The Associations Between Cognitive Prognosis and Kynurenines Are Modified by the Apolipoprotein ε4 Allele Variant in Patients With Dementia

International Journal of Tryptophan Research
Volume 12: 1–8

DOI: 10.1177/1178646919885637

Arne Olav Ervik[1], Stein-Erik Hafstad Solvang[1,2], Jan Erik Nordrehaug[1,2], Per Magne Ueland[3], Øivind Midttun[3], Audun Hildre[1], Adrian McCann[3], Ottar Nygård[2,4], Dag Aarsland[5] and Lasse Melvaer Giil[1,2]

[1]Department of Clinical Science, University of Bergen, Bergen, Norway. [2]Department of Internal Medicine, Haraldsplass Deaconess Hospital, Bergen, Norway. [3]Bevital A/S, Bergen, Norway. [4]Department of Heart Disease, Haukeland University Hospital, Bergen, Norway. [5]Department of Old Age Psychiatry, King's College London, London, UK.

ABSTRACT

BACKGROUND: The apolipoprotein E ε4 gene variant (APOEε4) confers considerable risk for dementia and affects neuroinflammation, brain metabolism, and synaptic function. The kynurenine pathway (KP) gives rise to neuroactive metabolites, which have inflammatory, redox, and excitotoxic effects in the brain.

AIM: To assess whether the presence of at least one APOEε4 allele modifies the association between kynurenines and the cognitive prognosis.

METHODS: A total of 152 patients with sera for metabolite measurements and APOE genotype were included from the Dementia *Study* of Western Norway. The participants had mild Alzheimer disease and Lewy body dementia. Apolipoprotein E ε4 gene variant allele status was classified as one or more ε4 versus any other. Mini-Mental State Examination (MMSE) was measured at baseline and for 5 consecutive years. Mann-Whitney *U* tests and linear mixed-effects models were used for statistical analysis.

RESULTS: There were no significant differences in serum concentrations of tryptophan and kynurenine according to the presence or absence of APOEε4. High serum concentrations of kynurenic acid, quinolinic acid, and picolinic acid, and a higher kynurenine-to-tryptophan ratio, were all associated with more cognitive decline in patients without APOEε4 compared to those with the APOEε4 allele (*P*-value of the interactions $< .05$).

CONCLUSIONS: Kynurenic acid, quinolinic acid, picolinic acid, and the kynurenine-to-tryptophan ratio were associated with a significant increase in cognitive decline when the APOEε4 variant was absent, whereas there was a relatively less decline when the APOEε4 variant was present.

KEYWORDS: Dementia, Alzheimer disease, Lewy body dementia, kynurenines, picolinic acid, kynurenic acid, apolipoprotein E ε4, APOEε4, APOE4, MMSE decline, cognitive decline, interaction, effect modification

RECEIVED: October 2, 2019. **ACCEPTED:** October 8, 2019.

TYPE: Original Research

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The study was funded by the Norwegian Health Association, Dementia Research Program (contract number: 7349), and the Kavli trust. We are grateful for their support and continuous work for patients, caregivers, and scientists. The funding source had no role in the study design, data gathering, or interpretation of the results.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: D.A. has received research support and/or honoraria from Astra-Zeneca, H. Lundbeck, Novartis Pharmaceuticals, and GE Health and serves as paid consultant for H. Lundbeck, Eisai, Heptares, and Axovant. D.A. is a Royal Society Wolfson Research Merit Awards Holder and would like to thank the Wolfson Foundation and the Royal Society for their support. P.M.U. is a member of the steering board of the nonprofit Foundation to Promote Research into Functional Vitamin B12 Deficiency, which owns Bevital, the company that carried out biochemical analyses. None of the remaining authors have any potential conflicts of interest to disclose.

CORRESPONDING AUTHOR: Lasse Melvaer Giil, Floenbakken 56, 5009 Bergen, Norway. Email: lassegiil@gmail.com; lasse.melver.giil@haraldsplass.no

Introduction

Determinants of disease progression in patients with dementia are few. The apolipoprotein E ε4 allele variant (APOEε4) of the *APOE* gene confers considerable risk for dementia, including Alzheimer disease (AD), vascular dementia (VaD), and Lewy body dementia (LBD).[1-3] Being a carrier of the APOEε4 allele is linked to increased amyloid-β (Aβ) aggregation, reduced Aβ-clearance, mitochondrial dysfunction, neurofibrillary tangle formation, neuroinflammation, synaptic pathology, and excitotoxicity.[3] Yet, its impact on cognitive prognosis in patients with dementia is moderate[4] or even beneficial.[5]

Apolipoprotein E is mostly expressed in the liver and the brain, but there is also significant APOE expression in monocytes.[6] This expression is regulated by retinoid X receptor (RXR) and liver X receptor (LXR), which induce transcription of the *APOE* gene, co-regulated by the peroxisome proliferator-activated receptor γ (PPAR-γ).[7] In monocytes, pro-inflammatory cytokines suppress APOE expression[6,8] and are linked to activation of the kynurenine pathway (KP).[9]

The KP (Figure 1) gives rise to neuroactive metabolites, which are related to inflammation, immunomodulation, oxidative stress, and excitotoxicity.[10] The enzymes tryptophan

