969)	**0.048**	0.809 (0.657, 0.998)	0.060	0.773 (0.591, 1.010)
XA	0.293	0.911 (0.766, 1.083)	0.385	0.925 (0.777, 1.102)	0.286	0.884 (0.704, 1.109)
AA	0.144	0.871 (0.723, 1.048)	0.141	0.869 (0.721, 1.048)	0.264	0.877 (0.696, 1.104)
3-HAA	0.232	0.901 (0.759, 1.069)	0.302	0.913 (0.769, 1.085)	0.312	0.893 (0.718, 1.112)
PIC	0.629	0.959 (0.811, 1.135)	0.576	0.953 (0.805, 1.128)	0.804	0.974 (0.790, 1.200)
QA	0.210	0.873 (0.706, 1.080)	0.276	0.888 (0.718, 1.099)	0.174	0.827 (0.628, 1.088)
KTR	0.173	0.867 (0.707, 1.064)	0.207	0.876 (0.713, 1.076)	0.392	0.893 (0.688, 1.158)
KA/QA	0.457	0.939 (0.795, 1.109)	0.519	0.946 (0.801, 1.119)	0.784	0.972 (0.794, 1.190)
KA/KYN	0.270	0.909 (0.768, 1.077)	0.266	0.908 (0.767, 1.076)	0.311	0.897 (0.728, 1.106)
KA/HK	0.917	0.991 (0.840, 1.169)	0.906	1.010 (0.855, 1.193)	0.664	1.046 (0.853, 1.282)
XA/QA	0.899	0.989 (0.832, 1.175)	0.947	0.994 (0.836, 1.182)	0.972	0.996 (0.801, 1.238)
	Sensitivity analysis (SA)		**Model 2 with SA conditions**		**Model 2 with model 3 conditions**	
TRP	0.611	1.079 (0.806, 1.444)	0.726	1.052 (0.793, 1.395)	0.684	0.961 (0.792, 1.166)
KYN	0.470	0.873 (0.604, 1.262)	0.444	0.870 (0.608, 1.244)	0.245	0.868 (0.684, 1.102)

Table S6. (Continue)

	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)
	Sensitivity analysis (SA)		**Model 2 with SA conditions**		**Model 2 with model 3 conditions**	
3-HK	**0.019**	0.618 (0.413, 0.924)	**0.033**	0.672 (0.467, 0.968)	0.065	0.793 (0.620, 1.015)
KA	**0.006**	0.559 (0.369, 0.849)	**0.028**	0.655 (0.450, 0.954)	0.179	0.840 (0.652, 1.083)
XA	0.157	0.791 (0.573, 1.094)	0.270	0.843 (0.622, 1.142)	0.441	0.916 (0.734, 1.144)
AA	0.256	0.823 (0.588, 1.152)	0.278	0.837 (0.606, 1.155)	0.314	0.891 (0.711, 1.115)
3-HAA	0.468	0.890 (0.650, 1.219)	0.615	0.928 (0.694, 1.242)	0.483	0.927 (0.751, 1.145)
PIC	0.336	0.853 (0.616, 1.180)	0.380	0.873 (0.645, 1.182)	0.785	0.972 (0.791, 1.194)
QA	0.259	0.803 (0.549, 1.176)	0.372	0.851 (0.597, 1.213)	0.314	0.877 (0.678, 1.133)
KTR	0.238	0.791 (0.537, 1.167)	0.304	0.826 (0.574, 1.189)	0.471	0.911 (0.707, 1.174)
KA/QA	0.307	0.866 (0.656, 1.142)	0.380	0.887 (0.680, 1.159)	0.866	0.983 (0.805, 1.200)
KA/KYN	**0.032**	0.711 (0.520, 0.971)	0.092	0.775 (0.577, 1.042)	0.595	0.947 (0.774, 1.158)
KA/HK	0.922	1.015 (0.746, 1.382)	0.723	1.052 (0.794, 1.395)	0.511	1.070 (0.875, 1.307)
XA/QA	0.643	0.932 (0.694, 1.254)	0.722	0.949 (0.713, 1.264)	0.995	1.001 (0.808, 1.240)

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Binary logistic regression was used to analyze the association. P-value less than 0.05 (in bold) was considered nominal significant and p-value less than 0.004 was considered statically significant after Bonferroni correction. *Model 1*: adjusted for age, gender, educational level, and eGFR; *Model 2*: adjusted for *model 1* and diagnosis status (SCD, MCI, and dementia); *Model 3*: adjusted for *model 2* and lifestyle (BMI, smoking status, and alcohol consumption); *Sensitivity analysis (SA)*: adjusted for *model 3* and PLP, RIBO, MMSE, cardiovascular disease, cerebrovascular disease, and hypertension; *Model 2 with SA condition*: only included patients with all factors; *Model 2 with model 3 conditions*: only included patients with all factors up to lifestyle. Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; eGFR, estimated glomerular filtration rate; TRP, tryptophan; KYN, kynurenine; 3-HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; AA, anthranilic acid; 3-HAA, 3-hydroxyanthranilic acid; PIC, picolinic acid; QA: quinolinic acid; KTR, kynurenine/tryptophan ratio; BMI, body mass index; PLP, pyridoxal 5'-phosphate; RIBO, riboflavin; NPIA, neuropsychiatric inventory anxiety.

Table S7. NPIA-DS

	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)
		Model 1		**Model 2**		**Model 3**
TRP	0.608	1.051 (0.869, 1.272)	0.615	1.051 (0.866, 1.275)	0.784	0.964 (0.744, 1.249)
KYN	0.109	0.810 (0.625, 1.048)	0.144	0.826 (0.639, 1.067)	0.228	0.817 (0.589, 1.134)
3-HK	0.288	0.893 (0.726, 1.100)	0.400	0.915 (0.743, 1.126)	0.267	0.853 (0.644, 1.130)
KA	0.107	0.825 (0.652, 1.042)	0.134	0.836 (0.661, 1.057)	0.146	0.784 (0.565, 1.088)
XA	0.655	0.955 (0.779, 1.170)	0.712	0.962 (0.785, 1.180)	0.477	0.898 (0.668, 1.208)
AA	0.325	0.888 (0.700, 1.125)	0.361	0.896 (0.709, 1.133)	0.383	0.873 (0.643, 1.185)
3-HAA	0.395	0.912 (0.736, 1.128)	0.515	0.931 (0.751, 1.154)	0.328	0.866 (0.649, 1.155)
PIC	0.594	0.947 (0.777, 1.156)	0.591	0.947 (0.776, 1.155)	0.179	0.846 (0.663, 1.080)
QA	0.181	0.846 (0.663, 1.081)	0.224	0.858 (0.671, 1.098)	0.374	0.865 (0.628, 1.191)
KTR	0.063	0.801 (0.634, 1.013)	0.086	0.815 (0.645, 1.029)	0.392	0.869 (0.629, 1.200)
KA/QA	0.833	0.979 (0.805, 1.191)	0.830	0.979 (0.804, 1.191)	0.715	0.954 (0.742, 1.227)
KA/KYN	0.644	0.956 (0.791, 1.156)	0.602	0.951 (0.786, 1.150)	0.616	0.940 (0.739, 1.196)
KA/HK	0.698	0.961 (0.784, 1.177)	0.633	0.952 (0.779, 1.164)	0.893	0.983 (0.769, 1.258)
XA/QA	0.674	1.045 (0.852, 1.281)	0.677	1.044 (0.852, 1.279)	0.972	0.995 (0.750, 1.319)
		Sensitivity analysis (SA)		**Model 2 with SA conditions**		
TRP	0.874	0.965 (0.622, 1.497)	0.736	1.070 (0.722, 1.586)		
KYN	0.320	0.781 (0.479, 1.272)	0.451	0.833 (0.517, 1.341)		
3-HK	**0.041**	0.621 (0.394, 0.980)	N/A	N/A		
KA	0.075	0.582 (0.320, 1.057)	0.220	0.736 (0.451, 1.201)		
XA	0.285	0.772 (0.480, 1.241)	0.576	0.895 (0.605, 1.322)		
AA	0.523	0.901 (0.654, 1.241)	0.389	0.826 (0.534, 1.277)		
3-HAA	0.362	0.869 (0.643, 1.175)	0.672	0.926 (0.649, 1.322)		
PIC	0.278	0.805 (0.543, 1.191)	0.243	0.815 (0.579, 1.149)		
QA	0.637	0.896 (0.568, 1.414)	0.721	0.917 (0.570, 1.475)		

Table S7. (Continue)

	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)
	Sensitivity analysis (SA)		**Model 2 with SA conditions**			
KTR	0.377	0.859 (0.614, 1.203)	0.340	0.807 (0.520, 1.253)		
KA/QA	0.596	0.889 (0.576, 1.373)	0.530	0.884 (0.602, 1.299)		
KA/KYN	0.836	0.973 (0.755, 1.256)	0.522	0.884 (0.606, 1.289)		
KA/HK	0.775	1.040 (0.793, 1.364)	0.847	1.041 (0.691, 1.568)		
XA/QA	0.703	0.919 (0.597, 1.416)	0.785	0.949 (0.651, 1.383)		

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Negative binomial regression was used to analyze the association. P-value less than 0.05 (in bold) was considered nominal significant and p-value less than 0.004 was considered statically significant after Bonferroni correction. * No cardiovascular; # No hypertension; N/A: not available due to error. *Model 1*: adjusted for age, gender, educational level, and eGFR; *Model 2*: adjusted for *model 1* and diagnosis status (SCD, MCI, and dementia); *Model 3*: adjusted for *model 2* and lifestyle (BMI, smoking status, and alcohol consumption); *Sensitivity analysis (SA)*: adjusted for *model 3* and PLP, RIBO, MMSE, cardiovascular disease, cerebrovascular disease, and hypertension; *Model 2 with SA condition*: only included patients with all factors. Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; eGFR, estimated glomerular filtration rate; TRP, tryptophan; KYN, kynurenine; 3-HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; AA, anthranilic acid; 3-HAA, 3-hydroxyanthranilic acid; PIC, picolinic acid; QA: quinolinic acid; KTR, kynurenine/tryptophan ratio; BMI, body mass index; PLP, pyridoxal 5'-phosphate; RIBO, riboflavin; NPIA-DS, neuropsychiatric inventory anxiety-domain score.

Table S8. BBACL characteristics in sensitivity analysis

	SCD (n = 104)	MCI (n = 118)	Dementia (n = 73)
Demographics			
Age (year)	66.62 ± 10.40	72.12 ± 7.54	75.60 ± 7.18
Gender (male/female)	64/40	82/36	37/36
Education (low/middle/high)	43/40/21	37/48/33	34/30/9
BMI (kg/m^2)	27.68 ± 4.66	27.37 ± 4.79	25.98 ± 4.03
eGFR	78.73 ± 14.22	75.42 ± 14.44	71.21 ± 14.14
Tobacco usage (never/ex/current)	43/43/18	40/59/19	31/33/9

Alcohol consumption (never/low/high)	32/62/10	27/72/19	21/42/10
Neuropsychiatric			
NPID (absent/present)	51/51	72/46	45/27
NPID-DS	2.57 ± 3.33 (n = 102)	1.80 ± 2.94 (n = 118)	1.39 ± 2.19 (n = 72)
NPIA (absent/present)	78/24	84/34	54/18
NPIA-DS	1.23 ± 2.58 (n = 102)	1.31 ± 2.41 (n = 118)	1.13 ± 2.22 (n = 72)
GDS15 score	4.03 ± 3.19 (n = 102)	3.04 ± 2.59 (n = 118)	2.52 ± 1.92 (n = 73)
GDS15 4/5 cutoff (no depression/depression)	69/32	94/24	61/12
Neurocognition			
MMSE	28.42 ± 1.34	26.82 ± 2.36	24.26 ± 2.27
TRP and KP metabolites			
TRP (μM)	59.98 ± 10.58	55.35 ± 11.41	55.58 ± 11.18
KYN (μM)	1.84 ± 0.42	1.81 ± 0.44	1.79 ± 0.45
HK (nM)	52.59 ± 16.69 (n = 101)	57.57 ± 40.32 (n = 117)	67.79 ± 58.32 (n = 71)
KA (nM)	59.72 ± 19.66	58.73 ± 23.79	52.07 ± 20.00
XA (nM)	17.47 ± 7.05	15.98 ± 8.03	14.26 ± 6.43
AA (nM)	16.35 ± 5.52 (n = 101)	17.04 ± 5.46 (n = 117)	16.91 ± 5.65 (n = 71)
HAA (nM)	46.81 ± 14.78 (n = 101)	44.71 ± 16.79 (n = 117)	43.15 ± 19.45 (n = 71)
PIC (nM)	51.68 ± 20.60	51.25 ± 17.50	55.80 ± 21.82
QA (nM)	510.01 ± 242.05	536.68 ± 333.06	523.32 ± 244.63

Table S8. (Continue)

	SCD (n = 104)	MCI (n = 118)	Dementia (n = 73)
KP metabolite ratios			
KTR	31.34 ± 8.96	33.66 ± 9.88	32.89 ± 8.81
KA/QA	0.13 ± 0.05	0.12 ± 0.04	0.11 ± 0.04
KA/KYN	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
KA/HK	1.18 ± 0.36 (n = 101)	1.13 ± 0.38 (n = 117)	0.92 ± 0.33 (n = 71)
XA/QA	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.02
Vitamin B			
PLP (nM)	65.56 ± 67.90	45.96 ± 36.51	49.88 ± 49.55
Riboflavin (nM)	20.12 ± 28.08	18.93 ± 21.26	23.47 ± 40.30

Data are presented as n or mean ± standard deviation (SD). The sample size of each measurements are equal to the group sample size, unless otherwise stated in Table 1-3. Abbreviations: SCD, subjective cognitive impairment; MCI, mild cognitive impairment; BMI, body mass index; eGFR, estimated glomerular filtration rate; MMSE, mini-mental state exam; NPID, neuropsychiatric inventory depression; NPID-DS, NPID-domain score; NPIA, NPI anxiety; NPIA-DS, NPIA-domain score; GDS15, 15-item geriatric depression scale; TRP, tryptophan; KYN, kynurenine; 3-HK, 3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; AA, anthranilic acid; 3-HAA, 3-hydroxyanthranilic acid; PIC, picolinic acid; QA: quinolinic acid; KTR, kynurenine/tryptophan ratio; PLP, pyridoxal 5'-phophate; RIBO, riboflavin.

CHAPTER 5

Associations between plasma inflammatory and endothelial markers and affective symptomatology in subjects with or at risk for dementia

Kyonghwan Choe[1], Daniël L.A. van den Hove[1, 2], Inez H.G.B. Ramakers[1, 3], Sebastian Köhler[1, 3], Lieke Bakker[1, 3], Frans R.J. Verhey[1, 3], Casper Schalkwijk[4], Bart P.F. Rutten[1], Myeong Ok Kim[5, 6], Gunter Kenis[1]

[1] *Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience (MHeNs) and European Graduate School of Neuroscience (EURON), Faculty of Health, Medicine and Life Sciences (FHML), Maastricht University, Maastricht, the Netherlands.*

[2] *Department of Psychiatry, Psychosomatic and Psychotherapy, University of Würzburg, Germany.*

[3] *Department of Psychiatry and Neuropsychology, Alzheimer Center Limburg, Maastricht University, Maastricht, the Netherlands.*

[4] *Department of Internal Medicine, School for Cardiovascular Diseases (CARIM), Maastricht University, Maastricht, the Netherlands.*

[5] *Division of Life Science and Applied Life Science (BK21 FOUR), College of Natural Sciences, Gyeongsang National University, Jinju 52828, Republic of Korea.*

[6] *Alz-Dementia Korea Co., Jinju 52828, Republic of Korea.*

Abstract
Inflammatory and endothelial dysfunctions are well documented in patients with affective disorders such as depressive- and anxiety-like symptoms, as well as in patients with or at risk of developing dementia. Strikingly, affective symptoms are commonly observed in the latter. Despite the two relationships, markers of systemic inflammation have, as of yet, only been separately assessed in either cognitive or affective disorders. Therefore, the aim of the present study was to investigate associations of inflammatory and endothelial markers with affective symptoms in patients with and without cognitive impairment recruited from the memory clinic setting. Plasma inflammatory (interferon gamma [IFNγ], tumor necrosis factor alpha [TNFα], interleukin 1 beta [IL1β], IL2, IL6, IL8, IL10, C-reactive protein [CRP], serum amyloid A [SAA]) and endothelial (soluble intercellular molecule-1 [sICAM-1], soluble vascular cell adhesion molecule-1 [sVCAM-1], soluble E-selectin [sE-selectin]) markers were quantified in patients with subjective cognitive decline (SCD; n = 293), mild cognitive impairment (MCI; n = 304), and dementia (n = 162). Depressive- and anxiety-like symptoms were assessed using the informant-based neuropsychiatric inventory (NPI-) depression (NPID) and anxiety (NPIA), and the self-reported 15-item geriatric depression scale (GDS15). Cross-section associations of systemic inflammation markers with affective symptoms were investigated using binary logistic and negative binomial regression analyses adjusting for covariates. Levels of TNFα, IL6, IL8, CRP, SAA, sVCAM-1, and sICAM-1 were positively associated with a higher GDS15 incidence rate ratio (IRR). Additionally, IL10 and sE-selectin showed an interaction effect with diagnosis. Subgroup analyses showed that IL10 in the dementia group and sE-selectin in the SCD and MCI groups were positively related to the GDS15 IRR. Lastly, subgroup analysis showed that levels of CRP and SAA in the MCI group, and elevated sE-selectin in the dementia group, were negatively related to NPIA IRR. In conclusion, several systemic inflammation markers were associated with NPIA and GDS15 in patients with or at risk for dementia. As systemic inflammation (in)directly influences many other biological, further research is needed to understand the full context of its role in cognitive and affective symptoms.

Keywords: Dementia, cognitive impairment, affective symptomatology, cytokine, systemic inflammation

Introduction

Dementia is a general term to characterize a person whose functioning in daily life is severely disrupted due to loss of memory, social abilities, and problem-solving performances [1]. Preceding the clinical diagnosis of dementia, several stages of cognitive deterioration can be determined such as mild cognitive impairment (MCI) and subjective cognitive decline (SCD). MCI patients have similar characteristics as dementia, although symptoms are milder and day-to-day functioning is reasonably intact, while SCD patients report a subjective decline in memory or cognitive function, yet no deficits can clinically be confirmed. Both MCI and SCD are considered prodromal stages of dementia [2, 3], although not all patients with either MCI or SCD will develop dementia. Annual conversion rates from SCD to MCI have been reported to reach 6.67% [4], from SCD to dementia 2.33% [4], and from MCI to dementia 18.4% [5]. Furthermore, studies have shown that affective symptomatology such as depressive- and anxiety-like symptoms were commonly observed in patients with dementia [6, 7]. For example, meta-analyses studies have reported the prevalence rates of depression and anxiety across dementia stages were 38% for mild, 41% for moderate, and 37% for severe for both symptoms [6] and the overall pooled prevalence of depression in MCI patient was 32% [7].

The pathophysiological mechanisms behind depression and dementia are still elusive. Interestingly, a role for inflammatory processes has been ascribed to both disorders. Multiple studies have investigated levels of circulating inflammatory mediators as well as markers of endothelial activation in both depression and dementia. For example, in multiple meta-analyses, both disorders have shown elevated levels of interleukin 6 (IL6) and C-reactive protein (CRP) [8-13], while tumor necrosis factor alpha (TNFα) is elevated in depression [8, 11], but lesser extent in dementia [14]. However, until now the relationship between cytokines and cognitive or affective symptoms have been addressed independently from each other. As such it remains unclear whether systemic inflammation marker associations are shared in either symptoms or specific for one symptom. Therefore, this cross-sectional study aimed to investigate associations between expression of affective symptoms and plasma levels of inflammatory and endothelial markers in a clinical population diagnosed with either dementia or its prodromal stages.

Materials & methods

Study participants

Cross-sectional data was used from individuals who participated in the Biobank Alzheimer Center Limburg (BBACL) study and for whom plasma was available. The BBACL study is an ongoing, prospective clinical cohort of patients referred to the Memory Clinic of the Maastricht University Medical Center+ (MUMC+, Maastricht, the Netherlands), for the evaluation of their cognitive complaints. Patients were diagnosed either with SCD (n = 296), MCI (n = 308), or dementia (n = 164). Inclusion criteria were a clinical dementia rating scale (CDR; Morris 1993) score from 0 to 1, and a Mini-Mental State Examination (MMSE; Folstein 1975) score ≥ 20, thereby including patients across the clinical spectrum of SCD, MCI and mild dementia. Exclusion criteria at baseline were non-degenerative neurological diseases, such as Normal Pressure Hydrocephalus, Morbus Huntington, brain tumor, epilepsy, encephalitis, recent transient ischemic attack (TIA)or cerebrovascular accident (CVA) (< 2 years), or TIA/CVA with concurrent (within three months) cognitive decline; a history of psychiatric disorders, current major depressive disorder (within 12 months) (DSM IV), or alcohol abuse. All patients underwent a physical, cognitive and neuropsychiatric evaluation. Additionally, a structural magnetic resonance imaging (MRI) cerebrum was made, and biomaterials were collected. Past or present history of cardiovascular disease, hypertension, and cerebrovascular disease were self-reported. The BBACL study protocol was approved by the local ethics committee (METC 15-4-100) at the MUMC+ (Maastricht, the Netherlands). All participants gave their written informed consent.

Neuropsychiatric assessment

The 15-item based geriatric depression scale (GDS15), a shorter version of the GDS30, is a self-reported measure to screen for depression and if present, determine its severity [15]. In general, the patient answers 15 questions (yes or no) in front of a clinician and depending on age, education, and complaints, scores from 0-4 are considered normal, 5-8 are mild depression, 9-11 are moderate, and 12-15 are severe. The test can be completed in a short period of time (approximately 5 to 7 minutes), which makes it an ideal test for patients who cannot concentrate for extensive periods of time or easily fatigued. Alternatively, a binary version of the GDS15 using a cut-off scale is widely employed. Here, the cut-off was set at 4/5 (0-4: not depressed; 5-15: depressed),

which has been reported to have the highest accuracy, sensitivity, and specificity [16-18].

The NPI-, an informant-based questionnaire, is a validated clinical instrument for evaluating neuropsychiatric symptoms [19]. Briefly, the informant indicates the presence or absence of 12 NPI symptoms: delusions, hallucinations, agitation/aggression, depression/dysphoria, anxiety, elation/euphoria, apathy/ indifference, disinhibition, irritability/lability, aberrant motor behavior, night-time restlessness, and eating disorder. For this study, only symptoms of depression (NPID) and anxiety (NPIA) were included. If an informant indicated the presence of symptoms of depression or anxiety, the frequency (1 = rarely, less than once per week; 2 = sometimes, about once per week; 3 = often, several times per week; and 4 = very often, once or more per day) and severity (1 = mild; 2 = moderate; 3 = severe) were rated. If an informant indicated absence of symptoms, they were rated 0 for frequency and severity. A symptom domain score (0 to 12 points) (NPID-DS, depression; NPIA-DS, anxiety) was calculated by multiplying its frequency and severity.

Cytokine analysis

Venous non-fasted EDTA anticoagulated blood samples were collected at baseline, spun down at 2000g for 10 minutes at 4°C, aliquoted into 0.5ml polypropylene vials, and stored at -80ºC until later use. Before cytokine analysis, samples were thawed and aliquoted into adequate volumes for the three different assays; human pro-inflammatory panel (interferon gamma [IFNγ], TNFα, IL1β, IL2, IL6, IL8, and IL10), human vascular injury panel (CRP, serum amyloid A [SAA], soluble intercellular adhesion molecule-1 [sICAM-1], and soluble vascular cell adhesion molecule-1 [sVCAM-1]) and human soluble E-selectin panel (sE-selectin) and stored at -80ºC until assays were performed.

Multiplex assays from Meso Scale Discovery (Rockville, MD, USA) were used to measure pro-inflammation (V-Plex Pro-inflammatory Panel 1 human kit, K15049D, kit lot #K0081327) and vascular injury (V-Plex Vascular Injury Panel 2 human kit, K15198D, kit lot #K0081278) markers in plasma. In addition, levels of sE-selectin were assessed using the Human CD62E / ELAM-1 ELISA set (batch #EL-062E-12T) from Diaclone SAS (Besancon Cedex, France). All assays were performed according to manufacturer's protocols with minor adjustments, and the samples were processed in duplicates. Minor adjustments

are as follows: in the pro-inflammatory panel, the highest standard concentration was replaced by an additional lowest standard concentration. For the sE-selectin assay, based on in-house optimization, shorter/longer incubation times and fewer/additional washing steps. As internal plate control, two independent pooled serum samples were used for both the pro-inflammatory and vascular injury panels in duplicate, and three independent pooled plasma samples for sE-selectin in duplicate. The plate control samples were non-fasted healthy pooled serum and EDTA plasma samples (n = 7) from volunteered staff members in the Department of Internal Medicine, Maastricht University (Maastricht, the Netherlands). The participants' age ranged from 25 to 50 years. Samples were aliquoted and stored at -80^{O}C until later use. Plasma samples were diluted 2-fold for the pro-inflammatory panel, 1000-fold for the vascular injury panel and 11-fold for sE-selectin. In addition, for the pro-inflammatory panel, all samples had two freeze-thaw cycles, while samples for the vascular injury and sE-selectin panels had either two (vascular: n = 649; sE-selectin: n = 559) or three (vascular: n = 111; sE-selectin: n = 201) freeze-thaw cycles. Although multiple freeze-thaw cycles may cause variation in concentration, multiple studies have shown that the vascular injury and sE-selectin markers were stable even after multiple freeze-thaw cycles [20, 21].

For the pro-inflammatory and vascular injury panels, plate reading was done using MESO QuickPlex SQ 120 instrument equipped with DISCOVERY WORKBENCH® data analysis software. Sample duplicates between 40% to 60% coefficient of variation (CV) were excluded. If one of the two duplicate concentration was "below detection limit", it received the lower limit of detection (LLOD) value and sample with "below fit curve" were given ½ of LLOD value [22]. A 4-parameter logistic model was used to calculate the concentrations. For sE-selectin, plate reading was done using *CLARIOstar® Plus* (software version 5.70R2) microplate reader equipped with CLARIOstar MARS data analysis software (version 3.42R4). Plates were measured at 450nm as the primary wavelength and 650nm as the reference wavelength. Concentrations higher than the highest standard (n = 3) were calculated based on the formula written in the software (maximum standard concentration * 1.5). A 4-parameter logistic model was used to calculate the concentrations.

The inter-assay percent CV for IFNγ, TNFα, IL6, IL8, IL10, CRP, sICAM-1, sVCAM-1, and sE-selectin were <10%, except for SAA which was 18%. IL1β

and IL2 calculations were invalid because 90% of IL1β and 55% of IL2 control samples were either below detection limit or below fit curve. The average intra-assay percent CV based on duplicates for each marker was <7%, except for IL1β (16%), IL2 (15%), and IL10 (10%) (Supplementary Table S1).

Potential covariates

Several factors have shown to influence cognitive performance and/or affect regulation, and or used as covariates in our statistical models (see below). Body mass index (BMI), smoking status (never, former [more than 6 months ago], and current smoker), and alcohol consumption (none, low [≤ 7 glasses per week for women, ≤14 glasses per week for men], high [> 7 for women, >14 for men]) are known to influence cognitive and affective symptoms [23-25]. In addition, cognitive function, measured by mini mental state examination (MMSE), is associated with affective symptoms [26, 27]. Lastly, cardiovascular disease, cerebrovascular disease, and hypertension have been associated with cognitive and affective symptomatology [28-33].

Statistical analysis

All statistical analyses were performed using SPSS (version 27). Based on the Shapiro-Wilk normality test, patient characteristics were statistically analyzed using Kruskal-Wallis with Bonferroni multiple testing correction or Pearson chi-square test. Furthermore, all inflammatory and endothelial markers were log transformed prior to any statistical association analysis. To investigate associations of plasma inflammatory and endothelial markers with affective symptomatology, binary logistic- and negative binomial- regression analyses were performed while controlling for covariates. All assumptions were tested and met for all statistical models and results are reported as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Before running any statistical model (see below), we performed an interaction analysis between age and baseline diagnosis for group comparisons because age is the main risk factor for developing dementia. Additionally, cognitive impairment influences inflammatory markers and therefore interaction analyses between baseline diagnosis and inflammatory concentration was performed. All factors and covariates were analyzed as main effects, unless otherwise stated. If an interaction effect was shown, then association studies were conducted in subgroups.

Various models were performed to adjust for the effect of covariates on the association between aforementioned markers with affective symptomatology tests. The models were adjusted as follows:

- *Model 1*: age, gender, and educational level (low, middle, high);
- *Model 2* (main model): model 1 + baseline diagnosis (SCD, MCI, dementia);
- *Model 3*: model 2 + lifestyle (BMI, smoking status, and alcohol consumption);
- *Sensitivity analysis (SA)*: model 3 + MMSE, cardiovascular disease, cerebrovascular disease, and hypertension.

Model 2 with SA conditions and *Model 2 with model 3 conditions* analyses were only performed if a cytokine was significantly associated with affective symptomatology in *model 2*, but lost its significance in *model 3* or in *SA* to examine if change in sample size was the issue. P-values less than 0.05 were considered as nominal significant, while q-values (obtained after bonferroni multiple testing correction) of less than 0.05 were considered as statistically significant. For association analyses, a p-value lower than 0.004 (0.05/12 variables) was considered statistically significant after Bonferroni correction.

Results

Characteristics of study population

In this study population, there was a significant age difference between the groups, with dementia patients being older than SCD and MCI patients, and patients with MCI were older than patients with SCD (Table 1). Additionally, significant differences between the groups were shown for BMI, MMSE, and cardiovascular disease. However, no significant differences were shown for gender, educational level, tobacco usage, alcohol consumption, hypertension, and cerebrovascular diagnosis (Table 1). In regard to affective symptoms, there was a significant difference between the groups in NPIA, NPIA-DS, GDS15, and GDS15 cut-off scale (Table 2). In the NPID group comparisons, once adjusted for age, gender, educational level, and multiple testing correction, informants of patients with dementia showed higher IRR of reporting presence of depressive-like symptom (NPID) than patients with SCD (Table 3). For NPIA, informants of patients with dementia showed higher IRR of reporting presence of anxiety-like symptom than patients with SCD and MCI (Table 3). No significant group

differences were shown for NPID-DS (p = 0.83), NPIA-DS (p = 0.17), GDS15 (p = 0.37), and GDS15 cut-off scale (p = 0.79) (Data not shown). Lastly, in the inflammatory and endothelial marker comparisons, significant differences were shown in plasma levels of TNFα, IL6, IL8, IL10, SAA, sICAM-1, and sVCAM-1 (Table 4) between the SCD, MCI and dementia group.

Table 1. Demographics

	SCD (n = 293)	MCI (n = 304)	Dementia (n = 162)	p-value
Demographics				
Age (year)	61.8 ± 10.9	71.2 ± 9.6[a****,]	75.5 ± 7.7[b****, c***]	0.000
Gender				
Male/Female	183/110	171/133	84/78	0.072
Educational level				
Low/Mid/High	92/122/79	108/121/75	74/55/33	0.052
BMI (kg/m²)	27.0 ± 5.2 (n = 227)	26.7 ± 4.5 (n = 236)	25.8 ± 4.0[b*] (n = 129)	0.028
Tobacco usage				
Never/Former/Current	132/104/47	123/120/45	79/59/18	0.384
Alcohol consumption				
None/Low/High	69/171/35	76/163/40	36/91/18	0.887
Cardiovascular disease				
Absent/Present	151/139	128/171	61/96	0.013
Hypertension				
Absent/Present	57/84	71/101	39/58	0.981
Cerebrovascular disease				
Absent/Present	267/26	252/40	135/22	0.139
Cognition				
MMSE	28.7 ± 1.4	26.9 ± 2.4[a****]	24.2 ± 2.4[b****, c****]	0.000

Data are presented as n or mean ± standard deviation (SD). The sample size of each measurements are equal to the group sample size, unless otherwise stated. Kruskal-Wallis with Bonferroni correction or Pearson chi-square tests were used to analyze the difference between subjective cognitive decline (SCD), mild cognitive impairment (MCI), and dementia. Significance after Bonferroni correction: [a]SCD vs MCI; [b]SCD vs dementia; [c]MCI vs dementia; [*]q < 0.05; [**]q < 0.01; [***]q < 0.001; [****]q < 0.0001. Abbreviations: BMI, body mass index; MMSE, mini-mental state exam.

Table 2. Scores on neuropsychiatric tests

	SCD (n = 293)	MCI (n = 304)	Dementia (n = 162)	p-value
Affective				
NPID				
Absent/Present	156/133	169/133	82/77	0.662
NPID-DS	2.0 ± 2.9 (n = 289)	1.8 ± 2.9 (n = 304)	1.8 ± 2.6 (n = 158)	0.709

Table 2. (Continue)

	SCD (n = 293)	MCI (n = 304)	Dementia (n = 162)	p-value
Affective				
NPIA				
Absent/Present	213/76	226/76	101/58	0.026
NPIA-DS	1.2 ± 2.4 (n = 29)	1.1 ± 2.2 (n = 301)	1.6 ± 2.7^{c*} (n = 159)	0.024
GDS15	4.0 ± 3.1 (n = 283)	3.2 ± 2.7^{a**} (n = 290)	2.8 ± 2.3^{b***} (n = 156)	0.000
GDS15 cut-off scale (4/5)				
No depression/Depression	197/86	226/64	126/30	0.014

Data are presented as n or mean ± standard deviation (SD). The sample size of each measurements are equal to the group sample size, unless otherwise stated. Kruskal-Wallis with Bonferroni correction or Pearson chi-square tests were used to analyze the difference between subjective cognitive decline (SCD), mild cognitive impairment (MCI), and dementia. Significance after Bonferroni correction: [a]SCD vs MCI; [b]SCD vs dementia; [c]MCI vs dementia; $^{*}q < 0.05$; $^{**}q < 0.01$; $^{***}q < 0.001$; $^{****}q < 0.0001$. Abbreviations: NPID, neuropsychiatric inventory depression; NPID-DS, NPID-domain score; NPIA, NPI anxiety; NPIA-DS, NPIA-domain score; GDS15, 15-item geriatric depression scale.

Table 3. Presence of depression and anxiety across the groups

	NPID		NPIA	
	IRR (95% CI)	**q-value**	**IRR (95% CI)**	**q-value**
SCD[a] vs MCI	1.312 (0.911, 1.890)	0.427	1.114 (0.742, 1.672)	1.00
SCD[a] vs Dementia	1.801 (1.145, 2.831)	0.030	2.067 (1.271, 3.361)	0.012
MCI[a] vs Dementia	1.372 (0.922, 2.042)	0.352	1.856 (1.211, 2.843)	0.017

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Binary logistic regression with Bonferroni correction (q-value) and adjusted for age, gender, and educational level was used to analyze the association between the groups. [a]Reference group. NPID (n = 750) and NPIA (n = 750). Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; NPID, neuropsychiatric inventory depression; NPIA, NPI anxiety.

Table 4. Plasma levels of inflammatory and endothelial markers

	SCD (n = 293)	MCI (n = 304)	Dementia (n = 162)	p-value
Inflammatory marker				
IFNγ (pg/ml)	7.0 ± 8.0 (n = 292)	8.3 ± 16.8 (n = 304)	8.2 ± 10.2 (n = 161)	0.404
TNFα (pg/ml)	2.3 ± 0.8 (n = 293)	2.7 ± 2.6^{a***} (n = 304)	2.6 ± 1.0^{b**} (n = 160)	0.000
IL1β (pg/ml)	0.1 ± 0.1 (n = 290)	0.1 ± 0.1 (n = 298)	0.1 ± 0.2 (n = 158)	0.184

Table 4. (Continue)

	SCD (n = 293)	MCI (n = 304)	Dementia (n = 162)	p-value
Inflammatory marker				
IL6 (pg/ml)	1.0 ± 1.6 (n = 293)	1.1 ± 1.0[a**] (n = 304)	1.3 ± 1.2[b****] (n = 161)	0.000
IL8 (pg/ml)	4.7 ± 5.9 (n = 293)	5.7 ± 9.8[a****] (n = 304)	5.3 ± 2.9[b****] (n = 161)	0.000
IL10 (pg/ml)	0.3 ± 0.4 (n = 292)	0.5 ± 3.8 (n = 300)	0.3 ± 0.3 (n = 157)	0.034
IL2 (pg/ml)	0.2 ± 0.2 (n = 288)	0.3 ± 1.4 (n = 302)	0.2 ± 0.2 (n = 159)	0.648
CRP (µg/ml)	3.68 ± 7.96	4.04 ± 7.04	3.83 ± 9.11	0.693
SAA (µg/ml)	7.14 ± 20.21 (n = 293)	12.02 ± 30.12[a***] (n = 303)	12.26 ± 34.36 (n = 162)	0.001
Endothelial marker				
sICAM-1 (ng/ml)	439.29 ± 122.70	463.15 ± 132.09[a*]	454.22 ± 142.18	0.041
sVCAM-1 (ng/ml)	470.69 ± 107.91	517.63 ± 182.90[a**]	528.96 ± 140.72[b****]	0.000
sE-selectin (ng/ml)	115.92 ± 55.09	118.26 ± 69.39	108.25 ± 58.53	0.244

Data are presented as mean ± standard deviation (SD). The sample size of each measurements are equal to the group sample size, unless otherwise stated. Kruskal-Wallis with Bonferroni correction or Pearson chi-square tests were used to analyze the difference between subjective cognitive decline (SCD), mild cognitive impairment (MCI), and dementia. Significance after Bonferroni correction: [a]SCD vs MCI; [b]SCD vs dementia; [c]MCI vs dementia; [*]$q < 0.05$; [**]$q < 0.01$; [***]$q < 0.001$; [****]$q < 0.0001$. Abbreviations: IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; SAA, serum amyloid A; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; sE-selectin; soluble E-selectin.

Associations between inflammatory and endothelial markers and self-reported depressive symptoms

In *model 2*, higher plasma levels of TNFα, IL6, IL8, CRP, SAA, sICAM-1, and sVCAM-1 were associated with higher IRR in GDS15. Furthermore, TNFα, IL6, and CRP were still significant after multiple testing correction (Table 5). However, when adjusting for lifestyle (*model 3*), only TNFα remained significant and no markers were significant in the SA (Supplementary Table S2). Additionally, IL10 ($p = 0.038$) and sE-selectin ($p = 0.027$) showed a significant interaction with diagnosis status. Therefore, in the subgroup analysis, higher levels of IL10 and sE-selectin were associated with higher IRR in GDS15 score for dementia subgroup and SCD and MCI subgroups, respectively (Table 6; Supplementary Table S3).

Table 5. Association between inflammatory and endothelial markers with GDS15

	GDS15	GDS15 cut-off
Inflammatory marker		
IFNγ		
Model 1	1.179 (1.001, 1.389)*	1.578 (0.959, 2.598)
Model 2	1.174 (0.997, 1.384)	1.565 (0.949, 2.579)
TNFα		
Model 1	2.437 (1.661, 3.576)#	7.521 (2.340, 24.172)#
Model 2	2.443 (1.665, 3.584)#	7.584 (2.360, 24.377)#
IL1β		
Model 1	1.117 (0.895, 1.393)	1.586 (0.816, 3.085)
Model 2	1.117 (0.896, 1.394)	1.590 (0.817, 3.094)
IL6		
Model 1	1.393 (1.143, 1.696)#	2.094 (1.156, 3.793)*
Model 2	1.391 (1.143, 1.694)#	2.088 (1.152, 3.783)*
IL8		
Model 1	1.394 (1.022, 1.902)*	1.759 (0.712, 4.346)
Model 2	1.410 (1.033, 1.926)*	1.796 (0.726, 4.441)
IL10		
Model 1	1.246 (1.000, 1.554)	1.982 (1.041, 3.772)*
Model 2	IE	IE
IL2		
Model 1	1.171 (0.949, 1.445)	1.391 (0.746, 2.593)
Model 2	1.166 (0.945, 1.439)	1.381 (0.739, 2.581)
CRP		
Model 1	1.224 (1.100, 1.361)#	1.537 (1.107, 2.134)*
Model 2	1.218 (1.095, 1.355)#	1.532 (1.102, 2.131)*
SAA		
Model 1	1.182 (1.044, 1.337)**	1.628 (1.129, 2.348)**
Model 2	1.182 (1.045, 1.339)**	1.640 (1.136, 2.369)**
Endothelial marker		
sICAM-1		
Model 1	2.078 (1.244, 3.471)*	4.562 (0.954, 21.825)
Model 2	2.100 (1.257, 3.509)*	4.638 (0.966, 22.270)
sVCAM-1		
Model 1	1.790 (1.027, 3.118)*	3.918 (0.740, 20.744)
Model 2	1.820 (1.045, 3.169)*	3.990 (0.751, 21.185)
sE-selectin		
Model 1	1.554 (1.190, 2.029)**	2.822 (1.239, 6.426)*
Model 2	IE	2.865 (1.254, 6.544)*

IRR (95% CI) as outcome of respective negative binomial regression and binary logistic regression. Further covariate adjusted models can be found in the Supplementary Table S2 and S4. IE, interaction effect; *$p < 0.05$; **$p \leq 0.01$. #Significance after Bonferroni correction ($q < 0.004$). Abbreviations: IRR, incidence rate ratio; 95% CI, 95% confidence interval; SCD, subjective cognitive decline; MCI, mild cognitive impairment; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; SAA, serum amyloid A; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; sE-selectin; soluble E-selectin; GDS15, 15-item geriatric depression scale.

Table 6. Association between IL10 and sE-selectin with GDS15 in subgroup population

	SCD	MCI	Dementia
IL10			
Model 2	0.949 (0.664, 1.356)	1.262 (0.908, 1.754)	2.093 (1.259, 3.480)**
sE-selectin			
Model 2	1.872 (1.224, 2.864)**	1.726 (1.153, 2.584)**	0.729 (0.381, 1.394)

IRR (95% CI) as outcome of negative binomial regression. *Model 1*: adjusted for age, gender, and educational level; *Model 2*: adjusted for *model 1* and diagnosis status. Further covariate adjusted models can be found in the Supplementary Table S3. *p < 0.05; **p ≤ 0.01. Abbreviations: IRR, incidence rate ratio; 95% CI, 95% confidence interval; SCD, subjective cognitive decline; MCI, mild cognitive impairment; IL, interleukin; sE-selectin; soluble E-selectin; GDS15, 15-item geriatric depression scale.

In the GDS15 cut-off scale analysis, similar to GDS15, higher plasma levels of TNFα, IL6, CRP, SAA, and sE-selectin were associated with increased IRR in *model 2* and only TNFα survived multiple testing correction (Table 5). However, no markers were significant after adjusting for lifestyle and SA (Supplementary Table S4). Furthermore, IL10 showed interaction with diagnosis status (p = 0.048), thus subgroup analysis showed higher levels of IL10 was associated with increased IRR in patients with dementia (Table 7; Supplementary Table S5).

Table 7. Association between IL10 with GDS15 cut-off in subgroup population

	SCD	MCI	Dementia
IL10			
Model 2	0.938 (0.329, 2.671)	2.258 (0.844, 6.042)	10.903 (1.986, 59.857)**

IRR (95% CI) as outcome of binary logistic regression. *Model 1*: adjusted for age, gender, and educational level; *Model 2*: adjusted for *model 1* and diagnosis status. Further covariate adjusted models can be found in the Supplementary Table S5. *p < 0.05; **p ≤ 0.01. Abbreviations: IRR, incidence rate ratio; 95% CI, 95% confidence interval; SCD, subjective cognitive decline; MCI, mild cognitive impairment; IL, interleukin; GDS15, 15-item geriatric depression scale.

Association between inflammatory and endothelial markers and informant reported depressive- and anxiety-like symptoms

In the NPID analysis, neither NPID nor NPID-DS showed significant association with inflammatory and endothelial markers in all models (Table 8; Supplementary Tables S6 and S7). In a similar manner, there was no association between NPIA and any of the inflammatory nor endothelial markers (Table 9). However, CRP (p = 0.035), SAA (p = 0.018), and sE-selectin (p = 0.013) showed

an interaction effect with diagnosis status, and, hence, subgroup analyses were conducted. The presence of anxiety-like symptoms was associated with lower levels of CRP and SAA in patients with MCI and with lower sE-selectin levels in patients with dementia (Table 10). However, these associations were no longer significant after adjusting for other factors (Supplementary Tables S8 and S9). Lastly, no associations were present in the NPIA-DS analysis (Table 9; Supplementary Table S10).

Table 8. Association between inflammatory and endothelial markers with NPI depression

	NPID	NPID-DS
Inflammatory marker		
IFNγ		
Model 1	0.857 (0.556, 1.320)	0.928 (0.624, 1.379)
Model 2	0.885 (0.573, 1.364)	0.933 (0.627, 1.388)
TNFα		
Model 1	0.889 (0.317, 2.492)	1.056 (0.390, 2.855)
Model 2	0.917 (0.327, 2.574)	1.067 (0.394, 2.891)
IL1β		
Model 1	0.582 (0.325, 1.042)	1.007 (0.594, 1.707)
Model 2	0.581 (0.324, 1.042)	1.002 (0.591, 1.699)
IL6		
Model 1	1.035 (0.614, 1.744)	1.143 (0.715, 1.827)
Model 2	1.042 (0.617, 1.760)	1.144 (0.715, 1.829)
IL8		
Model 1	1.374 (0.619, 3.048)	1.305 (0.582, 2.923)
Model 2	1.347 (0.605, 2.998)	1.304 (0.579, 2.938)
IL10		
Model 1	0.843 (0.484, 1.467)	0.989 (0.576, 1.700)
Model 2	0.839 (0.481, 1.462)	0.985 (0.573, 1.694)
IL2		
Model 1	0.923 (0.538, 1.584)	0.887 (0.548, 1.437)
Model 2	0.942 (0.548, 1.617)	0.889 (0.549, 1.441)
CRP		
Model 1	0.900 (0.679, 1.193)	0.929 (0.722, 1.196)
Model 2	0.925 (0.697, 1.227)	0.936 (0.726, 1.207)
SAA		
Model 1	0.951 (0.688, 1.316)	0.985 (0.728, 1.332)
Model 2	0.956 (0.690, 1.325)	0.992 (0.732, 1.343)
Endothelial marker		
sICAM-1		
Model 1	1.382 (0.357, 5.343)	1.170 (0.347, 3.952)
Model 2	1.451 (0.372, 5.654)	1.167 (0.344, 3.957)
sVCAM-1		
Model 1	1.374 (0.324, 5.836)	0.951 (0.246, 3.680)
Model 2	1.324 (0.310, 5.650)	0.967 (0.250, 3.750)

Table 8. (Continue)

	NPID	NPID-DS
Endothelial marker		
sE-selectin		
Model 1	1.584 (0.792, 3.167)	1.087 (0.580, 2.037)
Model 2	1.625 (0.811, 3.257)	1.089 (0.581, 2.042)

IRR (95% CI) as outcome of respective negative binomial regression and binary logistic regression. *Model 1*: adjusted for age, gender, and educational level; *Model 2*: adjusted for *model 1* and diagnosis status. Further covariate adjusted models can be found in the Supplementary Tables S6 and S7. $^{*}p < 0.05$; $^{**}p \leq 0.01$. Abbreviations: IRR, incidence rate ratio; 95% CI, 95% confidence interval; SCD, subjective cognitive decline; MCI, mild cognitive impairment; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; SAA, serum amyloid A; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; sE-selectin; soluble E-selectin; NPID, neuropsychiatric inventory depression; NPID-DS, NPID-domain score.