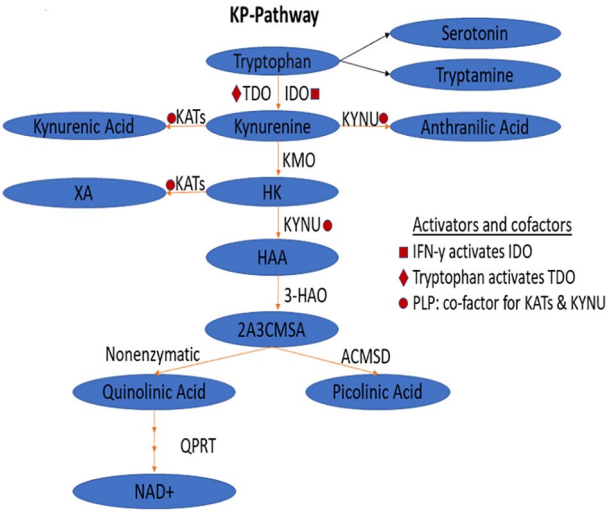

Figure 1. Summary of the kynurenine pathway. Most tryptophan (TRP) is metabolized via the kynurenine pathway (KP). Tryptophan 2,3-dioxygenase (TDO) and indolamine 2,3-dioxygenase (IDO) covert TRP to kynurenine (KYN). TRP activates TDO, and interferon gamma (IFN-y) activates IDO. Next, the pyridoxal 5′-phosphate (PLP)-dependent enzymes, kynurenine aminotransferases (KATs) and kynureninase (KYNU), generate kynurenic acid (KA) and anthranilic acid (AA), respectively, from KYN. Then, kynurenine 3-monooxygenase (KMO) converts KYN to 3-hydroxykynurenine (HK), which then converts KYNU to 3-hydroxyanthranilic acid (HAA). Also, KATs generate xanthurenic acid (XA) from HK. Furthermore, 3-hydroxyanthranilate dioxygenase (3-HAO) converts HAA to 2-amino-3-carboxymuconatesemialdehyde (ACMSA). Furthermore, aminocarboxymuconic semialdehyde decarboxylase (ACMSD) generates picolinic acid (PA) from ACMSA. There is also a spontaneous conversion to quinolinic acid (QA) from ACMSA. Finally, quinolinic acid phosphoribosyltransferase (QPRT) is involved in the production of NAD+ (nicotinamide adenine dinucleotide+).

2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO) convert the essential amino acid tryptophan (TRP) to formylkynurenine, which is quickly converted to kynurenine (KYN). Interferon-γ (IFN-γ) and other pro-inflammatory cytokines induce IDO, which is mostly expressed in monocytes. Kynurenine is converted to kynurenic acid (KA), 3-hydroxykynurenine (HK), or anthranilic acid (AA), where HK is a precursor of picolinic acid (PIC) and, via intermediates, to quinolinic acid (QA).[11] Kynurenine pathway metabolites and IDO inhibit T cell function and activate regulatory T cells, thereby participating in immune regulation.[12]

Tryptophan, KYN, and HK are able to pass the blood-brain barrier (BBB) and most kynurenines in the brain are derived from circulating kynurenines.[10,13] Kynurenic acid is a competitive antagonist of glutamate receptors, including the *N*-methyl-D-aspartate receptor (NMDAR), and of the α7 nicotinic acetylcholine receptor (α7nAChR). Quinolinic acid is an agonist of NMDAR.[10] Picolinic acid is able to block the neurotoxic actions of QA, although the mechanisms is unclear.[14] Reduced levels of TRP, KA, and other metabolites downstream of KYN have been reported in AD.[15] In contrast, KA increases regionally in the brain parenchyma in AD.[16] Effect modification of the APOEε4-associated risk for dementia has been shown for gender and obesity,[17-20] which have been linked to alterations in KP metabolites.[21] Apolipoprotein E–deficient mice display signatures of increased oxidative damage to the brain and significant alterations in peripheral and brain metabolism.[22-24] Furthermore, APOEε4 led to downregulation of NMDAR in a mouse model,[25] and there is considerable cross-talk between APOE, kynurenines, and the immune system.[12,26] As such, kynurenines and APOE are linked to IFN-γ, NMDAR, and oxidative stress.

We aimed to explore whether the association between circulating concentrations of kynurenines and cognitive prognosis in patients with mild dementia was modified by the presence of at least one APOEε4 allele.

Material and Methods

Subjects

A total of 152 patients with available sera for metabolite measurements and available ApoE gene sequencing were included from the Dementia *Study* of Western Norway. Dementia was diagnosed at study inclusion according to the *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition; *DSM*-IV) criteria based on structured interviews, clinical examination, a standardized neuropsychological test battery, and a neuropsychiatric assessment. Routine blood work, including assessment of thyroid function and Vitamin B_{12} status, was performed and structural neuroimaging was performed to rule out other causes of dementia such as tumors. The study procedures have been described in detail elsewhere.[27] The study included patients with mild dementia, excluding patients in a moderate-to-severe stage at the time of inclusion. Mild dementia was defined as a score of at least 20 on the Mini-Mental State Examination (MMSE) or not more than 1 on the Clinical Dementia Rating Scale (CDR). Patients with mild dementia were included from 2005 to 2012 and followed with annual testing of the MMSE. All those fulfilling the inclusion criteria from 2005 to 2007 were asked to join. From 2007 onwards, patients with LBD were selectively recruited, thus LBD is overrepresented in this study. This study considers only patients with either AD (N = 91) or LBD (N = 61). Lewy body dementia included both patients with dementia with Lewy bodies (DLB) and Parkinson disease with dementia (PDD) as both have similar α-synucleinopathy.[28] Patients with acute delirium, previous bipolar or psychotic disorder, terminal illness, or recently diagnosed major somatic illness were excluded from the study. The *APOE* gene was sequenced as previously described.[29] Self-reported smoking status was registered and for the purposes of this study classified as current smokers and current non-smokers.

Measurement of tryptophan and kynurenines in serum

At baseline, non-fasting blood samples were collected and processed, with aliquots of serum stored at −80°C until analysis. Tryptophan and 8 kynurenines [KYN, KA, AA, HK, 3-hydroxyanthranilic acid (HAA), xanthurenic acid (XA), PIC,

and QA] and creatinine were measured using liquid chromatography-tandem mass spectrometry. Limits of detection ranged from 0.2 to 7 nmol/L, while within- and between-day coefficients of variance (CVs) were 1.8 to 9.5 and 4.6 to 16.9, respectively.[30] The KYN-to-TRP ratio (KTR) was calculated as [KYN (nmol/L)/TRP (μmol/L)] × 100. The glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease (MDRD) study equation.[31] All biochemical analyses were performed at the laboratory Bevital (http://bevital.no) and laboratory staff was blinded to all participant data.

Potential confounders

A large community-based cohort found that men have 6% to 19% higher concentrations of TRP and most kynurenines, compared to women of the same age. Older (70-72 years) adults have higher concentrations (20%-30%) of kynurenines and higher KTR compared to younger adults (45-46 years). The lowest quartile of age-specific GFR was associated with a 18% to 26% increase in KTR and most kynurenines. Heavy smokers have a 4% to 14% lower concentration of Trp and most kynurenines. In this study, age and renal function were the strongest determinants.[21] Furthermore, circulating pyridoxal 5′-phosphate (PLP), a co-factor in the KP, is positively associated with most kynurenines but negatively associated with HK.[32] Based on these 2 studies on circulating kynurenines on more than 7000 individuals, we determined a priori that age, gender, GFR, smoking, and PLP were potential confounders.

Longitudinal assessment of cognition and clinical deterioration

The MMSE has satisfactory reliability, construct validity, and criterion validity for detecting cognitive impairment.[33] At the study's baseline, patients also completed an extensive neuropsychological test battery as part of the study protocol.[27] The annual rate of MMSE decline among patients with AD has been reported as 3.3 points per year.[34] A change of 2 to 4 points is considered a reliable change, and thus, our study was considered capable of measuring reliable change over 5 years.[35] Furthermore, several studies have found that patients who perform poorly antemortem on the MMSE have both increased amyloid plaque burden and neurofibrillary tangle burden.[36] Thus, we consider MMSE measures over 5 years as a suitable tool to measure disease progression. The CDR is an instrument used to assess the severity of dementia based on informant and patient interview and measures impairment in 6 cognitive domains. The sum of boxes (CDR-SB) is considered a valid outcome measuring both cognitive and functional impairment.[37] Although the primary outcome in this study was cognitive impairment, we aimed to assess whether interactions could be consistently identified using 2 different methods of assessing disease progression.

Statistics

Differences in serum concentrations of TRP and kynurenines according to the presence of at least one APOEε4 allele (hetero- and homozygosity) were tested using the Mann-Whitney *U* test. Prior to multivariate assessment, all kynurenines and KTR, except TRP, were log transformed. All continuous independent variables were standardized. Linear mixed-effects models with random intercepts and slopes were used to assess associations with cognitive prognosis, using MMSE and CDR-SB as outcomes. Age at baseline, gender, LBD vs AD, PLP, GFR, current smoking, and an interaction between age at baseline and time were included as covariates and potential confounders. The following terms were introduced to assess a 3-way interaction between the individual kynurenines, APOEε4, and follow-up-time: metabolite × APOEε4, metabolite × time, APOEε4 × time, and metabolite × APOEε4 × time. As differences over the trajectory were of interest, rather than at baseline, time was centered to estimate the intercept at 2.5 years in the study. Kynurenines were estimated in separate models to avoid collinearity and limit model complexity. A dose-response relationship was estimated for interpretative purposes using a linear mixed model with subsequent estimation of average marginal effects in APOEε4 non-carriers, heterozygotes, and homozygotes over time. Due to the small group of homozygotes and thus lack of statistical power and inability to adjust for confounders, we illustrated the effects sizes for interpretative purposes without formal hypothesis testing. The study was considered exploratory and the results were not adjusted for multiple comparisons. Statistical analyses were conducted using Stata 15 (https://www.stata.com).

Results

Subject demographics

The mean age of the participants (N = 152) at baseline was 75.1 years and 57% were female. The diagnoses were 58.9% with AD and 41.1% with LBD. Ninety of the participants had at least one APOEε4 variant, whereas 62 did not. The mean MMSE score at baseline was 23.7. Furthermore, 20% of the participants were current smokers, and the mean GFR was 79.1 (Table 1).

Kynurenines according to APOEε4

The allele frequency of heterozygotes carrying APOEε4 was 47.7% with 11.9% homozygotes. We identified no significant differences in serum levels of TRP or kynurenines according to the presence or absence of APOEε4 (Table 2).