Table 9. Association between inflammatory and endothelial markers with NPI anxiety

	NPIA	NPIA-DS
Inflammatory marker		
IFNγ		
Model 1	1.055 (0.659, 1.689)	0.847 (0.479, 1.497)
Model 2	1.099 (0.685, 1.762)	0.837 (0.473, 1.481)
TNFα		
Model 1	0.972 (0.311, 3.041)	0.931 (0.250, 3.470)
Model 2	1.041 (0.331, 3.276)	1.021 (0.274, 3.804)
IL1β		
Model 1	1.120 (0.599, 2.092)	1.103 (0.542, 2.248)
Model 2	1.150 (0.614, 2.155)	1.076 (0.533, 2.175)
IL6		
Model 1	0.556 (0.305, 1.012)	0.629 (0.304, 1.302)
Model 2	0.550 (0.300, 1.007)	0.601 (0.290, 1.245)
IL8		
Model 1	1.101 (0.458, 2.647)	1.396 (0.415, 4.698)
Model 2	1.109 (0.455, 2.705)	1.420 (0.431, 4.676)
IL10		
Model 1	0.709 (0.374, 1.344)	0.769 (0.347, 1.702)
Model 2	0.715 (0.375, 1.364)	0.791 (0.359, 1.742)
IL2		
Model 1	0.695 (0.379, 1.275)	0.627 (0.333, 1.179)
Model 2	0.718 (0.390, 1.323)	0.659 (0.352, 1.236)
CRP		
Model 1	0.767 (0.561, 1.049)	0.877 (0.598, 1.286)
Model 2	IE	0.878 (0.596, 1.293)
SAA		
Model 1	0.865 (0.600, 1.245)	1.023 (0.647, 1.619)
Model 2	IE	1.037 (0.656, 1.638)

Table 9. (Continue)

	NPIA	NPIA-DS
Endothelial marker		
sICAM-1		
Model 1	0.255 (0.055, 1.190)	0.526 (0.085, 3.254)
Model 2	0.283 (0.060, 1.328)	0.592 (0.096, 3.640)
sVCAM-1		
Model 1	0.508 (0.100, 2.581)	0.735 (0.098, 5.482)
Model 2	0.488 (0.095, 2.513)	0.878 (0.117, 6.572)
sE-selectin		
Model 1	1.103 (0.517, 2.357)	1.116 (0.499, 2.723)
Model 2	IE	1.334 (0.571, 3.112)

IRR (95% CI) as outcome of respective negative binomial regression and binary logistic regression. *Model 1*: adjusted for age, gender, and educational level; *Model 2*: adjusted for *model 1* and diagnosis status. Further covariate adjusted models can be found in the Supplementary Tables S8 and S10. IE, interaction effect; $^{*}p < 0.05$; $^{**}p \leq 0.01$. Abbreviations: IRR, incidence rate ratio; 95% CI, 95% confidence interval; SCD, subjective cognitive decline; MCI, mild cognitive impairment; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; SAA, serum amyloid A; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; sE-selectin; soluble E-selectin; NPIA, neuropsychiatric inventory anxiety; NPIA-DS, NPIA-domain score.

Table 10. Association between CRP, SAA, and sE-selectin with NPIA in subgroup population

	SCD	MCI	Dementia
CRP			
Model 2	1.356 (0.798, 2.302)	0.489 (0.291, 0.823)**	0.720 (0.362, 1.431)
SAA			
Model 2	1.453 (0.778, 2.713)	0.436 (0.227, 0.835)*	1.160 (0.575, 2.340)
sE-selectin			
Model 2	2.000 (0.544, 7.350)	2.093 (0.622, 7.039)	0.070 (0.011, 0.463)**

IRR (95% CI) as outcome of negative binomial regression. *Model 1*: adjusted for age, gender, and educational level; *Model 2*: adjusted for *model 1* and diagnosis status. Further covariate adjusted models can be found in the Supplementary Table S9. $^{*}p < 0.05$; $^{**}p \leq 0.01$. Abbreviations: IRR, incidence rate ratio; 95% CI, 95% confidence interval; SCD, subjective cognitive decline; MCI, mild cognitive impairment; NPIA, neuropsychiatric inventory anxiety; CRP, C-reactive protein; SAA, serum amyloid A; sE-selectin, soluble E-selectin.

Discussion

This large cross-sectional study investigated associations between plasma inflammatory and endothelial markers with affective symptoms in patients with cognitive complaints, cognitive impairment, and dementia. Overall, higher levels

of several inflammatory (TNFα, IL6, IL8, IL10, CRP, and SAA) and endothelial markers (sICAM-1, sVCAM-1, and sE-selectin) were associated with higher IRR of self-reported depressive symptoms (GDS15) and lower levels of CRP, SAA, and sE-selectin were associated with higher IRR of informants reporting anxiety-like symptoms (NPIA).

Although both NPID and GDS15 are designed to screen depressive symptoms, after adjusting for covariates, only NPID screened for depressive status between groups while GDS15 could not, but vice versa for association studies. This could be due to the nature of the study design. One study has shown that the content, validity, reliability and consistency of the NPI were valid and reliable, but due to their scoring design, parametric analysis was deemed not applicable [34]. Therefore, our NPIA data is too limited to make a conclusion, thus further research in anxiety symptom using clinical anxiety assessment tools is necessary. On the other hand, the GDS15 is deemed to be an effective, reliable, and accurate screening tool for depression, but lost its validity in a large population of patients with dementia of the Alzheimer's type [35]. However, more recent study concluded that GDS15 was able to assess depressive symptoms among very old people with an MMSE score of 10 or more, but not for older people with MMSE scores lower than 10 [36]. Our dementia patients had an average MMSE score of 24 and our *models 1 and 2* analyses showed that patient diagnosis had little-to-no influence in the association outcome. Since affective symptomatology are heterogeneous symptoms that vary between individuals, despite being clinically validated screening tool, each screening tool has its strengths and weaknesses. Therefore, it is important to assess the symptoms with multiple clinical tests as demonstrated in this study.

Pro-inflammatory cytokines and depressive symptoms

Although there are no studies similar to this study, there are studies investigating the association between systemic inflammation and GDS15 in different study population. For example, a prospective study investigated in older individuals and showed that, in cross-sectional analysis, CRP, IL6 and TNFα were significantly associated with depression at baseline. Additionally, longitudinal analysis controlling for covariates showed that IL6 and TNFα were associated with increased risk of depression at follow-ups [37]. Another prospective study of non-demented community-dwelling elderly participants reported positive association between IL6 and GDS15, whereas IL8 was positively associated with

depressive symptoms at baseline and at 2 years follow-ups, even after adjusting for various factors [38]. Lastly, one study looked into post-myocardial infarction patients and revealed that higher levels of CRP were associated with more depressive and apathy symptoms, and less dispositional optimism [39].

The relationship between depression and levels of circulating markers of inflammation is evidenced by four independent meta-analyses, reporting higher levels of TNFα [8, 11], IL6 [8-11], and CRP [9, 10] and no difference in IL1β [8, 10, 11] and IL2 [8, 11]. While we examined associations with depressive symptoms rather than clinical depression, our findings are grossly in agreement with these reports. Thus, we found depressive symptoms to be associated with higher concentrations of TNFα, IL6 and CRP. Depressive symptoms were also related to increased IL10 levels which was also reported by the meta-analysis of Köhler et al. [11].

The increase of pro-inflammatory cytokines in patients with depression may relate to 'the macrophage theory of depression', proposed by Ronald Smith in 1991 [40]. According to this theory, circulating monocytes migrate to the site of inflammation and differentiate into inflammatory dendritic cells and macrophages [41]. Macrophages can further differentiate, known as macrophage polarization, into M1 (acute defense against pathogens; secrete pro-inflammatory cytokines) or M2 (clearing damaged tissues and repairing activities; secrete anti-inflammatory cytokines) [42, 43]. Studies in both depression and AD have reported an increase in pro-inflammatory cytokines release (IL6 and TNFα) from microglia, suggesting a disturbance in the M1/M2 balance [44, 45]. At the same time, an increase in systemic inflammation can influence the neurotransmitters such as serotonin via the tryptophan-kynurenine pathway. Activation of pro-inflammatory cytokines, such as IFNγ and TNFα can induce the enzyme indoleamine 2,3-dioxygenase (IDO), which converts the serotonin precursor, tryptophan, to kynurenine and over-activation of IDO causes serotonin depletion [46]. Moreover, studies have shown that certain kynurenine pathway associated metabolites contribute to depression severity [47, 48]. Lastly, circulating pro-inflammatory cytokines stimulate the liver to release the acute phase reactants CRP and SAA. Multiple studies have reported elevated levels of CRP in patients with depression [9, 10]. Bryleva et al. (2017) reported plasma SAA concentration to be associated with depressive symptoms

and with a higher score on the Hamilton depression rating scale 17 (HAMD-17) [49].

Endothelial markers in depression

In regards to endothelial function in depression, fewer studies have investigated the role of sICAM-1, sVCAM-1 and sE-selectin. sICAM-1, an immunoglobulin (Ig)-like transmembrane glycoprotein, plays a role in leukocyte migration and activation, found in peripheral fluids [50-52]. Multiple studies have shown elevated levels of blood sICAM-1 in patients with depression [53-56]. sVCAM-1 is a transmembrane protein expressed by various cell types such as endothelial cells, neurons, smooth muscle cells, fibroblasts, and macrophages, and which expression is induced by IL1β, IL4, TNFα, and IFNγ [57, 58]. One study reported that serum sVCAM-1 was a partial mediator of depressive symptom associated with dementia [59]. Additionally, sVCAM-1 showed positive association with depressive symptom (Revised Center for Epidemiological Studies Depression Scale [CESD-R]) in community-dwelling participants [60] and patients with depression demonstrated elevated sVCAM-1 levels compared to controls [53]. Lastly, sE-selectin is a glycoprotein only expressed on endothelial cells and activated by IL1, TNFα, or bacterial lipopolysaccharide (LPS) and mediates leukocyte rolling on endothelium allowing neutrophil, monocyte, and T-cell recruitment to the inflammatory foci [61, 62]. Our data showed sE-selectin to have an interaction effect with diagnosis in the GDS15, but not in the GDS15 cut-off scale. Although no study investigated sE-selectin in SCD, MCI, and dementia patients with depressive symptoms, studies in patients with depression did report higher sE-selectin levels compared to non-depressed patients [56]. In another study, sE-selectin was positively associated with depressive symptoms and the presence of a depressive disorder [55]. Therefore, a large body of evidence demonstrates that endothelial damage is present in patients with depression.

Differences in inflammatory and endothelial levels across cognitive spectrum

Although this study investigated affective symptomatology, our study populations are patients with or at risk for dementia. As mentioned before, systemic inflammation is widely observed in patients with dementia, especially in Alzheimer's disease (AD) patients. Multiple studies, have shown that inflammatory makers were elevated in patients with AD when compared to

health controls. For example, studies reported higher levels of IL6 [13, 63, 64], and TNFα [63-65] in patients with AD compared to controls. Additionally, MCI patients had higher levels of IL6 and IL8 compared to controls [13]. Furthermore, IL6 was associated with 32-40% increased risk of all-cause dementia [12, 66, 67], TNFα had a 2-fold increase in rate of cognitive decline over a 6-month period [68], and CRP was associated with increased risk of dementia [12, 66, 67] have been reported as well. Lastly, studies have shown that peripheral immune response are associated with AD pathology. For example, IL6 induces phosphorylation of tau protein [69] and early-stage amyloid plaque formation in AD [70]. Besides inflammatory markers, endothelial markers such as sVCAM-1 were shown to be elevated in AD [63, 71-73], to reflect the severity of dementia [73], and are negatively associated with cognitive performance as measured by different tests for short- and long-term memory [71, 73]. Finally, post-mortem brain tissue of patients with AD have shown ICAM-1 accumulation in senile plaques [74]. Current evidence indicates that several inflammatory and endothelial markers are associated with cognitive and affective symptomatology.

Strengths and limitations

The major strength of this study is that, to our knowledge, this is the first large cross-sectional study to demonstrate associations between systemic inflammation and affective symptoms in patients with cognitive complaints, cognitive impairment, and dementia. Furthermore, the study used various clinically validated affective symptom tests while adjusting for several covariates. The main limitations of our study were not having a neurologically healthy control group and significant age difference between the groups. In order to compensate for age difference, it was adjusted in our model. Lastly, despite the uncertainty whether the systemic inflammation markers act independent from each other, we still corrected for multiple testing, which may be associated with and increased chance for type II errors. As such, it is also important to interpret our data based on the pattern of associations (e.g., strength, consistency, or directional expectation).

Conclusion

Overall, results from this large cross-sectional study confirmed previous findings such as IL6, IL8, CRP, and sVCAM-1, but also reported new findings such as TNFα, sICAM-1, sE-selectin, and interaction effects between IL10 and sE-

selectin with diagnosis in the association between plasma inflammatory and endothelial markers with depressive symptoms in patients with cognitive complaints, cognitive impairment, and dementia.

References

1. Wallin K, Bostrom G, Kivipelto M, Gustafson Y. Risk factors for incident dementia in the very old. Int Psychogeriatr. 2013;25(7):1135-43.
2. Petersen RC. Mild cognitive impairment as a diagnostic entity. J Intern Med. 2004;256(3):183-94.
3. Jessen F, Amariglio RE, Van Boxtel M, Breteler M, Ceccaldi M, Chételat G, et al. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. Alzheimer's & dementia. 2014;10(6):844-52.
4. Mitchell A, Beaumont H, Ferguson D, Yadegarfar M, Stubbs B. Risk of dementia and mild cognitive impairment in older people with subjective memory complaints: meta-analysis. Acta Psychiatrica Scandinavica. 2014;130(6):439-51.
5. Thaipisuttikul P, Jaikla K, Satthong S, Wisajun P. Rate of conversion from mild cognitive impairment to dementia in a Thai hospital-based population: A retrospective cohort. Alzheimer's & Dementia: Translational Research & Clinical Interventions. 2022;8(1):e12272.
6. Leung DKY, Chan WC, Spector A, Wong GHY. Prevalence of depression, anxiety, and apathy symptoms across dementia stages: A systematic review and meta-analysis. Int J Geriatr Psychiatry. 2021;36(9):1330-44.
7. Ismail Z, Elbayoumi H, Fischer CE, Hogan DB, Millikin CP, Schweizer T, et al. Prevalence of depression in patients with mild cognitive impairment: a systematic review and meta-analysis. JAMA psychiatry. 2017;74(1):58-67.
8. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, et al. A Meta-Analysis of Cytokines in Major Depression. Biological Psychiatry. 2010;67(5):446-57.
9. Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. Psychosom Med. 2009;71(2):171-86.
10. Haapakoski R, Mathieu J, Ebmeier KP, Alenius H, Kivimäki M. Cumulative meta-analysis of interleukins 6 and 1β, tumour necrosis factor α and C-reactive protein in patients with major depressive disorder. Brain Behav Immun. 2015;49:206-15.
11. Köhler CA, Freitas TH, Maes M, de Andrade NQ, Liu CS, Fernandes BS, et al. Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. Acta Psychiatrica Scandinavica. 2017;135(5):373-87.
12. Koyama A, O'Brien J, Weuve J, Blacker D, Metti AL, Yaffe K. The role of peripheral inflammatory markers in dementia and Alzheimer's disease: a meta-analysis. J Gerontol A Biol Sci Med Sci. 2013;68(4):433-40.
13. Shen X-N, Niu L-D, Wang Y-J, Cao X-P, Liu Q, Tan L, et al. Inflammatory markers in Alzheimer's disease and mild cognitive impairment: a meta-analysis and systematic review of 170 studies. Journal of Neurology, Neurosurgery & Psychiatry. 2019;90(5):590-8.
14. Ng A, Tam WW, Zhang MW, Ho CS, Husain SF, McIntyre RS, et al. IL-1β, IL-6, TNF- α and CRP in Elderly Patients with Depression or Alzheimer's disease: Systematic Review and Meta-Analysis. Scientific Reports. 2018;8(1):12050.
15. Yesavage JA, Sheikh JI. 9/Geriatric Depression Scale (GDS). Clinical Gerontologist. 1986;5(1-2):165-73.
16. Dias F, Teixeira AL, Guimarães HC, Barbosa MT, Resende EPF, Beato RG, et al. Accuracy of the 15-item Geriatric Depression Scale (GDS-15) in a community-dwelling oldest-old sample: the Pietà Study. Trends Psychiatry Psychother. 2017;39(4):276-9.
17. Marc LG, Raue PJ, Bruce ML. Screening performance of the 15-item geriatric depression scale in a diverse elderly home care population. Am J Geriatr Psychiatry. 2008;16(11):914-21.
18. Weintraub D, Oehlberg KA, Katz IR, Stern MB. Test characteristics of the 15-item geriatric depression scale and Hamilton depression rating scale in Parkinson disease. The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry. 2006;14(2):169-75.

19. Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J. The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. Neurology. 1994;44(12):2308-14.
20. Hartweg J, Gunter M, Perera R, Farmer A, Cull C, Schalkwijk C, et al. Stability of soluble adhesion molecules, selectins, and C-reactive protein at various temperatures: implications for epidemiological and large-scale clinical studies. Clin Chem. 2007;53(10):1858-60.
21. Gislefoss RE, Lauritzen M, Langseth H, Mørkrid L. Effect of multiple freeze-thaw cycles on selected biochemical serum components. Clinical Chemistry and Laboratory Medicine (CCLM). 2017;55(7):967-73.
22. Hornung RW, Reed LD. Estimation of Average Concentration in the Presence of Nondetectable Values. Applied Occupational and Environmental Hygiene. 1990;5(1):46-51.
23. Fluharty M, Taylor AE, Grabski M, Munafò MR. The Association of Cigarette Smoking With Depression and Anxiety: A Systematic Review. Nicotine Tob Res. 2017;19(1):3-13.
24. Luppino FS, de Wit LM, Bouvy PF, Stijnen T, Cuijpers P, Penninx BW, et al. Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. Arch Gen Psychiatry. 2010;67(3):220-9.
25. Cournot M, Marquié JC, Ansiau D, Martinaud C, Fonds H, Ferrières J, et al. Relation between body mass index and cognitive function in healthy middle-aged men and women. Neurology. 2006;67(7):1208-14.
26. Gotlib IH, Joormann J. Cognition and depression: current status and future directions. Annu Rev Clin Psychol. 2010;6:285-312.
27. Ma L. Depression, Anxiety, and Apathy in Mild Cognitive Impairment: Current Perspectives. Frontiers in Aging Neuroscience. 2020;12(9).
28. Alfaddagh A, Martin SS, Leucker TM, Michos ED, Blaha MJ, Lowenstein CJ, et al. Inflammation and cardiovascular disease: From mechanisms to therapeutics. American Journal of Preventive Cardiology. 2020;4:100130.
29. Hare DL, Toukhsati SR, Johansson P, Jaarsma T. Depression and cardiovascular disease: a clinical review. Eur Heart J. 2014;35(21):1365-72.
30. Patrick DM, Van Beusecum JP, Kirabo A. The role of inflammation in hypertension: novel concepts. Current Opinion in Physiology. 2021;19:92-8.
31. Seidel GA, Giovannetti T, Libon DJ. Cerebrovascular disease and cognition in older adults. Curr Top Behav Neurosci. 2012;10:213-41.
32. Stuart MJ, Baune BT. Depression and type 2 diabetes: inflammatory mechanisms of a psychoneuroendocrine co-morbidity. Neurosci Biobehav Rev. 2012;36(1):658-76.
33. Wang K, Li F, Cui Y, Cui C, Cao Z, Xu K, et al. The Association between Depression and Type 1 Diabetes Mellitus: Inflammatory Cytokines as Ferrymen in between? Mediators of Inflammation. 2019;2019:2987901.
34. Lai CKY. The merits and problems of Neuropsychiatric Inventory as an assessment tool in people with dementia and other neurological disorders. Clin Interv Aging. 2014;9:1051-61.
35. Burke WJ, Houston MJ, Boust SJ, Roccaforte WH. Use of the Geriatric Depression Scale in dementia of the Alzheimer type. J Am Geriatr Soc. 1989;37(9):856-60.
36. Conradsson M, Rosendahl E, Littbrand H, Gustafson Y, Olofsson B, Lövheim H. Usefulness of the Geriatric Depression Scale 15-item version among very old people with and without cognitive impairment. Aging Ment Health. 2013;17(5):638-45.
37. Shi R, Gwee X, Chua DQL, Tan CTY, Yap KB, Larbi A, et al. Inflammatory markers and incident depression: Evidence in a population-based prospective study. Psychoneuroendocrinology. 2022;142:105806.
38. Baune BT, Smith E, Reppermund S, Air T, Samaras K, Lux O, et al. Inflammatory biomarkers predict depressive, but not anxiety symptoms during aging: the prospective Sydney Memory and Aging Study. Psychoneuroendocrinology. 2012;37(9):1521-30.
39. Heeres RHM, Hoogeveen EK, Geleijnse JM, de Goede J, Kromhout D, Giltay EJ. Kidney dysfunction, systemic inflammation and mental well-being in elderly post-myocardial infarction patients. BMC Psychology. 2017;5(1):1.

40. Smith RS. The macrophage theory of depression. Med Hypotheses. 1991;35(4):298-306.
41. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. Science. 2010;327(5966):656-61.
42. Murray PJ. Macrophage Polarization. Annual Review of Physiology. 2017;79(1):541-66.
43. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nature Reviews Immunology. 2016;16(1):22-34.
44. Dey A, Hankey Giblin PA. Insights into Macrophage Heterogeneity and Cytokine-Induced Neuroinflammation in Major Depressive Disorder. Pharmaceuticals (Basel). 2018;11(3).
45. Wang WY, Tan MS, Yu JT, Tan L. Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. Ann Transl Med. 2015;3(10):136.
46. Tanaka M, Bohár Z, Vécsei L. Are Kynurenines Accomplices or Principal Villains in Dementia? Maintenance of Kynurenine Metabolism. Molecules. 2020;25(3).
47. Ogyu K, Kubo K, Noda Y, Iwata Y, Tsugawa S, Omura Y, et al. Kynurenine pathway in depression: A systematic review and meta-analysis. Neuroscience & Biobehavioral Reviews. 2018;90:16-25.
48. Marx W, McGuinness AJ, Rocks T, Ruusunen A, Cleminson J, Walker AJ, et al. The kynurenine pathway in major depressive disorder, bipolar disorder, and schizophrenia: a meta-analysis of 101 studies. Molecular Psychiatry. 2021;26(8):4158-78.
49. Bryleva EY, Keaton SA, Grit J, Madaj Z, Sauro-Nagendra A, Smart L, et al. The acute-phase mediator serum amyloid A is associated with symptoms of depression and fatigue. Acta Psychiatr Scand. 2017;135(5):409-18.
50. Hogg N, Henderson R, Leitinger B, McDowall A, Porter J, Stanley P. Mechanisms contributing to the activity of integrins on leukocytes. Immunol Rev. 2002;186:164-71.
51. Camacho SA, Heath WR, Carbone FR, Sarvetnick N, LeBon A, Karlsson L, et al. A key role for ICAM-1 in generating effector cells mediating inflammatory responses. Nat Immunol. 2001;2(6):523-9.
52. Müller N. The Role of Intercellular Adhesion Molecule-1 in the Pathogenesis of Psychiatric Disorders. Front Pharmacol. 2019;10:1251-.
53. Dimopoulos N, Piperi C, Salonicioti A, Mitsonis C, Liappas I, Lea RW, et al. Elevation of plasma concentration of adhesion molecules in late-life depression. Int J Geriatr Psychiatry. 2006;21(10):965-71.
54. Thomas AJ, Morris C, Davis S, Jackson E, Harrison R, O'Brien JT. Soluble cell adhesion molecules in late-life depression. Int Psychogeriatr. 2007;19(5):914-20.
55. van Dooren FE, Schram MT, Schalkwijk CG, Stehouwer CD, Henry RM, Dagnelie PC, et al. Associations of low grade inflammation and endothelial dysfunction with depression - The Maastricht Study. Brain Behav Immun. 2016;56:390-6.
56. Rajagopalan S, Brook R, Rubenfire M, Pitt E, Young E, Pitt B. Abnormal brachial artery flow-mediated vasodilation in young adults with major depression. The American Journal of Cardiology. 2001;88(2):196-8.
57. Muller WA. Mechanisms of leukocyte transendothelial migration. Annu Rev Pathol. 2011;6:323-44.
58. Cybulsky MI, Gimbrone MA, Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science. 1991;251(4995):788-91.
59. Royall DR, Al-Rubaye S, Bishnoi R, Palmer RF. Serum proteins mediate depression's association with dementia. PLoS One. 2017;12(6):e0175790.
60. Tchalla AE, Wellenius GA, Sorond FA, Travison TG, Dantoine T, Lipsitz LA. Elevated circulating vascular cell Adhesion Molecule-1 (sVCAM-1) is associated with concurrent depressive symptoms and cerebral white matter Hyperintensities in older adults. BMC Geriatr. 2015;15:62.
61. Mantovani A, Dejana E. Endothelium. In: Delves PJ, editor. Encyclopedia of Immunology (Second Edition). Oxford: Elsevier; 1998. p. 802-6.
62. Miyasaka M. Selectins (CD62-E/L/P). In: Delves PJ, editor. Encyclopedia of Immunology (Second Edition). Oxford: Elsevier; 1998. p. 2158-61.