MMSE decline and serum kynurenines, according to APOEε4

Serum levels of KA, PIC, QA, and KTR ($P < .05$) were more significantly associated with cognitive decline in patients without APOEε4 compared to patients with APOEε4

Table 1. Patients with mild dementia (N = 152) and kynurenine levels at baseline.

Demographics at baseline	
Age[a,b]	75.1 [7.3]
Female, %	57
Current smokers, %	20
Mini-Mental State Examination score[b]	23.7 [2.8]
Diagnosis	
Alzheimer disease, %	58.9
Lewy body dementia, %	41.1
Apolipoprotein E ε4 allele frequency	
Heterozygotes (ε4ε3 or ε4ε2), %	47.7
Homozygotes (ε4ε4), %	11.9
Hetero- and homozygotes, %	59.6
Potential confounders	
Glomerular filtration rate[b,c]	79.1 [20.7]
Pyridoxal 5′-phosphate[d,e]	31.7 [34.0]
Tryptophan and kynurenines	
Tryptophan[e,f]	66.5 [22.0]
Kynurenine[e,f]	1.73 [0.64]
3-hydroxykynurenine[d,e]	50.0 [33.7]
Kynurenic acid[d,e]	51.1 [23.9]
Xanthurenic acid[d,e]	12.4 [9.3]
Anthranilic acid[d,e]	21.4 [10.8]
3-hydroxyanthranilic acid[d,e]	36.1 [16.7]
Picolinic acid[d,e]	36.1 [21.8]
Quinolinic acid[d,e]	470 [313]
Kynurenine-to-tryptophan ratio[e]	2.58 [1.07]

The kynurenine-to-tryptophan ratio is multiplied by 100. Glomerular filtration rate (GFR) was calculated by the Modification of Diet in Renal Disease (MDRD) formula.[31]
[a]In years.
[b]Mean and [standard deviation].
[c]mL/min/1.73 m^2.
[d]nmol/L.
[e]Median and [interquartile range].
[f]μmol/L.

(Table 3). Figure 2 illustrates the simple slopes of the predicted fixed effects of MMSE according to tertiles of PIC and APOEε4. A crude correlation coefficient between MMSE and CDR-SB over all measurement periods was –0.77. The results of the same interactions were consistent using CDR-SB as the outcome, except that QA was insignificant, bearing in mind that increasing CDR-SB indicates more severe disease compared to decreasing MMSE. Please see Supplemental Table 1 and Supplemental Figure 1. Further analyses aimed to illustrate if there were signs of a dose-response relationship on MMSE with stronger interactions in homozygotes compared to heterozygote APOEε4 carriers. Inspecting the results graphically did not suggest the presence of a dose-response relationship (Supplemental Figure 2).

Discussion

In this exploratory study on cognitive prognosis and kynurenines in dementia according to APOEε4, the associations between PIC, KA, QA, and KTR with MMSE decline were significantly different in patients with and without APOEε4. Specifically, higher concentrations of PIC, KA, and QA and higher KTR were all associated with a significant increase in cognitive decline when APOEε4 was absent, whereas there was relatively less association with cognitive decline when APOEε4 was present.

We have previously observed a relationship between KTR, neopterin, and poor cognitive performance in healthy older adults[38] and a non-linear association between KYN and MMSE performance in this study, but not with MMSE decline rates.[39] Tryptophan deficiency has been widely reported in AD, whereas kynurenine concentrations have shown conflicting findings, where one study on neuropathologically confirmed cases found equivalent or decreased kynurenines.[40] Most circulating kynurenines are moderately to strongly correlated with their respective cerebrospinal fluid concentrations in AD, but are unchanged in the cerebrospinal fluid except for a marked reduction in KA.[41,42] The Apolipoprotein E ε4 gene variant induces several metabolic changes outside the brain such as an atherogenic cholesterol and lipid profile in peripheral blood but is associated with lower C-reactive protein levels.[24,43] Such processes might relate to kynurenine levels in serum, but no differences in kynurenine levels were observed between APOEε4 carriers versus non-carriers in our study. Experimental studies indicate that APOEε4 mice on a high-fat diet may develop more substantial cognitive deficits accompanied by significant changes in brain metabolism compared to APOEε3 mice.[44] Apolipoprotein E affects Aβ-clearance, neuroinflammation, and brain glucose metabolism[45] and leads to synaptic pathology.[46] Being a carrier of the APOEε4 allele is also associated with increased oxidative stress in the brain,[22,47] neuronal hyperactivity,[48] and down-regulation of the NMDAR.[25,49]

Higher concentrations of all kynurenines were beneficial for APOEε4 carriers and detrimental to non-carriers, leaving the question open as to whether these associations are specific to one metabolite, or reflect broader activities in the KP. We found largely consistent results using CDR-SB as the outcome, demonstrating that all interactions except the one involving QA were invariant to the method of measuring disease progression. Mini-Mental State Examination relies on patients performing

Table 2. Kynurenine levels according to APOEε4.