63. Lai KSP, Liu CS, Rau A, Lanctôt KL, Köhler CA, Pakosh M, et al. Peripheral inflammatory markers in Alzheimer's disease: a systematic review and meta-analysis of 175 studies. Journal of Neurology, Neurosurgery & Psychiatry. 2017;88(10):876-82.
64. Swardfager W, Lanctôt K, Rothenburg L, Wong A, Cappell J, Herrmann N. A meta-analysis of cytokines in Alzheimer's disease. Biol Psychiatry. 2010;68(10):930-41.
65. Fillit H, Ding WH, Buee L, Kalman J, Altstiel L, Lawlor B, et al. Elevated circulating tumor necrosis factor levels in Alzheimer's disease. Neurosci Lett. 1991;129(2):318-20.
66. Darweesh SKL, Wolters FJ, Ikram MA, de Wolf F, Bos D, Hofman A. Inflammatory markers and the risk of dementia and Alzheimer's disease: A meta-analysis. Alzheimer's & Dementia. 2018;14(11):1450-9.
67. Engelhart MJ, Geerlings MI, Meijer J, Kiliaan A, Ruitenberg A, van Swieten JC, et al. Inflammatory proteins in plasma and the risk of dementia: the rotterdam study. Arch Neurol. 2004;61(5):668-72.
68. Holmes C, Cunningham C, Zotova E, Woolford J, Dean C, Kerr S, et al. Systemic inflammation and disease progression in Alzheimer disease. Neurology. 2009;73(10):768-74.
69. Quintanilla RA, Orellana DI, González-Billault C, Maccioni RB. Interleukin-6 induces Alzheimer-type phosphorylation of tau protein by deregulating the cdk5/p35 pathway. Exp Cell Res. 2004;295(1):245-57.
70. Huell M, Strauss S, Volk B, Berger M, Bauer J. Interleukin-6 is present in early stages of plaque formation and is restricted to the brains of Alzheimer's disease patients. Acta Neuropathol. 1995;89(6):544-51.
71. Tchalla AE, Wellenius GA, Sorond FA, Gagnon M, Iloputaife I, Travison TG, et al. Elevated Soluble Vascular Cell Adhesion Molecule-1 Is Associated With Cerebrovascular Resistance and Cognitive Function. The Journals of Gerontology: Series A. 2016;72(4):560-6.
72. Zuliani G, Cavalieri M, Galvani M, Passaro A, Munari MR, Bosi C, et al. Markers of endothelial dysfunction in older subjects with late onset Alzheimer's disease or vascular dementia. J Neurol Sci. 2008;272(1-2):164-70.
73. Huang CW, Tsai MH, Chen NC, Chen WH, Lu YT, Lui CC, et al. Clinical significance of circulating vascular cell adhesion molecule-1 to white matter disintegrity in Alzheimer's dementia. Thromb Haemost. 2015;114(6):1230-40.
74. Verbeek MM, Otte-Höller I, Westphal JR, Wesseling P, Ruiter DJ, De Waal R. Accumulation of intercellular adhesion molecule-1 in senile plaques in brain tissue of patients with Alzheimer's disease. The American journal of pathology. 1994;144(1):104.

Supplementary Material

Table S1. Inter- and Intra-assay coefficient of variation

	Inter-assay (%CV)	Intra-assay (%CV)
IFNγ	7.26	6.49
TNFα	6.21	4.39
IL1β	N/A	16.22
IL2	N/A	15.02
IL6	9.95	5.64
IL8	5.79	3.31
IL10	7.91	10.31
CRP	6.36	4.43
SAA	18.43	5.35
sICAM-1	7.15	5.54
sVCAM-1	6.15	4.64
sE-selectin	4.57	2.31

Inter-assay <15% and intra-assay <10% are considered acceptable in the overall reliability of the immunoassay results. Abbreviations: CV, coefficient of variation; N/A, not available; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; SAA, serum amyloid A; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; sE-selectin; soluble E-selectin.

Table S2. GDS15

	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)
	Model 1		**Model 2**		**Model 3**	
IFNγ	**0.049**	1.179 (1.001, 1.389)	0.055	1.174 (0.997, 1.384)	0.375	1.093 (0.898, 1.330)
TNFα	**0.000005**	2.437 (1.661, 3.576)	**0.000005**	2.443 (1.665, 3.584)	**0.027**	1.791 (1.070, 2.997)
IL1β	0.327	1.117 (0.895, 1.393)	0.326	1.117 (0.896, 1.394)	0.292	1.149 (0.888, 1.487)
IL6	**0.001**	1.393 (1.143, 1.696)	**0.001**	1.391 (1.143, 1.694)	0.054	1.272 (0.995, 1.625)
IL8	**0.036**	1.394 (1.022, 1.902)	**0.030**	1.410 (1.033, 1.926)	0.149	1.333 (0.902, 1.968)
IL10	0.050	1.246 (1.000, 1.554)	IE	IE	0.354	1.136 (0.867, 1.489)
IL2	0.142	1.171 (0.949, 1.445)	0.151	1.166 (0.945, 1.439)	0.245	1.171 (0.898, 1.527)
CRP	**0.000203**	1.224 (1.100, 1.361)	**0.000287**	1.218 (1.095, 1.355)	0.070	1.139 (0.989, 1.312)
SAA	**0.008**	1.182 (1.044, 1.337)	**0.008**	1.182 (1.045, 1.339)	0.108	1.136 (0.972, 1.327)
sICAM-1	**0.005**	2.078 (1.244, 3.471)	**0.005**	2.100 (1.257, 3.509)	0.087	1.759 (0.922, 3.355)
sVCAM-1	**0.040**	1.790 (1.027, 3.118)	**0.034**	1.820 (1.045, 3.169)	0.400	1.331 (0.683, 2.595)
sE-selectin	**0.001**	1.554 (1.190, 2.029)	IE	IE	0.604	1.095 (0.777, 1.544)
	Sensitivity analysis (SA)		**Model 2 with SA conditions**		**Model 2 with model 3 conditions**	
IFNγ	0.271	1.159 (0.891, 1.508)	0.129	1.225 (0.943, 1.592)	0.136	1.161 (0.954, 1.412)
TNFα	0.080	1.738 (0.936, 3.226)	**0.015**	2.140 (1.161, 3.942)	**0.000342**	2.502 (1.514, 4.132)
IL1β	0.893	0.977 (0.700, 1.364)	0.854	0.969 (0.696, 1.351)	0.394	1.121 (0.862, 1.459)
IL6	0.153	1.251 (0.920, 1.701)	**0.027**	1.408 (1.040, 1.906)	**0.001**	1.489 (1.175, 1.888)
IL8	0.465	1.190 (0.746, 1.900)	0.321	1.270 (0.792, 2.036)	0.069	1.446 (0.971, 2.152)
IL10	0.471	1.130 (0.810, 1.576)	0.244	1.222 (0.872, 1.711)	0.157	1.220 (0.926, 1.607)
IL2	0.080	1.389 (0.962, 2.006)	0.084	1.386 (0.957, 2.007)	0.176	1.204 (0.920, 1.576)
CRP	0.240	1.117 (0.929, 1.342)	**0.045**	1.201 (1.004, 1.436)	**0.001**	1.254 (1.097, 1.434)
SAA	**0.021**	1.270 (1.037, 1.556)	**0.002**	1.370 (1.118, 1.679)	**0.021**	1.203 (1.028, 1.407)
sICAM-1	0.199	1.777 (0.740, 4.267)	**0.041**	2.401 (1.038, 5.556)	**0.002**	2.671 (1.434, 4.973)
sVCAM-1	0.734	1.161 (0.490, 2.750)	0.442	1.408 (0.589, 3.364)	0.090	1.785 (0.913, 3.489)
sE-selectin	0.713	0.918 (0.580, 1.451)	0.533	1.146 (0.746, 1.760)	**0.024**	1.452 (1.051, 2.007)

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Negative binominal regression was used to analyze the association. P-value less than 0.05 (in bold) was considered nominal significant and p-value less than 0.004 was considered statically significant after Bonferroni correction. *Model 1*: adjusted for age, gender, and educational level; *Model 2*: adjusted for *model 1* and diagnosis status (SCD, MCI, and dementia); *Model 3*: adjusted for *model 2* and lifestyle (BMI, smoking

status, and alcohol consumption); *Sensitivity analysis (SA)*: adjusted for *model 3* and MMSE, cardiovascular disease, cerebrovascular disease, and hypertension; *Model 2 with SA condition*: only included patients with all factors; *Model 2 with model 3 conditions*: only included patients with all factors up to lifestyle. Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; IE, interaction effect; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; SAA, serum amyloid A; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; sE-selectin; soluble E-selectin; GDS15, 15-item geriatric depression scale.

Table S3. GDS15 subgroup

	SCD		MCI		Dementia	
	p-value	**IRR (95% CI)**	**p-value**	**IRR (95% CI)**	**p-value**	**IRR (95% CI)**
Model 2						
IL10	0.774	0.949 (0.664, 1.356)	0.166	1.262 (0.908, 1.754)	**0.004**	2.093 (1.259, 3.480)
sE-selectin	**0.004**	1.872 (1.224, 2.864)	**0.008**	1.726 (1.153, 2.584)	0.339	0.729 (0.381, 1.394)
Model 2 with sensitivity analysis (SA) conditions						
IL10	0.605	0.851 (0.461, 1.570)	0.461	1.200 (0.739, 1.949)	0.060	1.805 (0.975, 3.341)
sE-selectin	0.960	1.020 (0.462, 2.254)	0.580	1.200 (0.630, 2.286)	0.483	0.744 (0.326, 1.699)
Model 2 with model 3 conditions						
IL10	0.737	0.927 (0.595, 1.444)	0.297	1.248 (0.823, 1.892)	**0.035**	1.987 (1.050, 3.761)
sE-selectin	0.055	1.648 (0.990, 2.743)	0.065	1.586 (0.971, 2.590)	0.666	0.840 (0.380, 1.857)

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Negative binominal regression was used to analyze the association. P-value less than 0.05 (in bold) was considered nominal significant and p-value less than 0.004 was considered statically significant after Bonferroni correction. *Model 1*: adjusted for age, gender, and educational level; *Model 2*: adjusted for *model 1* and diagnosis status (SCD, MCI, and dementia); *Model 3*: adjusted for *model 2* and lifestyle (BMI, smoking status, and alcohol consumption); *Sensitivity analysis (SA)*: adjusted for *model 3* and MMSE, cardiovascular disease, cerebrovascular disease, and hypertension; *Model 2 with SA condition*: only included patients with all factors; *Model 2 with model 3 conditions*: only included patients with all factors up to lifestyle. Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; IE, interaction effect; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; SAA, serum amyloid A; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; sE-selectin; soluble E-selectin; GDS15, 15-item geriatric depression scale.

Table S4. GDS15 cut-off (4/5) scale

	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)
	Model 1		**Model 2**		**Model 3**	
IFNγ	0.073	1.578 (0.959, 2.598)	0.079	1.565 (0.949, 2.579)	0.267	1.401 (0.773, 2.540)
TNFα	**0.001**	7.521 (2.340, 24.172)	**0.001**	7.584 (2.360, 24.377)	0.083	3.740 (0.842, 16.609)
IL1β	0.174	1.586 (0.816, 3.085)	0.172	1.590 (0.817, 3.094)	0.167	1.748 (0.792, 3.857)
IL6	**0.015**	2.094 (1.156, 3.793)	**0.015**	2.088 (1.152, 3.783)	0.246	1.563 (0.735, 3.324)
IL8	0.221	1.759 (0.712, 4.346)	0.205	1.796 (0.726, 4.441)	0.598	1.361 (0.433, 4.280)
IL10	**0.037**	1.982 (1.041, 3.772)	IE	IE	0.247	1.592 (0.725, 3.495)
IL2	0.300	1.391 (0.746, 2.593)	0.312	1.381 (0.739, 2.581)	0.608	1.234 (0.553, 2.754)
CRP	**0.010**	1.537 (1.107, 2.134)	**0.011**	1.532 (1.102, 2.131)	0.213	1.323 (0.852, 2.054)
SAA	**0.009**	1.628 (1.129, 2.348)	**0.008**	1.640 (1.136, 2.369)	0.068	1.548 (0.968, 2.475)
sICAM-1	0.057	4.562 (0.954, 21.825)	0.055	4.638 (0.966, 22.270)	0.155	4.236 (0.580, 30.938)
sVCAM-1	0.108	3.918 (0.740, 20.744)	0.104	3.990 (0.751, 21.185)	0.416	2.288 (0.311, 16.848)
sE-selectin	**0.013**	2.822 (1.239, 6.426)	**0.013**	2.865 (1.254, 6.544)	0.406	1.562 (0.546, 4.474)
	Sensitivity analysis (SA)		**Model 2 with SA conditions**		**Model 2 with model 3 conditions**	
IFNγ	0.204	1.754 (0.738, 4.172)	0.157	1.822 (0.793, 4.184)	0.127	1.575 (0.879, 2.820)
TNFα	0.111	4.593 (0.703, 29.990)	0.055	5.834 (0.961, 35.412)	**0.011**	6.486 (1.536, 27.394)
IL1β	0.384	1.688 (0.519, 5.491)	0.560	1.374 (0.472, 4.006)	0.182	1.691 (0.781, 3.663)
IL6	0.500	1.407 (0.521, 3.799)	0.317	1.605 (0.636, 4.055)	**0.028**	2.184 (1.087, 4.391)
IL8	0.603	1.477 (0.340, 6.414)	0.662	1.377 (0.328, 5.784)	0.366	1.680 (0.545, 5.174)
IL10	0.266	1.807 (0.637, 5.122)	IE	IE	IE	IE
IL2	0.070	3.105 (0.910, 10.600)	0.082	2.777 (0.877, 8.793)	0.506	1.304 (0.597, 2.851)
CRP	0.813	1.077 (0.585, 1.983)	0.540	1.198 (0.672, 2.136)	**0.019**	1.631 (1.085, 2.453)
SAA	0.103	1.710 (0.897, 3.257)	**0.039**	1.939 (1.034, 3.633)	**0.022**	1.704 (1.081, 2.684)
sICAM-1	0.233	5.930 (0.318, 110.645)	0.122	8.396 (0.567, 124.277)	**0.028**	8.376 (1.261, 55.651)
sVCAM-1	0.608	2.045 (0.133, 31.423)	0.427	2.898 (0.210, 39.982)	0.177	3.798 (0.547, 26.373)
sE-selectin	0.795	1.227 (0.264, 5.711)	0.330	2.002 (0.496, 8.084)	**0.043**	2.734 (1.032, 7.242)

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Binary logistic regression was used to analyze the association. P-value less than 0.05 (in bold) was considered nominal significant and p-value less than 0.004 was considered statically significant after Bonferroni correction. *Model 1*: adjusted for age, gender, and educational level; *Model 2*: adjusted for *model 1* and diagnosis status (SCD, MCI, and dementia); *Model 3*: adjusted for *model 2* and lifestyle (BMI, smoking status, and

alcohol consumption); *Sensitivity analysis (SA)*: adjusted for *model 3* and MMSE, cardiovascular disease, cerebrovascular disease, and hypertension; *Model 2 with SA condition*: only included patients with all factors; *Model 2 with model 3 conditions*: only included patients with all factors up to lifestyle. Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; IE, interaction effect; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; SAA, serum amyloid A; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; sE-selectin; soluble E-selectin; GDS15, 15-item geriatric depression scale.

Table S5. GDS15 cut-off (4/5) scale subgroup

	SCD		MCI		Dementia	
	p-value	**IRR (95% CI)**	**p-value**	**IRR (95% CI)**	**p-value**	**IRR (95% CI)**
Model 2						
IL10	0.904	0.938 (0.329, 2.671)	0.105	2.258 (0.844, 6.042)	**0.006**	10.903 (1.986, 59.857)
Model 2 with sensitivity analysis (SA) conditions						
IL10	0.413	0.442 (0.063, 3.117)	0.251	2.430 (0.534, 11.054)	**0.031**	18.841 (1.315, 269.984)
Model 2 with model 3 conditions						
IL10	0.555	0.675 (0.184, 2.481)	0.140	2.447 (0.745, 8.036)	**0.021**	11.669 (1.445, 94.231)

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Binary logistic regression was used to analyze the association. P-value less than 0.05 (in bold) was considered nominal significant and p-value less than 0.004 was considered statically significant after Bonferroni correction. *Model 1*: adjusted for age, gender, and educational level; *Model 2*: adjusted for *model 1* and diagnosis status (SCD, MCI, and dementia); *Model 3*: adjusted for *model 2* and lifestyle (BMI, smoking status, and alcohol consumption); *Sensitivity analysis (SA)*: adjusted for *model 3* and MMSE, cardiovascular disease, cerebrovascular disease, and hypertension; *Model 2 with SA condition*: only included patients with all factors; *Model 2 with model 3 conditions*: only included patients with all factors up to lifestyle. Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; IE, interaction effect; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; SAA, serum amyloid A; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; sE-selectin; soluble E-selectin; GDS15, 15-item geriatric depression scale.

Table S6. NPID

	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)
		Model 1		**Model 2**		**Model 3**
IFNγ	0.484	0.857 (0.556, 1.320)	0.579	0.885 (0.573, 1.364)	0.534	0.848 (0.504, 1.426)
TNFα	0.824	0.889 (0.317, 2.492)	0.869	0.917 (0.327 , 2.574)	0.934	1.056 (0.287, 3.883)
IL1β	0.068	0.582 (0.325, 1.042)	0.068	0.581 (0.324, 1.042)	0.078	0.539 (0.272, 1.070)
IL6	0.897	1.035 (0.614, 1.744)	0.877	1.042 (0.617, 1.760)	0.862	1.059 (0.556, 2.017)
IL8	0.435	1.374 (0.619, 3.048)	0.466	1.347 (0.605, 2.998)	0.577	1.330 (0.488, 3.626)
IL10	0.545	0.843 (0.484, 1.467)	0.536	0.839 (0.481, 1.462)	0.440	0.756 (0.372, 1.536)
IL2	0.771	0.923 (0.538, 1.584)	0.828	0.942 (0.548, 1.617)	0.487	0.779 (0.386, 1.575)
CRP	0.464	0.900 (0.679, 1.193)	0.587	0.925 (0.697, 1.227)	0.706	0.931 (0.642, 1.350)
SAA	0.763	0.951 (0.688, 1.316)	0.788	0.956 (0.690, 1.325)	0.874	0.967 (0.641, 1.459)
sICAM-1	0.639	1.382 (0.357, 5.343)	0.591	1.451 (0.372, 5.654)	0.100	4.255 (0.758, 23.880)
sVCAM-1	0.666	1.374 (0.324, 5.836)	0.705	1.324 (0.310, 5.650)	0.753	1.321 (0.233, 7.478)
sE-selectin	0.193	1.584 (0.792, 3.167)	0.171	1.625 (0.811, 3.257)	0.331	1.551 (0.640, 3.759)
		Sensitivity analysis (SA)				
IFNγ	0.627	0.828 (0.387, 1.771)				
TNFα	0.528	1.743 (0.311, 9.782)				
IL1β	0.194	0.526 (0.200, 1.385)				
IL6	0.160	0.521 (0.210, 1.292)				
IL8	0.800	1.185 (0.320, 4.388)				
IL10	0.727	0.839 (0.312, 2.252)				
IL2	0.325	0.578 (0.194, 1.722)				
CRP	0.259	0.731 (0.425, 1.260)				
SAA	0.911	1.034 (0.576, 1.855)				
sICAM-1	0.131	6.841 (0.566, 82.740)				
sVCAM-1	0.147	5.955 (0.534, 66.360)				
sE-selectin	0.792	0.840 (0.231, 3.055)				

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Binary logistic regression was used to analyze the association. P-value less than 0.05 (in bold) was considered nominal significant and p-value less than 0.004 was considered statically significant after Bonferroni correction. *Model 1*: adjusted for age, gender, and educational level; *Model 2*: adjusted for *model 1* and diagnosis status (SCD, MCI, and dementia); *Model 3*: adjusted for *model 2* and lifestyle (BMI, smoking status, and alcohol consumption); *Sensitivity analysis (SA)*: adjusted for *model 3* and MMSE, cardiovascular disease, cerebrovascular disease, and hypertension. Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; SAA, serum amyloid A; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; sE-selectin; soluble E-selectin; NPID, neuropsychiatric inventory depression.

Table S7. NPID-DS

	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)
		Model 1		**Model 2**		**Model 3**
IFNγ	0.710	0.928 (0.624, 1.379)	0.732	0.933 (0.627, 1.388)	0.532	0.858 (0.530, 1.388)
TNFα	0.915	1.056 (0.390, 2.855)	0.898	1.067 (0.394, 2.891)	0.590	0.707 (0.200, 2.501)
IL1β	0.978	1.007 (0.594, 1.707)	0.994	1.002 (0.591, 1.699)	0.602	1.175 (0.641, 2.152)
IL6	0.578	1.143 (0.715, 1.827)	0.575	1.144 (0.715, 1.829)	0.798	1.076 (0.616, 1.880)
IL8	0.518	1.305 (0.582, 2.923)	0.522	1.304 (0.579, 2.938)	0.734	1.195 (0.427, 3.350)
IL10	0.969	0.989 (0.576, 1.700)	0.957	0.985 (0.573, 1.694)	0.851	0.937 (0.479, 1.836)
IL2	0.627	0.887 (0.548, 1.437)	0.634	0.889 (0.549, 1.441)	0.807	0.917 (0.460, 1.831)
CRP	0.570	0.929 (0.722, 1.196)	0.611	0.936 (0.726, 1.207)	0.392	0.865 (0.621, 1.205)
SAA	0.921	0.985 (0.728, 1.332)	0.957	0.992 (0.732, 1.343)	0.724	0.935 (0.643, 1.358)
sICAM-1	0.800	1.170 (0.347, 3.952)	0.804	1.167 (0.344, 3.957)	0.967	0.969 (0.209, 4.482)
sVCAM-1	0.942	0.951 (0.246, 3.680)	0.962	0.967 (0.250, 3.750)	0.465	0.539 (0.102, 2.836)
sE-selectin	0.795	1.087 (0.580, 2.037)	0.790	1.089 (0.581, 2.042)	0.653	0.826 (0.359, 1.901)
		Sensitivity analysis (SA)				
IFNγ	0.632	0.835 (0.399, 1.748)				
TNFα	0.554	1.718 (0.286, 10.305)				
IL1β	0.392	1.465 (0.611, 3.510)				
IL6	0.686	0.839 (0.358, 1.965)				
IL8	0.740	1.278 (0.301, 5.423)				
IL10	0.974	1.017 (0.377, 2.740)				
IL2	0.954	0.968 (0.318, 2.946)				
CRP	0.066	0.616 (0.368, 1.032)				
SAA	0.520	0.819 (0.447, 1.503)				
sICAM-1	0.413	2.663 (0.255, 27.798)				
sVCAM-1	0.232	4.611 (0.376, 56.511)				
sE-selectin	0.332	0.549 (0.163, 1.847)				

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Negative binominal regression was used to analyze the association. P-value less than 0.05 (in bold) was considered nominal significant and p-value less than 0.004 was considered statically significant after Bonferroni correction. *Model 1*: adjusted for age, gender, and educational level; *Model 2*: adjusted for *model 1* and diagnosis status (SCD, MCI, and dementia); *Model 3*: adjusted for *model 2* and lifestyle (BMI, smoking status, and alcohol consumption); *Sensitivity analysis (SA)*: adjusted for *model 3* and MMSE, cardiovascular disease, cerebrovascular disease, and hypertension. Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; IE, interaction effect; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; SAA, serum amyloid A; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; sE-selectin; soluble E-selectin; NPID-DS, neuropsychiatric inventory depression-domain score.

Table S8. NPIA

	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)
	Model 1		**Model 2**		**Model 3**	
IFNγ	0.823	1.055 (0.659, 1.689)	0.696	1.099 (0.685, 1.762)	0.703	1.117 (0.632, 1.974)
TNFα	0.961	0.972 (0.311, 3.041)	0.946	1.041 (0.331, 3.276)	0.812	1.191 (0.283, 5.016)
IL1β	0.723	1.120 (0.599, 2.092)	0.662	1.150 (0.614, 2.155)	0.385	1.387 (0.663, 2.904)
IL6	0.055	0.556 (0.305, 1.012)	0.053	0.550 (0.300, 1.007)	0.186	0.602 (0.284, 1.277)
IL8	0.829	1.101 (0.458, 2.647)	0.820	1.109 (0.455, 2.705)	0.666	1.274 (0.423, 3.838)
IL10	0.292	0.709 (0.374, 1.344)	0.309	0.715 (0.375, 1.364)	0.074	0.462 (0.198, 1.077)
IL2	0.239	0.695 (0.379, 1.275)	0.288	0.718 (0.390, 1.323)	0.163	0.568 (0.257, 1.257)
CRP	0.097	0.767 (0.561, 1.049)	IE	IE	0.310	0.804 (0.528, 1.225)
SAA	0.435	0.865 (0.600, 1.245)	IE	IE	0.731	1.083 (0.687, 1.706)
sICAM-1	0.082	0.255 (0.055, 1.190)	0.110	0.283 (0.060, 1.328)	0.721	0.709 (0.107, 4.692)
sVCAM-1	0.414	0.508 (0.100, 2.581)	0.391	0.488 (0.095, 2.513)	0.727	1.409 (0.205, 9.682)
sE-selectin	0.800	1.103 (0.517, 2.357)	IE	IE	0.997	0.998 (0.375, 2.658)
	Sensitivity analysis (SA)		**Model 2 with SA conditions**		**Model 2 with model 3 conditions**	
IFNγ	0.838	0.919 (0.411, 2.056)	0.894	0.949 (0.436, 2.066)	0.663	1.132 (0.648, 1.979)
TNFα	0.656	1.511 (0.245, 9.302)	0.838	1.200 (0.209, 6.887)	0.840	1.152 (0.292, 4.555)
IL1β	0.172	0.465 (0.154, 1.397)	0.237	0.529 (0.185, 1.519)	0.342	1.427 (0.686 ,2.969)
IL6	0.096	0.425 (0.155, 1.163)	0.162	0.509 (0.198, 1.312)	0.295	0.687 (0.341, 1.386)
IL8	0.876	1.120 (0.270, 4.651)	0.878	1.116 (0.275, 4.521)	0.633	1.305 (0.438, 3.894)
IL10	0.118	0.397 (0.125, 1.262)	0.137	0.422 (0.135, 1.317)	0.093	0.489 (0.212, 1.128)
IL2	0.704	1.251 (0.395, 3.961)	0.774	1.178 (0.385, 3.604)	0.166	0.574 (0.262, 1.259)
CRP	0.323	0.745 (0.415, 1.336)	IE	IE	IE	IE
SAA	0.922	1.032 (0.549, 1.940)	IE	IE	IE	IE
sICAM-1	0.945	0.909 (0.061, 13.475)	0.925	1.129 (0.090, 14.135)	0.774	0.767 (0.125, 4.719)
sVCAM-1	0.669	1.759 (0.132, 23.452)	0.763	1.480 (0.116, 18.893)	0.697	1.456 (0.220, 9.621)
sE-selectin	0.957	1.039 (0.257, 4.201)	IE	IE	IE	IE

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Binary logistic regression was used to analyze the association. P-value less than 0.05 (in bold) was considered nominal significant and p-value less than 0.004 was considered statically significant after Bonferroni correction. *Model 1*: adjusted for age, gender, and educational level; *Model 2*: adjusted for *model 1* and diagnosis status (SCD, MCI, and dementia); *Model 3*: adjusted for *model 2* and lifestyle (BMI, smoking status, and

alcohol consumption); *Sensitivity analysis (SA)*: adjusted for *model 3* and MMSE, cardiovascular disease, cerebrovascular disease, and hypertension. Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; IE, interaction effect; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; SAA, serum amyloid A; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; sE-selectin; soluble E-selectin; NPIA, neuropsychiatric inventory anxiety.

Table S9. NPIA subgroup

	SCD		MCI		Dementia	
	p-value	**IRR (95% CI)**	**p-value**	**IRR (95% CI)**	**p-value**	**IRR (95% CI)**
Model 2						
CRP	0.260	1.356 (0.798, 2.302)	**0.007**	0.489 (0.291, 0.823)	0.348	0.720 (0.362, 1.431)
SAA	0.241	1.453 (0.778, 2.713)	**0.012**	0.436 (0.227, 0.835)	0.678	1.160 (0.575, 2.340)
sE-selectin	0.297	2.000 (0.544, 7.350)	0.233	2.093 (0.622, 7.039)	**0.006**	0.070 (0.011, 0.463)
Model 2 with sensitivity analysis (SA) conditions						
CRP	0.164	2.157 (0.731, 6.358)	0.254	0.620 (0.273, 1.410)	0.066	0.309 (0.088, 1.080)
SAA	**0.038**	3.410 (1.070, 10.863)	0.100	0.397 (0.132, 1.193)	0.992	1.006 (0.322, 3.146)
sE-selectin	0.461	2.679 (0.195, 36.780)	0.586	1.728 (0.241, 12.383)	0.044	0.043 (0.002, 0.916)
Model 2 with model 3 conditions						
CRP	**0.010**	2.539 (1.248, 5.169)	**0.024**	0.484 (0.258, 0.908)	0.496	0.726 (0.289, 1.826)
SAA	**0.006**	3.157 (1.386, 7.191)	0.088	0.512 (0.238, 1.105)	0.489	1.391 (0.546, 3.545)
sE-selectin	0.143	3.290 (0.667, 16.226)	0.329	2.079 (0.479, 9.025)	**0.009**	0.040 (0.004, 0.446)

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Binary logistic regression was used to analyze the association. P-value less than 0.05 (in bold) was considered nominal significant and p-value less than 0.004 was considered statically significant after Bonferroni correction. *Model 1*: adjusted for age, gender, and educational level; *Model 2*: adjusted for *model 1* and diagnosis status (SCD, MCI, and dementia); *Model 3*: adjusted for *model 2* and lifestyle (BMI, smoking status, and alcohol consumption); *Sensitivity analysis (SA)*: adjusted for *model 3* and MMSE, cardiovascular disease, cerebrovascular disease, and hypertension. Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; SAA, serum amyloid A; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; sE-selectin; soluble E-selectin; NPIA, neuropsychiatric inventory anxiety.

Table S10. NPIA-DS

	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value	IRR (95% CI)
	Model 1		**Model 2**		**Model 3**	
IFNγ	0.568	0.847 (0.479, 1.497)	0.541	0.837 (0.473, 1.481)	0.928	0.967 (0.474, 1.975)
TNFα	0.915	0.931 (0.250, 3.470)	0.976	1.021 (0.274, 3.804)	0.864	1.199 (0.150, 9.584)
IL1β	0.786	1.103 (0.542, 2.248)	0.838	1.076 (0.533, 2.175)	0.487	1.373 (0.562, 3.356)
IL6	0.212	0.629 (0.304, 1.302)	0.171	0.601 (0.290, 1.245)	0.247	0.576 (0.226, 1.466)
IL8	0.590	1.396 (0.415, 4.698)	0.564	1.420 (0.431, 4.676)	0.554	1.611 (0.333, 7.800)
IL10	0.517	0.769 (0.347, 1.702)	0.561	0.791 (0.359, 1.742)	0.317	0.580 (0.199, 1.688)
IL2	0.148	0.627 (0.333, 1.179)	0.194	0.659 (0.352, 1.236)	0.249	0.548 (0.197, 1.523)
CRP	0.502	0.877 (0.598, 1.286)	0.510	0.878 (0.596, 1.293)	0.645	0.881 (0.515, 1.508)
SAA	0.922	1.023 (0.647, 1.619)	0.878	1.037 (0.656, 1.638)	0.575	1.194 (0.642, 2.221)
sICAM-1	0.490	0.526 (0.085, 3.254)	0.571	0.592 (0.096, 3.640)	0.777	1.428 (0.121, 16.897)
sVCAM-1	0.764	0.735 (0.098, 5.482)	0.899	0.878 (0.117, 6.572)	0.508	2.349 (0.187, 29.454)
sE-selectin	0.723	1.166 (0.499, 2.723)	0.506	1.334 (0.571, 3.112)	0.982	1.014 (0.315, 3.263)
	Sensitivity analysis (SA)					
IFNγ	0.633	0.761 (0.249, 2.329)				
TNFα	0.674	1.932 (0.090, 41.454)				
IL1β	0.749	0.803 (0.210, 3.070)				
IL6	0.593	0.670 (0.154, 2.908)				
IL8	0.876	1.200 (0.121, 11.868)				
IL10	N/A	N/A				
IL2	0.834	1.187 (0.239, 5.906)				
CRP	0.811	0.911 (0.426, 1.950)				
SAA	0.392	1.577 (0.555, 4.479)				
sICAM-1	0.823	1.493 (0.045, 49.550)				
sVCAM-1	0.330	6.407 (0.153, 268.390)				
sE-selectin	0.881	1.140 (0.205, 6.329)				

Data are presented as incidence rate ratio (IRR) and 95% confidence interval (95% CI). Negative binominal regression was used to analyze the association. P-value less than 0.05 (in bold) was considered nominal significant and p-value less than 0.004 was considered statically significant after Bonferroni correction. *Model 1*: adjusted for age, gender, and educational level; *Model 2*: adjusted for *model 1* and diagnosis status (SCD, MCI, and dementia); *Model 3*: adjusted for *model 2* and lifestyle (BMI, smoking status, and alcohol consumption); *Sensitivity analysis (SA)*: adjusted for *model 3* and MMSE, cardiovascular disease, cerebrovascular disease, and hypertension. Abbreviations: SCD, subjective cognitive decline; MCI, mild cognitive impairment; N/A, not available; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; SAA, serum amyloid A; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; sE-selectin; soluble E-selectin; NPIA-DS, neuropsychiatric inventory anxiety-domain score.

CHAPTER 6

General Discussion

The primary aim of this academic thesis was to investigate the role of the kynurenine pathway (KP) and systemic inflammation in view of affective symptomatology in subjects with or at risk of developing dementia. As mentioned throughout the thesis, dementia is a syndrome mainly characterized by severe cognitive impairment impacting daily live functioning. Individuals with subjective cognitive decline (SCD) and mild cognitive impairment (MCI) are at risk of developing dementia [1-3]. Affective symptomatology such as depressive- and anxiety-like symptoms are commonly observed in these patients and both the KP and systemic inflammation have been shown to be implicated in both affect-related disorders and dementia [4-9].

The kynurenine pathway, aging, and dementia

Increasing numbers of clinical KP studies in both cognitive and psychiatric disorders are being published. Currently, there are few systematic reviews and meta-analyses comparing KP metabolites, also known as kynurenines, in patients with depression [6, 7], but none in dementia. Chapter 2 is the first systematic review and meta-analysis that investigated 1) the differences in kynurenines between healthy controls and patients with cognitive impairment and dementia, and 2) the evidence of the relationship between kynurenines and cognition in normal aging, since age is the main risk factor for developing dementia. Based on the overall (cerebrospinal fluid [CSF] and blood combined) meta-analysis, AD dementia patients had lower levels of tryptophan (TRP), kynurenic acid (KA), xanthurenic acid (XA), anthranilic acid (AA), and quinolinic acid (QA) when compared to controls. The same results were observed in blood (plasma and serum combined and separate), except for QA, which showed a tendency towards lower levels in AD dementia. Additionally, no differences were found for kynurenine (KYN), 3-hydroxykynurenine (3-HK), and the KYN/TRP ratio (KTR). Furthermore, the systematic review addressing the relationship between kynurenines and cognition in normal aging suggested that, in general, TRP was negatively associated with age, whereas KYN, KTR, KA, and QA were positively associated with age. The association between KP and cognitive performance was inconclusive and generally non-significant.

The neuroprotective properties of KA are well-documented. Thus, lower KA levels in AD dementia were expected, but a large amount of studies reported no difference while one study even reported increased KA level in serum. Additionally, most studies included in the systematic review reported an increase

of KA levels in AD dementia in CSF and in post-mortem brain tissue, although this increase was not supported by all studies. The reason for the latter could be the small sample sizes, age differences, or not controlling for important covariates, thus requiring further research. A plausible explanation for an increase in central KA levels could be the activation of counteractive mechanisms to balance the neurotoxicity. For instance, KA is an agonist of the aryl hydrocarbon receptor (AhR), which regulates the immune response by preventing over-activation of pro-inflammatory cytokines in response to inflammatory stimuli, amongst others [10]. Additionally, KA in the brain is mainly synthesized in astrocytes [11], and some evidence suggests that dementia is associated with astrocyte activation and astrogliosis. For example post-mortem brain imaging of AD patients showed increased activity of monoamine oxidase B (MAO-B), an enzyme primarily found in astrocytes and increased during reactive astrocytosis [12]. Clearly, further research is necessary to understand the exact role of KA in dementia.

Interestingly, several lines of evidence suggests that QA has neurotoxic properties, which could be related to the pathophysiology of AD. As such, an increase in QA levels was expected in patients with AD. Yet again, our meta-analysis showed otherwise. Post-mortem brain studies found no differences in QA levels between AD dementia and controls in several brain areas [13, 14] and studies reported no significant association between QA and cognitive function. In contrast to what is generally accepted, our systematic review and meta-analysis dismisses a negative association of QA with AD dementia. While this does not refute that the presumed neurotoxic effects of QA may contribute to the pathophysiology of AD, it does indicate that is likely not a suitable diagnostic biomarker for AD.

Lastly, in the systematic review, TRP was the most reported variable studied in the context of dementia and most studies reported either lower levels of TRP in AD dementia or no difference between AD dementia and controls. On top of its known role as a biochemical precursor for the kynurenine and serotonin pathways, studies have shown that TRP has antioxidant properties such as scavenging free radicals, as well as reactive oxygen and chlorine species, and has the highest antiradical activity compared to other amino acids [15-17]. As such, lower peripheral levels of TRP, as demonstrated in our meta-analysis, potentially indicates less antioxidant capacity in AD patients. As mammals

cannot synthesize TRP, food intake and intestinal wall absorption of amino acids is expected to impact its levels in AD. For instance, studies report that approximately 24% to 81% of AD patients have eating disturbances [18-20]. Additionally, serum TRP was inversely correlated with fecal calprotectin, a protein present in inflamed intestinal tissues, which suggests a disturbance in the intestinal barrier function (leaky gut), leading to inflammation [21].

A key player in the pathogenesis of AD is neuroinflammation [22]. As an example, studies reported a negative association between KTR, a marker for inflammation, and cognitive scores in healthy volunteers, patients with AD dementia [23, 24] and ischemic stroke [25]. However, our KP meta-analysis showed no difference for KTR between groups. The reason behind these contrasting results are not yet obvious and require further research, but discrepancies may be due to not controlling for important covariates. Therefore, we performed meta-regression analyses, which showed considerable heterogeneity in the included studies, especially for levels of TRP, KYN, 3-HK, KA, and KTR. We found that inter-study differences in KP metabolite levels between groups could be explained by several factors, including the 'analytical technique', 'year of publication', 'type of biomaterial', and 'gender'. The main strength of this study was that it systematically summarized all studies investigating the difference in TRP and kynurenines levels and the association between kynurenines with age and cognition. Additionally, the meta-analysis provided an overall effect of individual KP associated metabolites in AD dementia and control comparisons of various biological fluids. Furthermore, the systematic review and meta-analysis highlighted the neuroprotective properties of less widely studied downstream metabolites such as XA and AA. However, the limitation of this review is the relatively small sample size included in the meta-analysis, mainly because kynurenines reported in median and interquartile range were excluded in the analysis. Nevertheless, the current review demonstrated that some KP metabolites were dysregulated in patients with dementia and cognitive impairment when compared with neurologically healthy controls. In addition some metabolites showed an association with age and cognitive function.

The kynurenine pathway and the role of DNA (hydroxy)methylation

The TRP catabolic pathway is a very complex pathway that feeds into various precursor pathways involving kynurenine, serotonin, tryptamine, and protein

synthesis. While most studies address protein and/or metabolite levels only, investigating related transcriptional and epigenetic signatures is of great interest as well. Therefore, Chapter 3 switched gears and demonstrated the use of both transcriptomic- and DNA (hydroxy)methylomic- profiling, as well as that of gene regulatory network (GRN) and associated network perturbation analyses, and pyrosequencing validation on the TRP catabolic pathway - more specifically the TRP metabolic pathway and the nicotinic adenine dinucleotide (NAD) pathway - in patients with AD using post-mortem middle temporal gyrus (MTG) and in blood samples of two independent longitudinal AD cohorts, i.e., the Ageing, Cognition and Dementia in Primary Care Patients (AgeCoDe) cohort and the Biobank Alzheimer Center Limburg (BBACL) cohort. The MTG analysis revealed several TRP catabolic pathway-associated genes to be differentially expressed in AD with numerous loci displaying differential DNA (hydroxy)methylation, the levels of which correlated to the corresponding gene expressions on several occasions. As the GRN and network perturbation analyses hinted at the same genes playing a prominent role in AD *IDO2*, *SLC7A5*, and *PARP14* were selected as candidate genes to be further assessed in the AgeCoDe cohort. One CpG site, cg11251498 (*IDO2*; displaying differential levels of unmodified cytosine in the MTG), showed a difference in methylation levels between converters to AD dementia and non-converters at baseline as well as a tendency towards higher methylation in AD dementia after 4.5 years follow-up in the AgeCoDe cohort. Although the same CpG site did not show differences between groups in the BBACL study, it did show a significant negative association with age. Amongst the genes altered at both the transcriptional and epigenetic level, the gene encoding for the solute carrier family 7 member 5 (SLC7A5) protein, also known as the large amino acid transporter 1 (LAT1). LAT1 allows large neutral amino acids (LNAAs), but also KYN and 3-HK, to pass through the blood-brain-barrier (BBB) [26]. Although further research is necessary, DNA (hydroxy)methylation seems to play a role in its transcriptional regulation and could influence the abnormal degree of TRP and/or the delivery of other LNAAs to the CNS.

The nicotinamide adenine dinucleotide (NAD) pathway

Downstream of the KP is the NAD pathway, involving the *de novo* synthesis pathway for the production of NAD, which is key metabolite involved in a large array of cellular metabolic pathways and known to be decreased in various age-related diseases [27]. Quinolinate Phosphoribosyltransferase (QPRT) is the

initial enzyme to start the *de novo* synthesis pathway. Interestingly, QPRT levels decrease during aging or e.g., after lipopolysaccharide (LPS) stimulation, leading to diminished NAD. This in turn leads to a chain reaction, involving decreased mitochondrial electron-transport chain complex I activity and increased inflammation. Furthermore, aging is associated with increased inflammation and DNA damage, thus decreased NAD and sirtuin (SIRT) activity, as well as increased poly(ADP-Ribose) polymerase (PARP) activity. SIRTs and PARP are both part of the salvage pathway, a pathway to recycle NAD, consuming NAD to repair DNA damage caused by reactive oxygen species (ROS) [28]. In view of this pathway, our MTG analysis has shown altered gene expression for *QPRT*, *PARP- (1, 4, 9, 10, 14)*, and *SIRT- (1, 2, 5)*. Some isoforms additionally displayed altered DNA (hydroxy)methylation levels, suggesting transcriptional and epigenetic dysregulation of the NAD pathway is present in AD.

Indoleamine 2,3-dioxygenase 2 (IDO2)

As previously mentioned, neuroinflammation is a key player in the pathogenesis of AD. It is hypothesized that pro-inflammatory cytokine-induced activation of IDO stimulates the KP. Based on our data, on top of being significant both at the level of DNA (hydroxy)methylation and gene expression, *IDO2* was identified as a potentially critical player in the pathophysiology of AD through GRN and network perturbation analysis. While IDO1 has been shown to inhibit T cell activation and induce T regulatory cell development, IDO2 is a pro-inflammatory mediator of B and T cell activation [29, 30]. In the context of AD, the group of Guillemin et al. has reported that upon inflammatory cytokine and Aβ stimulation, expression of IDO was increased, and they confirmed increased IDO activity in the hippocampus of AD patients [31-34]. Notably, an association between age and cg11251498 (*IDO2*) methylation was observed in the BBACL cohort, an effect that was not seen in the MTG and AgeCoDe datasets. This apparent discrepancy may be due to the different age ranges in the various cohort studies (BBACL: 43 years-90 years; MTG: 70-95; AgeCoDe: 75-89). Moreover, unlike the MTG and AgeCoDe datasets, groups within the BBACL cohort were not age-matched, with patients suffering from SCD being about 10 years younger than MCI and dementia patients. Clearly, these findings warrant further investigation.

Despite these limitations, this work has demonstrated that numerous transcriptional and epigenetic differences were present in TRP catabolic pathway-associated genes when comparing patients with AD and controls, as well as the advantages of using an *in silico* approach and cohort validations for the development of novel biomarkers and treatment strategies for AD.

The KP, inflammation, and their role in affective symptoms in dementia

As mentioned throughout this thesis, cognitive and affective symptoms often go hand in hand and studies have shown that the KP and systemic inflammation are involved in, for example, both depression and AD. Despite the interplay between these disorders, the KP and systemic inflammation studies have only investigated their relationship independently. Therefore, Chapter 4 investigated the association between the KP and affective symptomatology in patients with or at risk of developing dementia using the BBACL cohort, while Chapter 5, in a similar manner, investigated the role of systemic inflammation. In both chapters, care partners in patients with dementia more commonly reported depressive-like symptoms than care partners in patients with SCD, while care partners in dementia patients reported more anxiety-like symptoms than care partners in SCD, and in MCI patients. In Chapter 4, higher plasma levels of XA, picolinic acid (PIC), as well as higher KQ/QA, KA/KYN, and XA/QA ratios were associated with a lower incidence rate ratio (IRR) of depressive symptoms on the self-rated 15-item geriatric depression scale (GDS15). Except for KA and PIC, the associations were robust.

Moreover, this is a unique study because it investigated kynurenines in both cognitive and affective symptoms and demonstrated that some kynurenines are involved in both symptoms while some are unique to one symptom. The association between depressive symptoms and KP metabolites has been extensively examined by others, with similar findings as described in Chapter 4. Additionally, our comparisons on metabolite levels and associated ratios between the groups showed similar trends as previously reported studies and were in line with the results from our systematic review and meta-analysis described in Chapter 2. Interestingly, in Chapter 2, XA levels were lower in AD dementia compared to control and were positively associated with cognitive function, while being negatively associated with depressive symptoms in Chapter 4. Moreover, this study also addressed the importance of investigating the KP ratios, particularly the KA/QA ratio, as indicators of the balance between

neuroprotective and neurotoxic metabolites. As such, in line findings of others, we observed a lower KA/QA ratio in dementia [35, 36]. In addition, the KA/KYN ratio showed associations with both cognitive and depressive symptoms. Furthermore, for the first time, the XA/QA ratio was investigated, with a similar purpose as the KA/QA ratio. In our analysis, KA/QA, KA/KYN, and XA/QA ratios were shown to represent extremely robust measures, suggesting that they may serve as potential diagnostic biomarkers for affective symptomatology regardless of cognitive impairment and other factors. Although more research is needed to validate these findings, these ratio markers can be used to develop prediction models of depressive-like symptom in patients with or without cognitive impairment.