	NO APOEE4 (N = 62)	APOEE4 (N = 90)	P[a]
Tryptophan[b,c]	66.7 [21.9]	66.6 [21.5	.564
Kynurenine[b,c]	1.80 [0.64]	1.72 [0.69]	.483
3-hydroxykynurenine[c,d]	52.1 [33.3]	48.6 [34.1]	.475
Kynurenic acid[c,d]	52.5 [23.9]	50.9 [25.2]	.178
Xanthurenic acid[c,d]	12.4 [10.5]	12.4 [8.2]	.697
Anthranilic acid[c,d]	22.1 [10.6]	21.1 [10.7]	.272
3-hydroxyanthranilic acid[c,d]	36.3 [15.1]	36.2 [17.6]	.934
Picolinic acid[c,d]	36.4 [21.3]	36.0 [22.7]	.688
Quinolinic acid[c,d]	487 [384]	442 [315]	.204
Kynurenine-to-tryptophan ratio[c]	2.72 [1.03]	2.47 [1.13]	.256

[a]Mann-Whitney *U* test.
[b]μmol/L.
[c]Median and [interquartile range].
[d]nmol/L.

Table 3. Effect modification between APOEε4 and kynurenines on MMSE over 5 years.[a]

MODEL	PREDICTORS	CENTERED INTERCEPT[b]			SLOPE[c]		
		EST.	*SE*	*P*	EST.	*SE*	*P*
TRP	TRP	0.16	0.91	.857	−0.01	0.40	.990
	TRP* APOEε4	0.06	1.11	.958	0.02	0.46	.964
	APOEε4[d]	−0.50	1.07	.637	−0.05	0.38	.890
KYN	KYN	−1.08	0.98	.272	−0.46	0.32	.152
	KYN* APOEε4	1.89	1.18	.111	0.74	0.39	.059
HK	HK	0.87	0.84	.297	0.21	0.27	.446
	HK* APOEε4	0.16	1.04	.878	−0.19	0.37	.960
KA	KA	−2.04	0.88	.021	−0.57	0.27	.035*
	KA* APOEε4	2.40	1.07	.025*	0.90	0.35	.009*
XA	XA	−0.65	0.73	.372	−0.13	0.27	.626
	XA* APOEε4	0.36	1.03	.725	0.08	0.36	.818
AA	AA	−0.02	0.74	.976	−0.25	0.31	.432
	AA* APOEε4	1.53	0.99	.121	0.72	0.38	.054
HAA	HAA	−0.87	0.73	.233	−0.21	0.25	.398
	HAA* APOEε4	1.15	0.96	.231	0.33	0.32	.302
PIC	PIC	−2.18	0.95	.023*	−0.80	0.35	.022*
	PIC* APOEε4	3.17	1.13	.005*	1.17	0.41	.004*
QA	QA	−1.48	1.09	.176	−0.65	0.34	.053
	QA* APOEε4	2.12	1.22	.083	0.94	0.39	.017*
KTR	KTR	−1.67	1.00	.095	−0.58	0.35	.095
	KTR* APOEε4	2.17	1.12	.052	0.83	0.40	.039*

Abbreviations: AA, anthranilic acid; APOEε4, the apolipoprotein E epsilon 4 gene variant (1 or 2); Est., estimate; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; KA, kynurenic acid; KTR, kynurenine-to-tryptophan ratio; KYN, kynurenine; MMSE, Mini-Mental State Examination; PIC, picolinic acid; QA, quinolinic acid; SE, standard error; TRP, tryptophan; XA, xanthurenic acid.
[a]Linear mixed effects model with random intercept and slope with centered time. Age, age × time, Lewy body dementia vs Alzheimer disease, glomerular filtration rate, and pyridoxal 5′-phosphate as covariates. All continuous covariates were standardized, as was log(kynurenines). Trp was on its original scale.
[b]When time is centered, the intercepts (or mean differences) are estimated at the middle of the trajectory, as opposed to baseline, when the time is not centered.
[c]All predictors listed under *Slope* include interactions with time (centered).
[d]The effect size of APOEε4 on the intercept was from −0.53 to −0.49 and on the slopes from −0.09 to 0.02. None were significant (only shown for TRP).

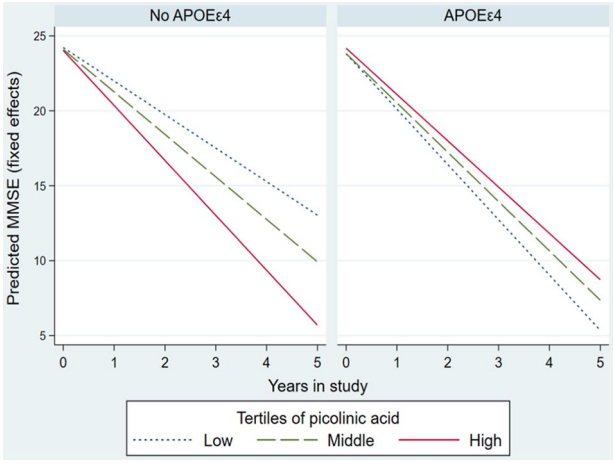

Figure 2. MMSE declines according to tertiles of picolinic acid and APOEε4. When picolinic acid is high, there is an increasing decline in MMSE test scores over time in patients without the ε4 allele variant, whereas this decline is slightly lower with high picolinic acid in patients with the ε4 allele. Predictions are based on the fixed effects from the model in Table 3. APOEε4 indicates the ε4 allelic variant of the apolipoprotein E gene; MMSE, Mini-Mental State Examination.

a psychometric test, whereas CDR-SB summarizes both cognitive and functional impairment based on interviews of both informants and patients.[37] Although this observational study cannot shed light on mechanisms, there are findings which may be of relevance. Carriers of APOEε4 could in theory benefit relatively more from the scavenging of free radicals performed by KA and PIC.[50] NAD biosynthetic pathways are downregulated in AD[51] and with APOEε4[52] and inversely related to PIC.[53] Kynurenic acid is considered neuroprotective due to its endogenous activity as an NMDAR antagonist.[10] Whether the observed protective association between both KA and PIC, and cognitive decline in APOEε4 carriers and detrimental associations in non-carriers relate to these observations remains to be investigated.