The BBB is the barrier that prevents toxic substances in the blood to cross into the extracellular fluid of the central nervous system (CNS) and filters toxic compounds from the brain back to the bloodstream [37]. It has shown that patients with AD exhibit BBB leakage due to severe damage in its structure [38]. A recent study on the human hippocampus indicated age-dependent deterioration of the BBB during normal aging and an accelerated degradation in patients with MCI compared to controls, thus suggesting that the damaged BBB structure may contribute to cognitive impairment as early as the prodromal stage of dementia [39]. Furthermore, a recent review by Wu et al. (2021) reported that, although the exact mechanism is still unclear, disruption of the tight junctions in the BBB may also play a role in the pathophysiology of depression and increase the susceptibility to depression [40]. As described in Chapter 1, peripheral TRP, KYN, and 3-HK can enter into the BBB by LAT1. One study measuring both plasma and CSF kynurenines in patients with AD and control and showed a significant positive correlation between plasma and CSF levels of KYN, 3-HK, AA, and PIC [41]. Another study performed serum and CSF correlation analysis in patients with AD, Parkinson's disease, and control, which showed positive correlation for KYN, 3-HK, KA, XA, and QA [42]. In addition, positive correlations between plasma and CSF were reported for KYN [43, 44], AA [44], and QA [44] in patients with major depressive disorder (MDD). Although further research is needed, these data suggests that BBB degradation may be a contributing factor for the positive correlation in levels that leads to cognitive and affective symptoms.

The hypothesized causal role for inflammation in depression was introduced in the early 90s by Ronald Smith as 'The macrophage theory of depression' [45]. He hypothesized that the observed increase in pro-inflammatory cytokines in patients with depression was due to the migration of monocytes to the site of inflammation, their subsequent differentiation into macrophages with excessive secretion of pro-inflammatory cytokines. In the meantime, numerous lines of evidence have supported this theory and have shown that patients with depression have more pro-inflammatory cytokines released from microglia, suggesting a disturbance in the M1/M2 ratio by having more M1-polarizations [46, 47]. Furthermore, our study revealed evidence of endothelial activation (see Chapter 5) which may be related to BBB disruption and thus, leading to BBB hyperpermeability [48, 49]. Because of this, peripheral inflammation may induce or aggravate neuroinflammation [50, 51], and may also facilitate exchange of KP metabolites from CNS to periphery or vice versa. In addition, BBB hyperpermeability could lead to infiltration of peripheral leukocytes [52, 53] and contribute to neuroinflammation in depression. In view of TRP metabolism, the increase in pro-inflammatory cytokines may cause a snowballing effect, by stimulating IDO and inducing the KP, thereby creating a neurotoxic challenge, and by impairing endothelial function. Indeed, accelerated BBB deterioration was shown in prodromal stages of dementia, and, in addition, chronic social stress alters BBB integrity resulting in depressive-like behaviors by inducing neurovascular pathology [49, 54]. Additionally, stress can induce the activation of tryptophan 2,3-dioxygenase (TDO2) in the liver that also activates the KP [55]. This may increase the production of downstream KP metabolites, which can affect the immune system, as described in Chapter 1, and start another snowball effect. On top of this, BBB leakage allows macrophage to infiltrate into the CNS, secreting pro-inflammatory cytokines, causing neuroinflammation. Also, macrophages are responsible for the production of neurotoxic metabolites such as 3-HK and QA. As a result, initiate a vicious circle to accelerate the pathophysiology of cognitive and affective disorders. Although more research is needed to determine the exact nature of the inflammation-KP relationship in cognitive and affective disorders, this study implemented various models to adjust for covariates and demonstrated that several kynurenines and inflammatory markers were associated with affective symptomatology, with certain KP biomarkers were robust and independent from various factors.

As described above, systemic inflammation is a key player in both cognitive and affective symptoms. As such, Chapter 5 demonstrated that higher plasma levels of TNFα, IL6, IL8, C-reactive protein (CRP), serum amyloid A (SAA), soluble vascular cell adhesion molecule-1 (sVCAM-1), and soluble intercellular molecule-1 (sICAM-1) were associated with increased IRR in the GDS15. However, these associations were lost once adjusted for lifestyle, except for TNFα, indicating that lifestyle is an important factor. Although more research is needed, the findings in this study validate that systemic inflammation is associated with depressive-like symptoms regardless of cognitive impairment.

Overall, this thesis has systematically gathered all available KP studies investigating in patients with cognitive disorders, and associations between kynurenines with age and cognitive function. As such, it has demonstrated via meta-analysis that certain metabolites were dysregulated in AD dementia patients compared to neurologically healthy controls, identified metabolites which were associated with age and cognition, highlighted the importance of correcting for various factors in the KP studies (see Chapter 2). Additionally, the work in this thesis, for the first time, identified several transcriptional and epigenetic differences between AD and controls in the entire TRP catabolic pathway and validated the findings in other datasets. Again, the findings in this study pointed towards (neuro)inflammation and highlighted that transcriptional and epigenetic dysregulation may play a role in KP metabolite levels, and the approaches taken in this study can serve as an important tool to identify potential biomarkers and treatment for AD (see Chapter 3). Lastly, numerous studies have validated the dysregulation of kynurenines and systemic inflammation in patients with cognitive and affective disorders. It is well known that affective symptoms are commonly observed in patients with cognitive disorder. Despite these links, studies have yet to investigate this matter until now. The work in this thesis have shown that several kynurenines and systemic inflammation markers were associated with depressive-like symptoms in patients with or at risk of developing dementia. Moreover, the work collected in this thesis highlighted the importance of adjusting for covariates in the KP studies, thus the association analyses implemented various models to adjust for covariates such as demographics, cognitive status, lifestyle, and comorbidities and demonstrated that some markers were robust and independent from factors while others were not. Additionally, the findings from this thesis showed that some of these markers are involved in both depressive- and anxiety-like symptoms, while

others are specific for one symptom. Taken together, although further researches are needed, this may suggest that kynurenines and systemic inflammation markers have the potential to be used as a (trans)diagnostic biomarker for both cognitive and affective symptoms (see Chapters 4 and 5). Finally, it is important to emphasize that inflammation is a key activator of the KP and that kynurenines affect the immune system (see Chapter 1). Therefore, the associations reported in this thesis may be independent, but may also be mediated by one another.

Limitations

The main limitation encountered in this thesis is the significant age difference present in the BBACL cohort since TRP and KP metabolites have shown age associations, as described in Chapter 2, and aging is the main risk factor for dementia [56]. On the one hand, this cannot be avoided since SCD patients are the earliest prodromal stage of cognitive impairment, thus SCD patients are, in general, much younger than patients with either MCI or dementia. The best option is to include age-matched neurologically healthy controls, but since BBACL does not include healthy controls, the best option was to adjust for age in the analysis. Additionally, BBACL participants did not fast overnight before CSF and/or blood collection. Although it is unclear how much this will affect the metabolite concentrations, TRP is an essential amino acid, thus unfasted samples may influence the outcome and it is impossible to adjust in the analysis. It is also important to be aware of the fact that the association analyses were cross-sectional studies.

Future perspectives

The study of the KP has recently shed new light on the pathophysiology of cognitive and affective disorders, but our current knowledge on its mechanisms and (neuro)properties are still in its infant stage. Nevertheless, preclinical and clinical studies have identified several factors such as analytical techniques, fasting, demographics, lifestyle, and comorbidities that may influence the metabolite levels, and which should thus be taken into account during the when investigating kynurenines, especially in the context of clinical studies. This will allow for a better between-study comparison. Additionally, more downstream metabolites and its ratios should be explored in KP studies. Moreover, in general, multi-omics and *in silico* approaches should be considered in pathophysiological

studies, as they can identify central players in disease mechanism (as reported in Chapter 3), transcriptomic and DNA (hydroxy)methylomic dysregulations may contribute to protein/metabolite levels. Lastly, in order to assess the validity of the potential (trans)diagnostic application, more longitudinal studies are necessary. BBACL is an on-going longitudinal cohort that follows patients to the point that dementia develops. All patients not diagnosed with dementia at baseline were invited for follow-up assessments after 1, 2, 3, 5, and 10 years. The cohort also has KP levels in CSF, magnetic resonance imaging (MRI), and follow-up cognitive and affective tests.

To conclude, the studies in this thesis offer novel insights into the association between kynurenines and markers of systemic inflammation in view of affective symptoms in patients with or at risk of developing dementia. Based on these findings, KP metabolites and its ratios, in combination with current biomarkers of dementia, will provide better personalized (trans)diagnostic tools and could lead to the development of novel therapeutic drugs to help relieve cognitive and affective symptoms.

Reference

1. Petersen RC. Mild cognitive impairment as a diagnostic entity. J Intern Med. 2004;256(3):183-94.
2. Jessen F, Amariglio RE, Van Boxtel M, Breteler M, Ceccaldi M, Chételat G, et al. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. Alzheimer's & dementia. 2014;10(6):844-52.
3. Mitchell A, Beaumont H, Ferguson D, Yadegarfar M, Stubbs B. Risk of dementia and mild cognitive impairment in older people with subjective memory complaints: meta-analysis. Acta Psychiatrica Scandinavica. 2014;130(6):439-51.
4. Liew TM. Subjective cognitive decline, anxiety symptoms, and the risk of mild cognitive impairment and dementia. Alzheimer's Research & Therapy. 2020;12(1):107.
5. Leung DKY, Chan WC, Spector A, Wong GHY. Prevalence of depression, anxiety, and apathy symptoms across dementia stages: A systematic review and meta-analysis. Int J Geriatr Psychiatry. 2021;36(9):1330-44.
6. Ogyu K, Kubo K, Noda Y, Iwata Y, Tsugawa S, Omura Y, et al. Kynurenine pathway in depression: A systematic review and meta-analysis. Neurosci Biobehav Rev. 2018;90:16-25.
7. Marx W, McGuinness AJ, Rocks T, Ruusunen A, Cleminson J, Walker AJ, et al. The kynurenine pathway in major depressive disorder, bipolar disorder, and schizophrenia: a meta-analysis of 101 studies. Mol Psychiatry. 2021;26(8):4158-78.
8. Willette AA, Pappas C, Hoth N, Wang Q, Klinedinst B, Willette SA, et al. Inflammation, Negative Affect, and Amyloid Burden in Alzheimer's Disease: Insights from the Kynurenine Pathway. Brain Behav Immun. 2021.
9. Giil LM, Midttun O, Refsum H, Ulvik A, Advani R, Smith AD, et al. Kynurenine Pathway Metabolites in Alzheimer's Disease. Journal of Alzheimer's Disease. 2017;60(2):495-504.
10. Kimura A, Naka T, Nohara K, Fujii-Kuriyama Y, Kishimoto T. Aryl hydrocarbon receptor regulates Stat1 activation and participates in the development of Th17 cells. Proc Natl Acad Sci U S A. 2008;105(28):9721-6.
11. Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain: when physiology meets pathology. Nat Rev Neurosci. 2012;13(7):465-77.
12. Gonzalez-Reyes RE, Nava-Mesa MO, Vargas-Sanchez K, Ariza-Salamanca D, Mora-Munoz L. Involvement of Astrocytes in Alzheimer's Disease from a Neuroinflammatory and Oxidative Stress Perspective. Front Mol Neurosci. 2017;10:427.
13. Mourdian MM, Heyes MP, Pan JB, Heuser IJ, Markey SP, Chase TN, et al. No changes in central quinolinic acid levels in Alzheimer's disease. Neurosci Lett. 1989;105(1-2):233-8.
14. Sofic E, Halket J, Przyborowska A, Riederer P, Beckmann H, Sandler M, et al. Brain quinolinic acid in Alzheimer's dementia. Eur Arch Psychiatry Neurol Sci. 1989;239(3):177-9.
15. Bitzer-Quintero OK, Dávalos-Marín AJ, Ortiz GG, Meza AR, Torres-Mendoza BM, Robles RG, et al. Antioxidant activity of tryptophan in rats under experimental endotoxic shock. Biomed Pharmacother. 2010;64(1):77-81.
16. Weiss G, Diez-Ruiz A, Murr C, Theur I, Fuchs D. Tryptophan Metabolites as Scavengers of Reactive Oxygen and Chlorine Species. Pteridines. 2002;13(4):140-3.
17. Pazos M, Andersen ML, Skibsted LH. Amino acid and protein scavenging of radicals generated by iron/hydroperoxide system: an electron spin resonance spin trapping study. J Agric Food Chem. 2006;54(26):10215-21.
18. Taemeeyapradit U, Udomittipong D, Tepparak N. Characteristics of behavioral and psychological symptoms of dementia, severity and levels of distress on caregivers. J Med Assoc Thai. 2014;97(4):423-30.
19. Charernboon T, Phanasathit M. Prevalence of neuropsychiatric symptoms in Alzheimer's disease: a cross-sectional descriptive study in Thailand. J Med Assoc Thai. 2014;97(5):560-5.
20. Kai K, Hashimoto M, Amano K, Tanaka H, Fukuhara R, Ikeda M. Relationship between Eating Disturbance and Dementia Severity in Patients with Alzheimer's Disease. PLOS ONE. 2015;10(8):e0133666.
21. Leblhuber F, Geisler S, Steiner K, Fuchs D, Schütz B. Elevated fecal calprotectin in patients with Alzheimer's dementia indicates leaky gut. Journal of Neural Transmission. 2015;122(9):1319-22.
22. Leng F, Edison P. Neuroinflammation and microglial activation in Alzheimer disease: where do we go from here? Nature Reviews Neurology. 2021;17(3):157-72.
23. Wissmann P, Geisler S, Leblhuber F, Fuchs D. Immune activation in patients with Alzheimer's disease is associated with high serum phenylalanine concentrations. Journal of the Neurological Sciences. 2013;329(1-2):29-33.
24. Widner B, Leblhuber F, Walli J, Tilz GP, Demel U, Fuchs D. Tryptophan degradation and immune activation in Alzheimer's disease. Journal of Neural Transmission. 2000;107(3):343-53.

25. Gold AB, Herrmann N, Swardfager W, Black SE, Aviv RI, Tennen G, et al. The relationship between indoleamine 2,3-dioxygenase activity and post-stroke cognitive impairment. Journal of Neuroinflammation. 2011;8 (no pagination).
26. Boado RJ, Li JY, Nagaya M, Zhang C, Pardridge WM. Selective expression of the large neutral amino acid transporter at the blood-brain barrier. Proc Natl Acad Sci U S A. 1999;96(21):12079-84.
27. Wang W, Zhao F, Ma X, Perry G, Zhu X. Mitochondria dysfunction in the pathogenesis of Alzheimer's disease: recent advances. Molecular Neurodegeneration. 2020;15(1):30.
28. Camacho-Pereira J, Tarragó MG, Chini CC, Nin V, Escande C, Warner GM, et al. CD38 dictates age-related NAD decline and mitochondrial dysfunction through an SIRT3-dependent mechanism. Cell metabolism. 2016;23(6):1127-39.
29. Merlo LMF, DuHadaway JB, Montgomery JD, Peng W-D, Murray PJ, Prendergast GC, et al. Differential Roles of IDO1 and IDO2 in T and B Cell Inflammatory Immune Responses. Frontiers in Immunology. 2020;11.
30. Metz R, Smith C, DuHadaway JB, Chandler P, Baban B, Merlo LMF, et al. IDO2 is critical for IDO1-mediated T-cell regulation and exerts a non-redundant function in inflammation. International Immunology. 2014;26(7):357-67.
31. Guillemin GJ, Brew BJ. Implications of the kynurenine pathway and quinolinic acid in Alzheimer's disease. Redox Rep. 2002;7(4):199-206.
32. Pemberton LA, Kerr SJ, Smythe G, Brew BJ. Quinolinic acid production by macrophages stimulated with IFN-gamma, TNF-alpha, and IFN-alpha. J Interferon Cytokine Res. 1997;17(10):589-95.
33. Guillemin GJ, Smythe GA, Veas LA, Takikawa O, Brew BJ. A beta 1-42 induces production of quinolinic acid by human macrophages and microglia. Neuroreport. 2003;14(18):2311-5.
34. Guillemin GJ, Brew BJ, Noonan CE, Takikawa O, Cullen KM. Indoleamine 2,3 dioxygenase and quinolinic acid immunoreactivity in Alzheimer's disease hippocampus. Neuropathol Appl Neurobiol. 2005;31(4):395-404.
35. Marx W, McGuinness AJ, Rocks T, Ruusunen A, Cleminson J, Walker AJ, et al. The kynurenine pathway in major depressive disorder, bipolar disorder, and schizophrenia: a meta-analysis of 101 studies. Mol Psychiatr. 2021;26(8):4158-78.
36. Ogyu K, Kubo K, Noda Y, Iwata Y, Tsugawa S, Omura Y, et al. Kynurenine pathway in depression: A systematic review and meta-analysis. Neuroscience & Biobehavioral Reviews. 2018;90:16-25.
37. Persidsky Y, Ramirez SH, Haorah J, Kanmogne GD. Blood-brain barrier: structural components and function under physiologic and pathologic conditions. J Neuroimmune Pharmacol. 2006;1(3):223-36.
38. Zipser BD, Johanson CE, Gonzalez L, Berzin TM, Tavares R, Hulette CM, et al. Microvascular injury and blood-brain barrier leakage in Alzheimer's disease. Neurobiol Aging. 2007;28(7):977-86.
39. Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, et al. Blood-brain barrier breakdown in the aging human hippocampus. Neuron. 2015;85(2):296-302.
40. Wu S, Yin Y, Du L. Blood–Brain Barrier Dysfunction in the Pathogenesis of Major Depressive Disorder. Cellular and Molecular Neurobiology. 2021.
41. Jacobs KR, Lim CK, Blennow K, Zetterberg H, Chatterjee P, Martins RN, et al. Correlation between plasma and CSF concentrations of kynurenine pathway metabolites in Alzheimer's disease and relationship to amyloid-β and tau. Neurobiology of Aging. 2019;80:11-20.
42. Sorgdrager FJH, Vermeiren Y, Van Faassen M, van der Ley C, Nollen EAA, Kema IP, et al. Age- and disease-specific changes of the kynurenine pathway in Parkinson's and Alzheimer's disease. Journal of Neurochemistry. 2019.
43. Hestad KA, Engedal K, Whist JE, Farup PG. The Relationships among Tryptophan, Kynurenine, Indoleamine 2,3-Dioxygenase, Depression, and Neuropsychological Performance. Front Psychol. 2017;8:1561.
44. Haroon E, Welle JR, Woolwine BJ, Goldsmith DR, Baer W, Patel T, et al. Associations among peripheral and central kynurenine pathway metabolites and inflammation in depression. Neuropsychopharmacology. 2020;45(6):998-1007.
45. Smith RS. The macrophage theory of depression. Med Hypotheses. 1991;35(4):298-306.
46. Dey A, Hankey Giblin PA. Insights into Macrophage Heterogeneity and Cytokine-Induced Neuroinflammation in Major Depressive Disorder. Pharmaceuticals (Basel). 2018;11(3).
47. Wang WY, Tan MS, Yu JT, Tan L. Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. Ann Transl Med. 2015;3(10):136.
48. Sántha P, Veszelka S, Hoyk Z, Mészáros M, Walter FR, Tóth AE, et al. Restraint stress-induced morphological changes at the blood-brain barrier in adult rats. Frontiers in molecular neuroscience. 2016;8:88.
49. Menard C, Pfau ML, Hodes GE, Kana V, Wang VX, Bouchard S, et al. Social stress induces neurovascular pathology promoting depression. Nat Neurosci. 2017;20(12):1752-60.
50. Rochfort KD, Cummins PM. The blood-brain barrier endothelium: a target for pro-inflammatory cytokines. Biochem Soc Trans. 2015;43(4):702-6.

51. Cheng Y, Desse S, Martinez A, Worthen RJ, Jope RS, Beurel E. TNFα disrupts blood brain barrier integrity to maintain prolonged depressive-like behavior in mice. Brain Behav Immun. 2018;69:556-67.
52. Enache D, Pariante CM, Mondelli V. Markers of central inflammation in major depressive disorder: A systematic review and meta-analysis of studies examining cerebrospinal fluid, positron emission tomography and post-mortem brain tissue. Brain, Behavior, and Immunity. 2019;81:24-40.
53. Demir S, Atli A, Bulut M, İbiloğlu AO, Güneş M, Kaya MC, et al. Neutrophil-lymphocyte ratio in patients with major depressive disorder undergoing no pharmacological therapy. Neuropsychiatr Dis Treat. 2015;11:2253-8.
54. Dion-Albert L, Cadoret A, Doney E, Kaufmann FN, Dudek KA, Daigle B, et al. Vascular and blood-brain barrier-related changes underlie stress responses and resilience in female mice and depression in human tissue. Nature Communications. 2022;13(1):164.
55. Badawy AA. Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects. Int J Tryptophan Res. 2017;10:1178646917691938.
56. 2022 Alzheimer's disease facts and figures. Alzheimer's & Dementia. 2022;18(4):700-89.

CHAPTER 7

Summary

CHAPTER 1 introduced the reader to the different types of dementia and the prodromal stages, i.e., subjective cognitive decline (SCD) and mild cognitive impairment (MCI). Moreover, it introduced the notion of affective symptomatology, such as depressive- and anxiety-like symptoms, commonly observed in patients with or at risk of developing dementia. Additionally, the chapter described the role of the kynurenine pathway (KP) associated metabolites, also known as kynurenines, and systemic inflammation in dementia and affective disorders. Furthermore, the interplay between kynurenines, inflammation, and cognitive and affective symptoms was discussed. Finally, an overview of the general structure of the thesis was presented.

CHAPTER 2 presented the first systematic review and meta-analysis investigating 1) the differences in tryptophan (TRP) and kynurenines levels in patients with evident cognitive impairment and healthy controls, as well as 2) the evidence of the relationship between levels of TRP and kynurenines in normal aging and cognition. After a careful and extensive screening process, 103 studies were deemed qualified for our systematic review, of which 68 studies compared metabolite levels between patients with cognitive impairment and neurologically healthy controls, 41 studies in metabolite associations with normal aging, 19 studies in metabolite associations with cognitive functions, and 26 studies met the meta-analysis criteria. In the systematic review, we have identified and summarized various cohort studies investigating TRP and kynurenines in cerebrospinal fluid (CSF), plasma, serum, post-mortem brain tissue, saliva, fecal, and urine samples. In all the biomaterial samples, TRP was the most measured amino acid followed by kynurenine (KYN) and kynurenic acid (KA). In the post-mortem brain tissue, TRP level was either higher or no difference and quinolinic acid (QA) showed no difference in Alzheimer's disease (AD) dementia patients compared to controls, while in saliva TRP was inconclusive. Lastly, urine samples showed either lower or no difference in TRP, lower KA and xanthurenic acid (XA), and either higher or no difference in KYN/TRP ratio (KTR) between AD and control, while no differences were reported for KYN and KA in the fecal samples between AD and control. Additionally, with regards to the relation between metabolite levels and age, age was associated with lower blood levels of TRP and a higher KTR and KYN level. Finally, higher KTR levels were associated with lower scores on several cognitive tests. Furthermore, our main meta-analysis, which compared AD dementia patients with controls, showed that AD dementia patients had lower overall (CSF and blood combined) levels of

TRP, anthranilic acid (AA), KA, XA, and QA. Additionally, no differences were observed for KYN, 3-hydroxykynurenine (3-HK), and the KTR. Our KA finding was in line with its hypothesized neuroprotective properties. Interestingly, while QA has been postulated to exert neurotoxic effects and thereby contributing to the pathogenesis of AD, our meta-analysis provided evidence of lower overall levels in AD dementia. Finally, meta-regression was performed to explain the inter-study differences in the meta-analysis. The results indicated that 'analytical technique' was the most frequently showed covariate and it explained the heterogeneity in KA and KTR. Additionally, the 'type of biomaterial' (CSF, blood, plasma, or serum), 'year of publication', and 'gender' were other covariates that caused the heterogeneity. Therefore, this study was shown to both validate the role of certain KP metabolites, while contradicting other current views on KP metabolite concentrations in AD dementia. Moreover, it has shown that KP metabolites were associated with aging and cognitive function and identified covariates that may influence the outcome.

In **CHAPTER 3**, for the first time, we demonstrated the use of both transcriptomic- and DNA (hydroxy)methylomic- profiling, as well as that of gene regulatory network (GRN) and associated network perturbation analyses, and pyrosequencing validation on the TRP catabolic pathway - more specifically the TRP metabolic pathway and the nicotinic adenine dinucleotide (NAD) pathway in patients with AD using post-mortem middle temporal gyrus (MTG) and in blood samples of two independent longitudinal AD cohorts, i.e., the Ageing, Cognition and Dementia in Primary Care Patients (AgeCoDe) cohort and the Biobank Alzheimer Center Limburg (BBACL) cohort. In the MTG analysis, we identified several TRP- and NAD-pathway-associated genes to be dysregulated at the transcriptomic- and/or DNA (hydroxy)methylomic- levels. In combination with GRN and associated network perturbation analysis, we identified three AD candidate genes, i.e., *IDO2*, *SLC7A5*, and *PARP14*, for further validation. In the AgeCoDe cohort, CpG site cg11251498 of *IDO2* was identified as a candidate methylation mark predicting future conversion to AD in healthy aged individuals. While pyrosequencing on the same CpG site in the BBACL cohort displayed no significant difference in methylation between SCD, MCI, and dementia patients, a significant negative age association was observed. As such, we have identified cg11251498 (*IDO2*) as a potential candidate biomarker for AD and have demonstrated that the use of transcriptional and DNA (hydroxy)methylomic profiling and associated *in silico* modeling

approaches represent a powerful tool to gain more insight in the development and course of disorders like AD.

CHAPTERS 4 and **5** represent large cross-sectional molecular-epidemiology studies making use of the BBACL cohort. Both chapters looked into affective symptomatology such as depressive- and anxiety-like symptoms through the informant-based neuropsychiatric inventory (NPI) depression (NPID) and NPI anxiety (NPIA) questionnaires, as well as making use of the self-administered 15-item geriatric depression scale (GDS15). The aim of **CHAPTER 4** was to investigate the association between plasma KP metabolites and affective symptomatology in patients with or at risk of developing dementia, while **CHAPTER 5** investigated the association between systemic inflammation markers and affective symptomatology in a similar context. In both **CHAPTERS 4** and **5**, caregivers in patients with dementia more commonly reported depressive-like symptoms than in patients with SCD and caregivers in patients with dementia more commonly reported anxiety-like symptoms than in patients with SCD and MCI. Moreover, in both **CHAPTERS 4** and **5,** our modeling showed that patient diagnosis status had little-to-no influence on the association analyses.

In **CHAPTER 4**, although unadjusted for factors, but adjusted for multiple testing, both patients with dementia and MCI had lower levels of TRP, XA, KA/QA ratio, and XA/QA ratio compared to patients with SCD, while KA/3-HK ratio was only lower in patients with dementia compared to patients with SCD. On the contrary, patients with dementia and MCI had higher levels of AA, QA, and KTR, while only patients with dementia had higher levels of KYN, 3-HK, and picolinic acid (PIC) compared to patients with SCD. No differences were shown for KA, 3-hydroxyanthranilic acid (3-HAA), and KA/KYN ratio between the groups. In the affective symptom associations, higher plasma levels of XA and PIC, as well as higher KA/QA, KA/KYN, and XA/QA ratios were associated with a lower incidence rate ratio (IRR) of developing depressive symptoms on the self-rated GDS15. Furthermore, the associations of XA, KYN, KA/QA, and XA/QA with depressive symptoms were robust and independent from various factors such as demographics, cognitive diagnosis, lifestyles, cofactors, and comorbidities, where KA/QA and XA/QA also remained significant after correcting for multiple testing. Finally, higher levels of KA were associated with a lower IRR of informant-reported anxiety-like symptoms

(NPIA). Altogether, we have demonstrated various kynurenines and associated ratios to be associated with affective symptoms in patients with dementia and its prodromal stages.

Since systemic inflammation plays a key role in mediating both affective and cognitive symptomatology, **CHAPTER 5** investigated the association between plasma inflammatory and endothelial markers and affective symptomatology in patients with or at risk of developing dementia. When comparing the systemic inflammation markers between the groups, although unadjusted for factors, but adjusted for multiple testing, both patients with dementia and MCI had higher levels of tumor necrosis factor alpha (TNFα), interleukin 6 (IL6), IL8, and soluble vascular adhesion molecule 1 (sVCAM-1) than patients with SCD. Only MCI patients had higher soluble intercellular molecule 1 (sICAM-1) and lower serum amyloid A (SAA) levels when compared to patients with SCD. Lastly, no differences were shown for interferon gamma (IFNγ), IL1β, IL10, IL2, C-reactive protein (CRP) and soluble E-selectin (sE-selectin). In the affective symptom association analysis, elevated levels of TNFα, IL6, IL8, CRP, SAA, sICAM-1, and sVCAM-1 were associated with higher IRR of developing depressive symptoms on the self-rated GDS15. Additionally, elevated IL10 levels in dementia patients and elevated sE-selectin in SCD and MCI patients were associated with a higher IRR on the GDS15. Lastly, higher levels of CRP and SAA in MCI patients and higher levels of sE-selectin in patients with dementia were associated with a lower IRR of informant-reported anxiety-like symptoms. Overall, we both validated previous observations and reported new findings in the association between systemic inflammation and affective symptomatology in patients with or at risk of developing dementia.

HOOFDSTUK 7

Nederlandse samenvatting

HOOFDSTUK 1 liet de lezer kennismaken met de verschillende vormen van dementie en de prodromale stadia, dat wil zeggen subjectieve cognitieve achteruitgang (subjective congitive decline – SCD) en milde cognitieve stoornis (mild cognitive impairment – MCI). Bovendien introduceerde het de notie van affectieve symptomatologie, zoals symptomen van depressie en angst, die vaak worden waargenomen bij patiënten of mensen met een verhoogd risico op het ontwikkelen van dementie. Daarnaast beschreef het hoofdstuk de rol van kynurenine pathway (KP) geassocieerde metabolieten, ook bekend als kynurenines, en systemische inflammatie bij dementie en affectieve stoornissen. Verder werd het verband tussen kynurenines, inflammatie en cognitieve en affectieve symptomen besproken. Ten slotte werd een overzicht gegeven van de algemene structuur van het proefschrift.

HOOFDSTUK 2 presenteerde de eerste systematische review en meta-analyse die 1) de verschillen in tryptofaan (tryptophan – TRP) en kynurenineniveaus bij patiënten met duidelijke cognitieve stoornissen en gezonde controles onderzocht, evenals 2) het bewijs van de relatie tussen niveaus van TRP en kynurenines bij normale veroudering en cognitie. Na een zorgvuldig en uitgebreid screeningproces werden 103 onderzoeken gekwalificeerd geacht voor onze systematische review, waarvan 68 onderzoeken metabolietniveaus vergeleken tussen patiënten met cognitieve stoornissen en neurologisch gezonde controles, 41 onderzoeken naar metabolietassociaties met normale veroudering, 19 onderzoeken naar metabolietassociaties met cognitieve functies, waarvan in totaal 26 studies voldeden aan de meta-analysecriteria. In de systematische review hebben we verschillende cohortstudies geïdentificeerd en samengevat die TRP en kynurenines in cerebrospinale vloeistof (cerebrospinal fluid – CSF), plasma, serum, post-mortem hersenweefsel, speeksel, en fecale en urinemonsters onderzochten. In alle biomateriaalmonsters was TRP het meest gemeten aminozuur, gevolgd door kynurenine (kynurenine – KYN) en kynureninezuur (kynurenic acid – KA). In het post-mortem hersenweefsel was het TRP-niveau ofwel hoger of er was geen verschil waarneembaar. Chinolinezuur (quinolinic acid – QA) vertoonde geen verschil bij patiënten met de ziekte van Alzheimer (Alzheimer's disease - AD) vergeleken met controles, terwijl TRP in speeksel geen uitsluitsel gaf. Tenslotte vertoonden urinemonsters een lager niveau van of geen verschil in TRP, lagere niveaus van KA en xanthureenzuur (xanthurnic acid – XA), en ofwel een hoger niveau of geen verschil in de KYN/TRP-verhouding (KYN/TRP ratio – KTR) tussen AD patiënten en controles, terwijl er geen

verschillen werden gerapporteerd voor KYN en KA in de fecale monsters tussen AD en controle. Bovendien, met betrekking tot de relatie tussen metabolietniveaus en leeftijd, was leeftijd geassocieerd met lagere bloedspiegels van TRP en een hoger KTR- en KYN-niveau. Ten slotte waren hogere KTR-niveaus geassocieerd met lagere scores op verschillende cognitieve tests. Bovendien toonde onze belangrijkste meta-analyse, waarin patiënten met AD-dementie werden vergeleken met controles, aan dat patiënten met AD-dementie lagere algemene (CSF en bloed gecombineerd) niveaus van TRP, antranilzuur (anthranilic acid – AA), KA, XA en QA hadden. Bovendien werden er geen verschillen waargenomen voor KYN, 3-hydroxykynurenine (3-HK) en de KTR. Onze KA-bevinding was in overeenstemming met de veronderstelde neuroprotectieve eigenschappen. Interessant is dat, hoewel wordt aangenomen dat QA neurotoxische effecten uitoefent en daardoor bijdraagt aan de pathogenese van AD, onze meta-analyse bewijs leverde van lagere algemene niveaus bij AD-dementie. Ten slotte werd een meta-regressie uitgevoerd om de verschillen tussen de studies in de meta-analyse te verklaren. De resultaten gaven aan dat 'analytische techniek' de meest voorkomende covariabele was en het verklaarde de heterogeniteit in KA en KTR. Bovendien waren het 'type biomateriaal' (CSF, bloed, plasma of serum), 'jaar van publicatie' en 'geslacht' andere covariabelen die de heterogeniteit veroorzaakten. Daarmee werd aangetoond dat deze studie zowel de rol van bepaalde KP-metabolieten valideert, als andere huidige opvattingen over KP-metabolietconcentraties bij AD-dementie tegenspreekt. Bovendien heeft het aangetoond dat KP-metabolieten geassocieerd waren met veroudering en cognitieve functie en werden covariaten geïdentificeerd die de uitkomst kunnen beïnvloeden.

In **HOOFDSTUK 3** hebben we voor het eerst het nut aangetoond van zowel transcriptoom- als DNA (hydroxy)methyloomprofilering, gene regulatory network (GRN) en network perturbation analysis, en pyrosequencing-validatie op de TRP-katabolische pathway - meer specifiek de TRP metabole pathway en de nicotine adenine dinucleotide (NAD) pathway bij patiënten met AD met behulp van post-mortem middlee temporal gyrus (MTG) en in bloedmonsters van twee onafhankelijke longitudinale AD-cohorten, namelijk de veroudering, Ageing, Cognition and Dementia in Primary Care Patients (AgeCoDe) en het cohort Biobank Alzheimer Centrum Limburg (BBACL). In de MTG-analyse identificeerden we verschillende TRP- en NAD-pathway-geassocieerde genen die ontregeld waren op transcriptomische en/of DNA (hydroxy)methylomische

niveau(s). In combinatie met een GRN en network perturbation analysis identificeerden we drie AD-kandidaatgenen, namelijk *IDO2* , *SLC7A5* en *PARP14*, voor verdere validatie. In het AgeCoDe-cohort werd de CpG-site cg11251498 van *IDO2* geïdentificeerd als een kandidaat-methyleringsmarkering die toekomstige conversie naar AD bij gezonde oudere personen voorspelde. Hoewel pyrosequencing op dezelfde CpG-plaats in het BBACL-cohort geen significant verschil in methylering vertoonde tussen SCD-, MCI- en patiënten met dementie, werd een significant negatief leeftijdsassociatie waargenomen. Als zodanig hebben we cg11251498 (*IDO2*) geïdentificeerd als een potentiële kandidaat-biomarker voor AD en hebben we aangetoond dat het gebruik van transcriptionele en DNA (hydroxy)methylomische profilering en geassocieerde *in silico* modeling een krachtig hulpmiddel vormen om meer inzicht te krijgen in de ontwikkeling en verloop van aandoeningen zoals AD.

HOOFDSTUK 4 en **5** vertegenwoordigen grote moleculair-epidemiologische studies die gebruik maken van het BBACL-cohort. In beide hoofdstukken werd ingegaan op affectieve symptomatologie, zoals symptomen van depressie en angst, onder andere met behulp van neuropsychiatrische vragenlijsten (neuropsychiatric inventory – NPI; depressie [NPID] en NPI angst [NPIA]) en de geriatrische depressieschaal (geriatric depression scale 15 – GDS15). Het doel van **HOOFDSTUK 4** was om de associatie tussen plasma KP-metabolieten en affectieve symptomatologie te onderzoeken bij patiënten met of een risico op het ontwikkelen van dementie, terwijl **HOOFDSTUK 5** de associatie tussen systemische ontstekingsmarkers en affectieve symptomatologie in een vergelijkbare context onderzocht. Zowel in **HOOFDSTUK 4** als in **HOOFDSTUK 5** rapporteerden zorgverleners bij patiënten met dementie vaker depressieve symptomen dan bij patiënten met SCD en zorgverleners bij patiënten met dementie rapporteerden vaker angstachtige symptomen dan bij patiënten met SCD en MCI. Bovendien toonde onze modellen zowel in **HOOFDSTUK 4** als in **HOOFDSTUK 5** aan dat de status van de diagnose van de patiënt weinig tot geen invloed had op de associatieanalyses.

In **HOOFDSTUK 4**, gecorrigeerd voor multiple testing, hadden zowel patiënten met dementie als MCI lagere TRP en XA waardes, alsmede een lagere KA/QA- en XA/QA-ratio vergeleken met patiënten met SCD, terwijl de KA/3-HK-ratio alleen lager was bij patiënten met dementie in vergelijking met patiënten met SCD. Integendeel, patiënten met dementie en MCI hadden hogere niveaus van

AA, QA en KTR, terwijl alleen patiënten met dementie hogere niveaus van KYN, 3-HK en picolinezuur (picolinic acid – PIC) hadden in vergelijking met patiënten met SCD. Er werden geen verschillen aangetoond voor KA, 3-hydroxyantranilzuur (3-hydroxyanthranilic acid – 3-HAA) en de KA/KYN-verhouding tussen de groepen. In de associaties met affectieve symptomen waren hogere plasmaspiegels van XA en PIC, evenals hogere KA/QA-, KA/KYN- en XA/QA-ratio's geassocieerd met een lagere incidentieratio (incidence rate ratio – IRR) van het ontwikkelen van depressieve symptomen op de zelfgerapporteerde GDS15. Bovendien waren de associaties van XA, KYN, en de KA/QA en XA/QA ratio's met depressieve symptomen robuust en onafhankelijk van verschillende factoren zoals demografie, cognitieve diagnose, levensstijl, cofactoren en comorbiditeiten, waarbij ook de KA/QA en XA/QA ratio's significant bleven na correctie voor multiple testing. Ten slotte waren hogere niveaus van KA geassocieerd met een lagere IRR van door informant gerapporteerde angstachtige symptomen (NPIA). Al met al hebben we aangetoond dat verschillende kynurenines en bijbehorende ratio's geassocieerd zijn met affectieve symptomen bij patiënten met dementie en de prodromale stadia ervan.

Aangezien systemische ontsteking een sleutelrol speelt bij het mediëren van zowel affectieve als cognitieve symptomatologie, onderzocht **HOOFDSTUK 5** de associatie tussen de niveaus van inflammatoire en endotheliale markers in het plasma en affectieve symptomatologie bij patiënten met of mensen met een risico op het ontwikkelen van dementie. Bij het vergelijken van de markers voor systemische ontsteking tussen de groepen, gecorrigeerd voor multiple testing, hadden zowel patiënten met dementie als MCI hogere niveaus van tumornecrosefactor-alfa (tumor necrosis factor alpha – TNFα), interleukine 6 (interleukin 6 – IL6), IL8 en oplosbaar vasculaire adhesiemolecuul 1 (soluble vascular cell adhesion molecule-1 – sVCAM-1) dan patiënten met SCD. Alleen MCI-patiënten hadden hogere oplosbare intercellulaire molecule 1 (soluble intercellular adhesion molecule-1 – sICAM-1) en lagere serumamyloïde A (serum amyloid A – SAA)-spiegels in vergelijking met patiënten met SCD. Ten slotte werden geen verschillen aangetoond voor interferon-gamma (IFNγ), IL1β, IL10, IL2, C-reactief proteïne (C-reactive protein – CRP) en oplosbaar E-selectine (soluble E-selectin – sE-selectine). In de affectieve symptoomassociatie-analyse waren verhoogde niveaus van TNFα, IL6, IL8, CRP, SAA, sICAM-1 en sVCAM-1 geassocieerd met een hogere IRR van het

ontwikkelen van depressieve symptomen op de GDS15. Bovendien waren verhoogde IL10-spiegels bij patiënten met dementie en verhoogd sE-selectine bij SCD- en MCI-patiënten geassocieerd met een hogere IRR op de GDS15. Ten slotte waren hogere niveaus van CRP en SAA bij MCI-patiënten en hogere niveaus van sE-selectine bij patiënten met dementie geassocieerd met een lagere IRR van door informanten gerapporteerde angstachtige symptomen. Over het algemeen hebben we zowel eerdere observaties gevalideerd als nieuwe bevindingen gerapporteerd in de associatie tussen systemische ontsteking en affectieve symptomatologie bij patiënten met of mensen met een risico op het ontwikkelen van dementie.

CHAPTER 8

Impact paragraph

Scientific Impact

As mentioned throughout this thesis, dementia is a syndrome characterized by loss of memory, language, problem-solving, and social abilities severe enough to interfere with daily life. It is a heterogeneous syndrome, with individuals being affected differently based on the type of dementia and e.g., those region(s) of the brain affected by pathology. The underlying cause of dementia is still unclear, but certain risk groups, such as patients with mild cognitive impairment (MCI) and subjective cognitive decline (SCD), and associated biological factors, such as age or genetics, are associated with an increased risk of developing dementia. Besides cognitive issues, those suffering from SCD, MCI, and dementia often experience affective symptoms including depressive- and anxiety-like symptomatology.

For decades, the amyloid and tau hypotheses have been investigated and, at this moment, the best method to diagnose Alzheimer's disease (AD) is by measuring the protein concentrations of amyloid beta 1-42 ($A\beta_{1\text{-}42}$), total tau, and phosphorylated tau biomarkers in the cerebrospinal fluid (CSF). Despite lumbar puncture being extremely useful in measuring biomarkers for dementia, its use is accompanied with complications. Thus, an alternative and less invasive diagnostic method is necessary. Currently, blood-based early diagnostic tools for AD have been developed and commercialized by companies such as C_2N Diagnostics and QuantaMatrix. However, these kits mainly measure Aβ and/or Apolipoprotein E, which only represents the tip of the (pathological) iceberg. As shown in this thesis, other mechanisms such as inflammation and metabolic dysregulation are likely to be involved as well and more insight into these mechanisms and the identification of associated signatures may contribute to better diagnostic (and prognostic) tools for disorders like AD.

In recent years, the tryptophan (TRP) metabolic pathway has gained attention because of its involvement in dementia, emotional dysregulation, and systemic inflammation. TRP is an essential amino acid and serves as precursor to e.g., the kynurenine pathway (KP), the serotonin pathway, and the tryptamine pathway. Multiple (pre)clinical studies have shown neuroactive properties of KP-associated metabolites. Additionally, activity of the KP is upregulated in the brain during systemic inflammation, a phenomenon often occurring in dementia and affective disorders. Although many cross-sectional studies have compared

KP metabolite levels in e.g., AD dementia patients and healthy controls, the findings are often different for every study. Therefore, **CHAPTER 2**, for the first time, systematically collected, summarized and re-analyzed all articles published on the relationship between levels of kynurenines in patients with evident cognitive decline and in normal aging, as well as the associations of kynurenines with age or cognition, up to April 21 2021. As such, we presented via meta-analysis, which kynurenine concentrations were lower, higher or showed no difference between patients with AD dementia and neurologically healthy controls in various biomaterials such as CSF, blood, plasma, serum, and CSF and blood combined. Additionally, we have identified various kynurenines to show an association with age or cognition. Lastly, through meta-regression, we have identified factors that influence the concentrations, thus demonstrated the importance of controlling covariates in clinical studies. The findings from this systematic review and meta-analysis could potentially serve as biomarkers for AD and hints at treatment targets to halt neurotoxic or to stimulate neuroprotective contributors.

While protein and metabolite levels may provide valuable insights into e.g., disease phenotypes and associated causal factors, additionally studying associated transcriptional and epigenetic profiles yields an even better understanding of the pathophysiology of disorders like AD. Therefore, **CHAPTER 3** described both a transcriptomic- and DNA (hydroxy)methylomic-profiling, as well as associated gene regulatory network (GRN) and network perturbation analyses on the TRP catabolic pathway making use of middle temporal gyrus (MTG) of AD and control brain tissue. Additionally, these findings were validated in two independent blood-based cohorts. From the approach taken in this chapter, we have demonstrated the scientific importance of applying various -omics approaches as well as using *in silico* models such as GRN and network perturbation analyses to select candidate gene(s) in AD pathology. Preclinical studies have investigated different KP enzyme inhibitors as neurotherapeutics, such as targeting indoleamine 2,3-dioxygenase (IDO), tryptophan 2,3-dioxygeanse (TDO2) kynurenine aminotransferase (KAT), and kynurenine-3-monooxygenase (KMO) enzymes. **CHAPTER 3** has shown dysregulation of several KP genes, including IDO and TDO2, and these findings, in combination with **CHAPTER 2**, could serve as a pillar to validate our current knowledge in KP involvements in cognitive disorders and open to new discoveries into biomarker application and, potentially, drug targets.

The KP associated metabolites and inflammatory dysfunctions are well documented in cognitive and affective disorders. Although dementia represents a syndrome primarily associated with cognitive decline, it is not uncommon for patients to exhibit depressive- and anxiety-like symptoms. However, the relationship between KP and inflammation with cognitive or affective disorder have been addressed independently from each other. As such it remains unclear whether these associations are shared between or specific for one disorder. Therefore, making use of a large cross-section study, **CHAPTERS 4** and **5** respectively investigated the association between affective symptoms and kynurenines or systemic inflammation in patients with or at risk for dementia. Both chapters used various models to adjust for different covariates. In **CHAPTER 4**, we have shown several kynurenines and their ratios to be associated with self-reported depressive symptoms, informant reported anxiety-like symptoms. In a similar manner, **CHAPTER 5** also showed several inflammatory and endothelial markers to be associated with self-reported depressive- and informant-reported anxiety-like symptoms. Moreover, the majority of these markers lost its association once adjusted for lifestyle-related covariates, demonstrating that lifestyle is an important factor to consider when investigating systemic inflammation. The findings in both chapters showed that several identical KP metabolites and inflammation markers showed difference in concentration between patients with dementia or its prodromal stages and were associated with affective symptoms while other markers were involved in one disorder. These findings could be implemented for biomarker purposes for individual cognitive and affective symptoms, but also used as a transdiagnostic tool and, potentially, develop a transdiagnostic treatment.