Indoleamine 2,3-dioxygenase is induced by pro-inflammatory cytokines, which also suppress APOE expression in monocytes.[6,9] Circulating levels of KTR and QA are increased in human monocytes exposed to INF-gamma ex vivo.[9] In our study, KTR and QA were also associated with less cognitive decline in APOEε4 carriers and more cognitive decline in non-carriers. One can speculate whether the observed associations could be explained by an inflammation-induced downregulation of APOE which could benefit patients with APOEε4 but where the underlying inflammation would do more harm in others.

Several clinical trials have been undertaken in dementia where the treatment target is metabolic dysfunction in the brain, examples being pioglitazone,[54] targeting PPAR-γ, and intranasal insulin, targeting central insulin resistance.[55] This highlights the importance of studying APOE-metabolic interactions on cognitive prognosis in dementia, as this could be translated to personalize therapeutic interventions in the future.

There are limitations to our study. The study has a small sample size considering its aim to investigate gene-biomarker interactions with 90 APOEε4 carriers and 62 non-carriers. Considering both the explorative nature of the study, sample size and the correlation between kynurenines, the results were not adjusted for multiple comparisons. Thus, the topic of differential associations of metabolites on cognitive prognosis in dementia according to APOEε4 carrier status should be followed up in independent studies. A clear dose-response relationship was not identified in our study but could not be appropriately identified due to lack of statistical power. Similarly, we did not have the power to assess whether the interactions differed according to clinical diagnosis, and thus we cannot determine whether these findings are specific to AD or LBD. Brain kynurenines are mostly formed from peripheral KYN and HK. As such, cerebrospinal fluid measurements would have been highly informative.[10] Repeated measurements of metabolites at each annual follow-up would have been useful to more accurately characterize the associations over time, as would similar studies on cognition in healthy persons. Interactions, or effect modification, do not assess causal directions. Underlying immunological activity could be a residual confounder, due to extensive cross-talk between both APOE and kynurenines, and the immune system.[12,26]

In conclusion, the associations between MMSE decline and several kynurenines (KA, QA, PIC, and KTR) are modified by the presence of APOEε4. Still, APOEε4 has no relationship with kynurenine levels, suggesting that one needs to investigate whether the biological processes underpinning our findings relate to APOE expression or APOEε4 effects in the brain. In our data, KA, QA, PIC, and KTR predict a negative prognosis in patients without APOEε4 but are protective in patients with APOEε4.

Acknowledgements

We wish to thank all the study participants, researchers, and staff in DemVest.

Author Contributions

All authors have approved the final manuscript to be published and agrees to be accountable for all aspects of the work. The first author drafted the manuscript and all authors have critically revised the manuscript.

AOE contributed to the planning of the study, participated in statistical analyses and interpretation and presentation of the results.

SE-HS participated in the planning of the study, the interpretation and presentation of the results.

JEN participated in study planning, statistical analysis, and interpretation.

PMU participated in study planning, measurement and quality control of biomarkers, and interpretation of the findings.

ØM performed measurements and quality control of metabolic biomarkers and participated in the interpretation of the results.

AH contributed to the planning of the study, participated in analysis and in the presentation of the findings.
AM was involved in measurements and quality control of metabolic biomarkers in sera and participated in the interpretation of the results.
ON contributed to study planning, analytic approach and interpretation of the findings.
DA is the principal investigator of the DV study and was involved in the conception of the study, and interpretation of the results. DA provided critical input on cognitive prognosis.
LMG was involved in the conception of the study, assessed and checked all statistical analyses and their presentation, and critically assessed findings and coordinated the collaborating researchers in this study.

ORCID iD

Lasse Melvaer Giil https://orcid.org/0000-0003-3520-7530

Supplemental Material

Supplemental material for this article is available online.