Societal Impact

Worldwide, 55 million people currently suffer from dementia, with 10 million new cases every year. Moreover, the global societal costs for dementia were estimated to represent a total of 1.3 trillion US dollars in 2019 illustrating the profound socio-economic impact dementia has. In addition, there is currently no treatment for e.g., AD, while the exact cause of this neurodegenerative disorder is still unclear. On top of this, AD remains underdiagnosed, underreported, and the diagnosis is delayed by an average of 2-3 years after onset of the symptoms [1, 2]. Since the pathophysiological changes due to AD may occur many years before symptoms appear, early detection of AD through measuring biomarkers

and monitoring lifestyle interventions such as education, diet, cognitive stimulation, and comorbidities in the prodromal stages of AD would facilitate improved diagnosis, decrease the risk of developing AD, and introduce early treatment options. Furthermore, preventing or treating affective symptoms in the early stage is an important intervention to prevent further deterioration since it is common for patients with dementia or its prodromal stages to have depressive- and anxiety-like symptoms. While this thesis provides valuable insights into new potential biomarkers in this respect, it is important to note that the findings in this thesis requires further validation before being able to have a direct impact on society. Nevertheless, the work described in this thesis adds to the foundation that will improve early diagnostics and treatment for dementia in the near future.

Reference

1. 2022 Alzheimer's disease facts and figures. Alzheimer's & Dementia. 2022;18(4):700-89.
2. Sabbagh MN, Lue LF, Fayard D, Shi J. Increasing Precision of Clinical Diagnosis of Alzheimer's Disease Using a Combined Algorithm Incorporating Clinical and Novel Biomarker Data. Neurol Ther. 2017;6(Suppl 1):83-95.

CHAPTER 9

Curriculum vitae

Kyonghwan (Chris) Choe was born on February 15, 1992 in Daejeon, South Korea. Chris grew up around the world. He lived in Germany, Qatar, Korea, France, and graduated as class of 2010 in The American School of Doha, Qatar. In the same year, he started his undergraduate study at Purdue University in West Lafayette, Indiana, U.S.A and graduated as class of 2014 majoring in biochemistry and minoring in biology. During his 2 years undergraduate internship at Dr. Kavita Shah's labs, Chris experienced first-hand the field of neuroscience and lab work. His project aimed towards developing specific activators and inhibitors of any G protein of interest in Alzheimer's disease.

After completing his undergraduate, Chris wanted to improve his lab skills, thus worked as a lab technician under the guidance of Dr. Shu Chen at Case Western Reserve University in Cleveland, Ohio, U.S.A for 1.5 years. He used caenorhabditis elegans (*C. elegans*) model to study Parkinson's disease. He took part in two projects: 1) investigating the regulation of DJ-1 by glutaredoxin 1 in Parkinson's disease and 2) investigating the effect of alpha-synuclein aggregation in aged C. elegans, of which both projects were published.

During his lab technician period, Chris was accepted into the master's in Biomedical Sciences program at Maastricht University, Maastricht, the Netherlands. In 2018, he successfully obtained his degree, specialized in neuroscience. During his junior internship, under the supervision of Dr. Theo Gorgels and Wouter Hubens, he investigated apoptosis and amyloid-beta plaques in the retinal ganglion cell layer of the eye in genetically modified AD mouse model. Afterwards, during his senior internship, he investigated DNA methyltransferase isoform expression in the temporal lobe of epilepsy patients with a history of febrile seizures, under the supervision of Dr. Laurence de Nijs and Prof. Dr. Bart Rutten, and the study was published.

After successfully obtaining his Master's degree, Chris was given a PhD position in the Department of Psychiatry and Neuropsychology, School of Mental Health and Neuroscience (MHeNs), Maastricht University, Maastricht, the Netherlands, under the primary supervision of Prof. Dr. Bart Rutten and Prof. Dr. Daniël van den Hove, and co-supervised by Dr. Gunter Kenis and Dr. Sebastian Köhler. Chris and Lieke Bakker were the first PhD candidates to join the MHeNs Transdivisional project between division 1 (Lieke, cognitive neuropsychiatry & clinical neuroscience) and division 3 (Chris, translational neuroscience) to

investigate the association between the kynurenine pathway and cognitive and affective symptomatology in patients with or at risk of developing dementia using the BioBank Alzheimer Center Limburg (BBACL) cohort.

During his PhD, Chris bisulfite converted over 200 BBACL DNA samples for his pyrosequencing project, but also for future pyrosequencing projects of others. He obtained the European Graduate School of Neuroscience (EURON) mobility grant to perform pyrosequencing in Hasselt University, Hasselt, Belgium. Furthermore, he measured 12 proinflammatory and endothelial markers in 800+ plasma and 100+ cerebrospinal fluid (CSF) samples in the BBACL cohort and helped in the Cognitive and Affect after Stroke: a Prospective Evaluation of Risks (CASPER) cohort (n = 200+) as well.

Currently, Chris is working as a post-doc in Gyeongsang National University, Jinju, South Korea, in the group of Prof. Dr. Myeong Ok Kim in the field of early diagnostic biomarker for Alzheimer's disease. In addition, he is still affiliated with Maastricht University and acts as a bridge for international collaborations and continue his work in the BBACL projects.

CHAPTER 10

List of publications

Published

- Kim, M. W.†, **Choe, K.**†, Park, J. S.†, Lee, H. J., Kang, M. H., Ahmad, R., & Kim, M. O. (2022). Pharmacological Inhibition of Spleen Tyrosine Kinase Suppressed Neuroinflammation and Cognitive Dysfunction in LPS-Induced Neurodegeneration Model. *Cells*, *11*(11), 1777. †The authors have contributed equally in this study.

- **Choe, K.**†, Park, H. Y.†, Ikram, M.†, Lee, H. J.†, Park, T. J., Ullah, R., & Kim, M. O. (2022). Systematic Review of the Common Pathophysiological Mechanisms in COVID-19 and Neurodegeneration: The Role of Bioactive Compounds and Natural Antioxidants. *Cells*, *11*(8), 1298. †The authors have contributed equally in this study.

- Ikram, M.†, Jo, M. H.†, **Choe, K.**†, Khan, A., Ahmad, S., Saeed, K., Kim, M. W., & Kim, M. O. (2021). Cycloastragenol, a Triterpenoid Saponin, Regulates Oxidative Stress, Neurotrophic Dysfunctions, Neuroinflammation and Apoptotic Cell Death in Neurodegenerative Conditions. *Cells*, *10*(10), 2719. †The authors have contributed equally in this study.

- Giovagnoni, C., Ali, M., Eijssen, L., Maes, R., **Choe, K.**, Mulder, M., Kleinjans, J., Del Sol, A., Glaab, E., Mastroeni, D., Delvaux, E., Coleman, P., Losen, M., Pishva, E., Martinez-Martinez, P., & van den Hove, D. (2021). Altered sphingolipid function in Alzheimer's disease; a gene regulatory network approach. *Neurobiology of aging*, *102*, 178–187.

- Khan, M., Ullah, R., Rehman, S. U., Shah, S. A., Saeed, K., Muhammad, T., Park, H. Y., Jo, M. H., **Choe, K.**, Rutten, B., & Kim, M. O. (2019). 17β-Estradiol Modulates SIRT1 and Halts Oxidative Stress-Mediated Cognitive Impairment in a Male Aging Mouse Model. *Cells*, *8*(8), 928.

- de Nijs, L., **Choe, K.**, Steinbusch, H., Schijns, O., Dings, J., van den Hove, D., Rutten, B., & Hoogland, G. (2019). DNA methyltransferase isoforms expression in the temporal lobe of epilepsy patients with a history of febrile seizures. *Clinical epigenetics*, *11*(1), 118.

- Chen, S. G., Stribinskis, V., Rane, M. J., Demuth, D. R., Gozal, E., Roberts, A. M., Jagadapillai, R., Liu, R., **Choe, K.**, Shivakumar, B., Son, F., Jin, S., Kerber, R., Adame, A., Masliah, E., & Friedland, R. P. (2016). Exposure to the Functional Bacterial Amyloid Protein Curli Enhances Alpha-Synuclein Aggregation in Aged Fischer 344 Rats and Caenorhabditis elegans. *Scientific reports*, *6*, 34477.

- Johnson, W. M., Golczak, M., **Choe, K.**, Curran, P. L., Miller, O. G., Yao, C., Wang, W., Lin, J., Milkovic, N. M., Ray, A., Ravindranath, V., Zhu, X., Wilson, M. A., Wilson-Delfosse, A. L., Chen, S. G., & Mieyal, J. J. (2016). Regulation of DJ-1 by Glutaredoxin 1 in Vivo: Implications for Parkinson's Disease. *Biochemistry*, *55*(32), 4519–4532.

Submitted

- **Choe, K.**†, Bakker, L.†, van den Hove, D., Eussen, S., Kenis, G., Ramakers, I., Rutten, B., Verhey, F., & Köhler, S. (2022). Relation of the kynurenine pathway with cognitive functioning and differences in patients with cognitive impairment or dementia: a systematic review and meta-analysis. *Submitted*. †The authors have contributed equally in this study.

- **Choe, K.**, Ali, M., Lardenoije, R., Pishva, E., Leber, M., Wagner, H., Hoffmann, P., Luck, T., Riedel-Heller, S., Jessen, F., Maier, W., Wagner, M., Hurlemann, R., Mastroeni, D., Delvaux, E., Coleman, P., Ramirez, A., Ramakers, I., Verhey, F., Rutten, B., Kenis, G., & van den Hove, D. (2022). Alzheimer's disease-specific transcriptomic and epigenomic changes in the tryptophan catabolic pathway. *Submitted*.

- **Choe, K.**, Kenis, G., Ramakers, I., Köhler, S., Bakker, L., Eussen, S., Kim, M. O., Ueland, P., Midttun, Ø., Veryhey, F., van den Hove, D., & Rutten, B. (2022). Associations between plasma metabolites of the kynurenine pathway and affective symptomatology in subjects with or at risk for dementia. *Submitted*.

- Kim, M. W.†, **Choe, K.**†, Park, J. S.†, Lee, H. J., Rutten, B., & Kim, M. O. (2022). Osmotin regulates MAPK-mediated neurodegeneration in Parkinson's disease-associated pathology. *Submitted*. †The authors have contributed equally in this study.

- Lee, H. J.†, **Choe, K.**†, Khan, A., Kim, M. W., Park, J. S., Park, T. J., & Kim, M. O. (2022). O-cyclic phytosphingosine-1-phosphate protects against motor dysfunctions and glial cell mediated neuroinflammation in the Parkinson's disease mouse models. *Submitted.* †The authors have contributed equally in this study.

In preparation

- **Choe, K.**, van den Hove, D., Ramakers, I., Köhler, S., Bakker, L., Verhey, F., Schalkwijk, C., Rutten, B., Kim, M. O., & Kenis, G. (2022). Associations between plasma inflammatory and endothelial markers and affective symptomatology in subjects with or at risk for dementia. *In preparation*.

- Bakker, L.†, **Choe, K.**†, van den Hove, D., Eussen, S., Kenis, G., Ramakers, I., Rutten, B., Verhey, F., & Köhler, S. (2022). Relation of the kynurenine pathway with normal aging: a systematic review. *In preparation*. †The authors have contributed equally in this study.

CHAPTER 11

Acknowledgments

First and foremost, I would like to thank my promotion team for not only guiding me to become a better researcher, but also providing a fun and exciting work environment.

Bart, I still remember our first encounter when you were the division III leader. I walked into your big office at the end of my Master's junior internship asking if I can have my senior internship in your group. Thank you for giving me the opportunity to pursue my PhD degree. I really enjoyed having our small meetings time-to-time and I really appreciated all the inputs you gave for my research and my future career in academia. Again, thank you for giving me this opportunity and supporting me throughout my Master's and PhD years.

Daniël, you really showed me what a professor should be like. You are the supervisor every PhD students dreamed to have. You knew everything about everyone's research, gave valuable inputs in every meeting, cared about our work-life balance, and, most importantly, showed that science is fun. Because of your positive energy, I always looked forward in our meetings and never felt scared to enter your office. If I do become a professor, I wish I could become someone like you. Thank you for all the memorable times during my PhD, guiding me to become who I am today, and showing me what kind of person I want to become in the future.

Gunter, you also showed me what a professor should be like. You showed me that a professor should be intelligent and knowledgeable, but also care about teaching, networking, and enjoying the little things. I was most impressed with your time management. Even though you were extremely busy with EURON and teaching, you still cared about my PhD and I was never tossed aside for later. Also, when I was panicking because something didn't go as planned, your calm attitude really kept me back on track. Thank you for believing me and supporting me to improve day-by-day.

Sebastian, my sincerest appreciation for guiding me with all the epidemiology works. You were always available when I had questions about statistical models and SPSS related troubles. It has been a truly enriching experience and your support has significantly contributed to my skillets, which I will benefit throughout my academic career. Therefore, I want to express my sincerest gratitude.

Next, I would like to acknowledge the transdivisional group.

Inez, thank you for setting up, organizing, and giving me the opportunity to work with the BBACL cohort. **Frans**, thank you for your inputs, especially during our transdivisional meetings. It has been a great honor and pleasure to be able to work with you. **Simone**, thank you for sharing your knowledge in the kynurenines and the field of epidemiology. I have learned so much. Also, thank you for organizing the mini-symposium with Bevital and giving me the opportunity to present my work.

Lieke, the other better half of the PhD student in the group. Without your help, I do not know if I could have successfully finished my PhD. I really enjoyed our zoom meetings that always lasted longer than planned. I really appreciated your honest and extremely helpful feedback. Thank you for being patient and teaching me SPSS. I am sure it was not easy working with someone who had zero knowledge in epidemiology, but you did a great job in teaching me and thank you for being a great colleague throughout my PhD.

I am equally thankful to **Prof. Dr. Jos Prickaerts, Prof. Dr. Pilar Martinez-Martinez, Dr. Martin van Boxtel, Prof. Dr. Ellen Nollen, Dr. Livia Picker** for being on my assessment committee. Thank you for taking the time to read and to assess my thesis.

A warm and special thank you also goes to **Prof. Dr. Casper Schalkwijk** in the department of internal medicine for the collaboration in the cytokine project. Also, I would like to express my sincerest gratitude to **Marjo** for helping me setup the cytokine lab works and teaching me all the necessary skills to successfully measure the cytokines.

I would like to thank **Rachelle** for organizing all the administration of my PhD thesis!

Then, I would like to thank my paranymphs for being a great colleague and helping me with my thesis defense. Without their support, my defense would have been a disaster. Therefore, it is safe to say that I could not have asked for better paranymphs. Thank you for your hard work and it was a truly memorable

moment. For those who are looking for paranymphs, I definitely recommend both of them!

Jackson, your American personality always reminds me the good, the bad, and the weird times I had in the States. You always gave me an honest answer without sugarcoating. It may seem hurtful, but truthful and I always appreciated it. Additionally, Friday drinks were always more fun when you brought up variety of topics and fact checking everything. It always amazed me how much facts and topics you knew and that you can start a conversation with anyone about anything. The thing that amazed me the most was your enthusiasm in your work and the speed you can design and write a manuscript. Thank you for all the great memories, providing me my childhood American candies, and just being a great and fun colleague. Also, thank you for donating your fridge to our office. Without that fridge, Fridays would have been very different.

Renzo, the forever officemate, I realized you have been always sitting next to me or maybe it is the other way around. I still remember when I first met you in EURON EPI-AD conference in Barcelona. I thought you were very smart and friendly and I was not wrong. When you first came to our office, you were very quiet, a bit goofy, and always serious, but now the office has molded you into the master of spotting others when they arrive late/leave early and making everything into mom jokes. Nevertheless, somethings never change, you are still super smart and friendly to everyone, hence I always see students/colleagues stopping by the office to ask you questions. Also, thank you for all the help and fun memories during my PhD, especially for teaching me pyrosequencing, and answering all my endless questions.

A huge thank you to the Neuroepigenetics group. Each and every one helped me become a better scientist. Thank you for listening to my (boring) presentations, giving valuable feedback, and just being a great colleague.

Laurence, you were my daily supervisor during my Master's senior internship and gave me the opportunity to finish and publish the project during my PhD. Thank you for all the valuable inputs during my PhD and it has been a pleasure to be working with you.

Ehsan, I still remember my first impression of you and I was scared. It was during the division III BBQ party in front of the university building and I just started my PhD. You approached me to ask about my PhD project and I wasn't able to answer, so you looked unsatisfied and walked away. However, as time passed by, I realized my impression of you was completely wrong. You were kind and extremely helpful. Thank you for explaining bioinformatics in a way I could understand, which helped me tremendously for my project.

Muhammad and **Roy**, I asked so many questions and both of you were very patient and clearly explained everything. Thank you both for teaching me basic R and all your bioinformatics inputs in my project. It was a pleasure working with both of you!

Philippos, the core of the Neuroepigenetics group. I could really see why you are the Mr. Popular in the division. Your presence in the office, group, and department just makes everything better and fun. Your (dark) humors and (mom) jokes never gets old. You didn't hesitate to take over my goldfish when their faith was "down the toilet". Also, Friday beers in the office was just better when you were around. Thank you for being a great office-mate and a colleague.

Dean, MORGEN! Sometimes you were in our office more often than in your own, but it did not bother me at all because it was always fun having you around. It always surprised me that one minute you are super serious with your work and the next you run into our office playing carnival songs. Scientific discussions with you was fun, but showing off each other's cat was definitely the best moments. I still remember both of us hungover and listening to a sales person just to get a doll (Cap'n T cell and Stemmy). Who can forget our attempt to fix the coffee machine using reverse engineering? Thank you for all the great memories and I know you will be a wise and fun dad!

Martin, you were here for a very short time, but you made my time in the office fun. I am sure sitting next to me got you no work done, but I really enjoyed your company. When you went back to Argentina, your pacman decoration on the window always reminded me of our fun times. Unfortunately the packman had to go for window cleaning. People still talk about your farewell party, organized by me, and how epic it was. No office party, so far, can win that one. By the way, we are still waiting for the strippers. Thank you for the fun memories.

Katherine, Ghazi, Rick and **Valentin**, thank you for being a wonderful colleague. **Katherine**, aka DJ Katherine, your ethics discussions were extremely helpful and got me thinking all the time. You brought up sensitive, but necessary topics to the group which I really admired. I really hope you continue this line of work! **Ghazi**, aka the candy man, thank you for your positive attitude in everything. Also, thank you for sharing your -80°C freezer spaces. Without your help, I cannot image how I would have managed to store my 2700 samples. **Rick**, aka hyperactive guy, and **Valentin**, aka I have Chris's bike but I still walk to work guy, you guys joined at the end of my PhD, but we still managed to have some great memories. Good luck with your project. Please continue the office tradition to always fill the fridge with "liquid gold".

Last, but not least: **Sarah**, **Alix**, **Clara**, **Assia, Melissa**, **Ben**, **Xander**, **Lars**, **David**, **Tim** and **Nicole**, thank you for your help in one way or another during my PhD. You have been an amazing colleague and I could not ask for a better colleague.

A special mention to all the technician team. **Sandra**, **Barbie**, **Theodora**, **Hellen**, **Denise**, and **Wouter**, thank you all for your support and inputs during my internship and PhD periods.

Now, I would like to thank all my other colleagues, PhD students, post-docs and senior scientists: **Gowoon**, **Manon**, **Igor**, **Rose**, **Lonne**, **Roel**, **Imke**, **Nynke**, **Maarten**, **Mathilde**, **Jeroen**, **Faisal**, **Sylvana**, **Faris**, **Jana**, **Roman**, **Amée**, **Sarah**, **Christian**, **Daan**, **Caterina**, **Marina**, **Shenghua**, **Tanya**, **Britt**, **Nikita**, **Qian**, **Shuo, Ralph**, **Wenting**, **Suryan**, **Stijn**, **Mark**, **Jolien**, **Anita**, **Els**, **David**, **Tom**, **Marie-Thérèse**, **Anki**, **Damaris**, **Mario**, **Harry**, **Ali**, **Govert**, **Theo**, and **everybody else** that I might have forgotten. Thank you for all your valuable inputs and it was a pleasure to work with you all!

In particular, I would like to thank:

Perla and **Glenn,** first **Perla**, my thesis cover designer and advisor, best colleague, and go-to-person for chats. I could not have asked for a better cover design. You really did a great job and thank you for all the help/advices with my thesis. You made my thesis writing less stressful. Also, I really enjoyed our chats in the hallway and giving each other dinner menu ideas. It was nice having your

around. Now **Glenn**, aka my challenge of the month buddy, I really enjoyed your company, especially during lunch time. I really enjoyed and miss our challenges, especially the no coffee challenge, and random discussions since you knew everything. I am sure I bothered you too many times, but you were always happy to chat with me. Thank you both for all the great memories in the office.

Margot, the PhD, the myth, the legend, the non-coffee drinker in the division. I really enjoyed our chats on the hallways and in your office. I cannot really remember any of our conversation topics, but I know it was great. Also, we had a blast preparing for Glenn's defense. I still have the video of you speaking Korean. Again, thank you for all the great memories and introducing me to the wonders of the tea world, although it only lasted for a month =P.

Ellis, thank you for all the nice chats and scientific advices in the office and hallways. I really enjoyed our small talks about anything and everything.

Martijn and **Thomas,** the perfect duo, thank you for always welcoming me whenever I barged into the office and start chatting. I always had a great time talking with you both and appreciated the positive vibes you guys bring.

Christian, my ex-neighbor, but still the person I ask for tools and help. I really enjoyed sending time with you and **Aline**. You were always there when I needed help. I really enjoyed our Friday drinks, dinner sessions, BBQs, and Zeeland trip. I cannot imagine how I would have managed all the fixups in my house without your help. I could not ask for a better neighbor, a colleague, and a friend. Thank you for all these years.

Wouter, my ex-intern supervisor, colleague, and go-to-person for (long) chats. You were a good supervisor and a great colleague. I would stop by your office next-door every day to ask (mostly random) questions, but occasionally some scientific questions and your answers were always very helpful. You mentioned in your acknowledgment that you had fun coming to the office because of me and I could say the same thing. I really enjoyed and miss your company. Thank you for all the great memories.

Ghalib, you were my Master's student at Case Western Reserve University and a great friend to hangout, especially at Barking Spider Tavern. After you

graduated and went back to Saudi Arabia, I thought that was the end of our friendship, but we met again at Maastricht University. Although we did not hangout as much as we did in Cleveland, I really enjoyed having lunch with you at the hospital. Whenever I needed help, you were always there. Thank you for being a great friend!

Now, I would like to thank my new professor and colleagues in Gyeongsang National University, Jinju, South Korea. **Prof. Dr. Myeong Ok Kim**, thank you for giving me the opportunity to work with you and your group. **Jun Sung, Min Woo, Hyeon Jin, Min Hwa, Ikram, Amjad, Rahat, Riaz, Anny, Atiq, Tahir**, and **Ali**, thank you for welcoming me and I look forward in working with you all.

Of course, I would like to thank my **parents** and my **brother**. This journey would have not been possible without your support and guidance. You showed me the world and encouraged me to push myself. I know I am not the best at expressing my feelings, but thank you for everything you have done for me and believing in me.

A special thank you to my brother-in-law, **Taeju**, his wife **Yeongji**, and their beautiful and very cute daughter **Jihoo** for their supports and encouragements.

Then a special shout-out to my cat **Choco**. She is the fluffiest, cutest, smartest, and kindest cat and I live up to the quote, "I work hard so my cat can have a better life". Furthermore, shout-out to the Pu…"cat" lovers; **Dean**, **Wouter**, **Daan**, **Rick**, **Valentin**, **Thomas**, **Jos**, and **Gunter**. It is always great to see your cats. Cat people are the smartest! I said it. No regrets.

Finally, **Hailey**, my lovely wife, my inseparable best friend, and my partner in crime, we have been together for more than 10 years and you have always given me the best support. I really cannot image my life without you by my side. You make me happy simply by being with you. When I was down, you were there to help me stand backup, when I was sad, you were there to cheer me up, and of course, when I was out of energy, you provided me with the most delicious food in the world! Thank you for everything and motivating me to keep on pushing over my limit. I will always be by your side and support you in every way possible.