REFERENCES

1. Rohn TT. Is apolipoprotein E4 an important risk factor for vascular dementia? *Int J Clin Exp Pathol*. 2014;7:3504-3511.
2. Borroni B, Grassi M, Costanzi C, Archetti S, Caimi L, Padovani A. APOE genotype and cholesterol levels in Lewy body dementia and Alzheimer disease: investigating genotype-phenotype effect on disease risk. *Am J Geriatr Psychiatry*. 2006;14:1022-1031.
3. Liu C-C, Liu C-C, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat Rev Neurol*. 2013;9:106-118.
4. Vijayaraghavan S, Darreh-Shori T, Rongve A, et al. Association of butyrylcholinesterase-K allele and apolipoprotein E varepsilon4 allele with cognitive decline in dementia with Lewy bodies and Alzheimer's disease. *J Alzheimers Dis*. 2016;50:567-576.
5. Hoyt BD, Massman PJ, Schatschneider C, Cooke N, Doody RS. Individual growth curve analysis of APOE epsilon 4-associated cognitive decline in Alzheimer disease. *Arch Neurol*. 2005;62:454-459.
6. Braesch-Andersen S, Paulie S, Smedman C, Mia S, Kumagai-Braesch M. ApoE production in human monocytes and its regulation by inflammatory cytokines. *PLoS ONE*. 2013;8:e79908.
7. Yamazaki Y, Painter MM, Bu G, Kanekiyo T. Apolipoprotein E as a therapeutic target in Alzheimer's disease: a review of basic research and clinical evidence. *CNS Drugs*. 2016;30:773-789.
8. Brand K, Mackman N, Curtiss LK. Interferon-gamma inhibits macrophage apolipoprotein E production by posttranslational mechanisms. *J Clin Invest*. 1993;91:2031-2039.
9. Jones SP, Franco NF, Varney B, et al. Expression of the kynurenine pathway in human peripheral blood mononuclear cells: implications for inflammatory and neurodegenerative disease. *PLoS ONE*. 2015;10:e0131389.
10. Schwarcz R, Bruno JP, Muchowski PJ, Wu H-Q. Kynurenines in the mammalian brain: when physiology meets pathology. *Nat Rev Neurosci*. 2012;13:465-477.
11. Lovelace MD, Varney B, Sundaram G, et al. Recent evidence for an expanded role of the kynurenine pathway of tryptophan metabolism in neurological diseases. *Neuropharmacology*. 2017;112:373-388.
12. Mandi Y, Vecsei L. The kynurenine system and immunoregulation. *J Neural Transm (Vienna)*. 2012;119:197-209.
13. Fukui S, Schwarcz R, Rapoport SI, Takada Y, Smith QR. Blood-brain barrier transport of kynurenines: implications for brain synthesis and metabolism. *J Neurochem*. 1991;56:2007-2017.
14. Guillemin GJ, Cullen KM, Lim CK, et al. Characterization of the kynurenine pathway in human neurons. *J Neurosci*. 2007;27:12884-12892.
15. Gulaj E, Pawlak K, Bien B, Pawlak D. Kynurenine and its metabolites in Alzheimer's disease patients. *Adv Med Sci*. 2010;55:204-211.
16. Baran H, Jellinger K, Deecke L. Kynurenine metabolism in Alzheimer's disease. *J Neural Transm (Vienna)*. 1999;106:165-181.
17. Beydoun MA, Boueiz A, Abougergi MS, et al. Sex differences in the association of the apolipoprotein E epsilon 4 allele with incidence of dementia, cognitive impairment, and decline. *Neurobiol Aging*. 2012;33:720-731.e4.
18. Lim AS, Yu L, Kowgier M, Schneider JA, Buchman AS, Bennett DA. Modification of the relationship of the apolipoprotein E epsilon4 allele to the risk of Alzheimer disease and neurofibrillary tangle density by sleep. *JAMA Neurol*. 2013;70:1544-1551.
19. Zade D, Beiser A, McGlinchey R, et al. Apolipoprotein epsilon 4 allele modifies waist-to-hip ratio effects on cognition and brain structure. *J Stroke Cerebrovasc Dis*. 2013;22:119-125.
20. van den Kommer TN, Dik MG, Comijs HC, et al. The role of extracerebral cholesterol homeostasis and ApoE e4 in cognitive decline. *Neurobiol Aging*. 2012;33:622.e17-622.e28.
21. Theofylaktopoulou D, Midttun O, Ulvik A, 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;173:121-130.
22. Shea TB, Rogers E, Ashline D, Ortiz D, Sheu MS. Apolipoprotein E deficiency promotes increased oxidative stress and compensatory increases in antioxidants in brain tissue. *Free Radic Biol Med*. 2002;33:1115-1120.
23. Wu L, Zhang X, Zhao L. Human ApoE isoforms differentially modulate brain glucose and ketone body metabolism: implications for Alzheimer's disease risk reduction and early intervention. *J Neurosci*. 2018;38:6665-6681.
24. Karjalainen JP, Mononen N, Hutri-Kahonen N, et al. The effect of apolipoprotein E polymorphism on serum metabolome—a population-based 10-year follow-up study. *Sci Rep*. 2019;9:458.
25. Liu DS, Pan XD, Zhang J, et al. APOE4 enhances age-dependent decline in cognitive function by down-regulating an NMDA receptor pathway in EFAD-Tg mice. *Mol Neurodegener*. 2015;10:7.
26. Zhang H, Wu L-M, Wu J. Cross-talk between apolipoprotein E and cytokines. *Mediators Inflamm*. 2011;2011:949072.
27. Aarsland D, Rongve A, Nore SP, et al. Frequency and case identification of dementia with Lewy bodies using the revised consensus criteria. *Dement Geriatr Cogn Disord*. 2008;26:445-452.
28. Dodel R, Csoti I, Ebersbach G, et al. Lewy body dementia and Parkinson's disease with dementia. *J Neurol*. 2008;255:39-47.
29. Berge G, Sando SB, Rongve A, Aarsland D, White LR. Apolipoprotein E epsilon2 genotype delays onset of dementia with Lewy bodies in a Norwegian cohort. *J Neurol Neurosurg Psychiatry*. 2014;85:1227-1231.
30. Midttun O, 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;23:1371-1379.
31. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604-612.
32. Theofylaktopoulou D, Ulvik A, Midttun O, et al. Vitamins B2 and B6 as determinants of kynurenines and related markers of interferon-gamma-mediated immune activation in the community-based Hordaland Health Study. *Br J Nutr*. 2014;112:1065-1072.
33. Tombaugh TN, McIntyre NJ. The mini-mental state examination: a comprehensive review. *J Am Geriatr Soc*. 1992;40:922-935.
34. Han L, Cole M, Bellavance F, McCusker J, Primeau F. Tracking cognitive decline in Alzheimer's disease using the mini-mental state examination: a meta-analysis. *Int Psychogeriatr*. 2000;12:231-247.
35. Hensel A, Angermeyer MC, Riedel-Heller SG. Measuring cognitive change in older adults: reliable change indices for the mini-mental state examination. *J Neurol Neurosurg Psychiatry*. 2007;78:1298-1303.
36. Nelson PT, Braak H, Markesbery WR. Neuropathology and cognitive impairment in Alzheimer disease: a complex but coherent relationship. *J Neuropathol Exp Neurol*. 2009;68:1-14.
37. Coley N, Andrieu S, Jaros M, Weiner M, Cedarbaum J, Vellas B. Suitability of the clinical dementia rating-sum of boxes as a single primary endpoint for Alzheimer's disease trials. *Alzheimers Dement*. 2011;7:602-610.e2.
38. Solvang SH, Nordrehaug JE, Tell GS, et al. The kynurenine pathway and cognitive performance in community-dwelling older adults. *Brain Behav Immun*. 2019;75:155-162.
39. Hafstad Solvang S-E, Nordrehaug JE, Aarsland D, et al. Kynurenines, neuropsychiatric symptoms, and cognitive prognosis in patients with mild dementia. *Int J Tryptophan Res*. 2019;12:1-9.
40. Giil LM, Midttun O, Refsum H, et al. Kynurenine pathway metabolites in Alzheimer's disease. *J Alzheimers Dis*. 2017;60:495-504.
41. Jacobs KR, Lim CK, Blennow K, et al. Correlation between plasma and CSF concentrations of kynurenine pathway metabolites in Alzheimer's disease and relationship to amyloid-beta and tau. *Neurobiol Aging*. 2019;80:11-20.
42. Sorgdrager FJH, Vermeiren Y, Van Faassen M, et al. Age- and disease-specific changes of the kynurenine pathway in Parkinson's and Alzheimer's disease [published online ahead of print August 3, 2019]. *J Neurochem*. doi:10.1111/jnc.14843.

43. Martiskainen H, Takalo M, Solomon A, et al. Decreased plasma C-reactive protein levels in APOE epsilon4 allele carriers. *Ann Clin Transl Neurol*. 2018;5:1229-1240.
44. Johnson LA, Torres ERS, Impey S, Stevens JF, Raber J. Apolipoprotein E4 and insulin resistance interact to impair cognition and alter the epigenome and metabolome. *Sci Rep*. 2017;7:43701.
45. Liao F, Yoon H, Kim J. Apolipoprotein E metabolism and functions in brain and its role in Alzheimer's disease. *Curr Opin Lipidol*. 2017;28:60-67.
46. Lane-Donovan C, Herz J. ApoE, ApoE receptors, and the synapse in Alzheimer's disease. *Trends Endocrinol Metab*. 2017;28:273-284.
47. Xu H, Finkelstein DI, Adlard PA. Interactions of metals and Apolipoprotein E in Alzheimer's disease. *Front Aging Neurosci*. 2014;6:121-121.
48. Nuriel T, Angulo SL, Khan U, et al. Neuronal hyperactivity due to loss of inhibitory tone in APOE4 mice lacking Alzheimer's disease-like pathology. *Nat Commun*. 2017;8:1464.
49. Chen Y, Durakoglugil MS, Xian X, Herz J. ApoE4 reduces glutamate receptor function and synaptic plasticity by selectively impairing ApoE receptor recycling. *Proc Natl Acad Sci U S A*. 2010;107:12011-12016.
50. Reyes Ocampo J, Lugo Huitron R, Gonzalez-Esquivel D, et al. Kynurenines with neuroactive and redox properties: relevance to aging and brain diseases. *Oxid Med Cell Longev*. 2014;2014:646909.
51. Cai H, Cong W-N, Ji S, Rothman S, Maudsley S, Martin B. Metabolic dysfunction in Alzheimer's disease and related neurodegenerative disorders. *Curr Alzheimer Res*. 2012;9:5-17.
52. Theendakara V, Patent A, Peters Libeu CA, et al. Neuroprotective Sirtuin ratio reversed by ApoE4. *Proc Natl Acad Sci U S A*. 2013;110:18303-18308.
53. Katsyuba E, Mottis A, Zietak M, et al. De novo NAD+ synthesis enhances mitochondrial function and improves health. *Nature*. 2018;563:354-359.
54. Geldmacher DS, Fritsch T, McClendon MJ, Landreth G. A randomized pilot clinical trial of the safety of pioglitazone in treatment of patients with Alzheimer disease safety of pioglitazone in AD treatment. *Arch Neurol*. 2011;68:45-50.
55. Craft S, Baker LD, Montine TJ, et al. Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: a pilot clinical trial. *Arch Neurol*. 2012;69:29-38.