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Research report

Components of the choline oxidation pathway modify the association between the apolipoprotein ε4 gene variant and cognitive decline in patients with dementia

Audun Skjaerseth Hildre[a], Stein-Erik Hafstad Solvang[a,b], Dag Aarsland[c], Øivind Midtun[d], Adrian McCann[d], Arne Olav Ervik[a], Ottar Nygård[e], Per Magne Ueland[d], Jan Erik Nordrehaug[a,b], Lasse Melvaer Giil[a,b,*]

[a] Department of Clinical Science, University of Bergen, Bergen, Norway
[b] Department of Internal Medicine, Haraldsplass Deaconess Hospital, Bergen, Norway
[c] Department of Old Age Psychiatry, King's College University, London, UK
[d] Bevital A/S, Bergen, Norway
[e] Department of Heart Disease, Haukeland University Hospital, Bergen, Norway

HIGHLIGHTS

- One-carbon metabolism and the APOEε4 allele variant could interact in dementia.
- This potential interaction has not been addressed in prognostic dementia studies.
- We measured metabolites in sera in a longitudinal study on cognition in dementia.
- Choline oxidation metabolites seem to improve cognitive prognosis in APOEε4 carriers.
- In comparison, they may be detrimental to cognitive prognosis in non-carriers.

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ABSTRACT

Background: Metabolites involved in one-carbon metabolism (OCM) may predict cognitive prognosis in dementia. The link between OCM, apolipoprotein E (APOE), and DNA methylation creates a biologically plausible mechanism of interaction.

Aim: To assess OCM metabolites as predictors of 5-year cognitive prognosis in patients with mild dementia, and in subgroups defined by the APOEε4 allele variant.

Methods: We followed one-hundred and fifty-two patients with mild dementia (86 with Alzheimer's disease, 66 with Lewy body dementia, including 90 with at least one APOEε4 *allele*) for 5 years with annual Mini-Mental State Examinations (MMSE). Total homocysteine, methionine, choline, betaine, dimethylglycine, sarcosine, folate, cobalamin and pyridoxal 5′-phoshate were measured in serum at baseline. We used linear mixed models to assess metabolite-MMSE associations, including 3-way interactions between metabolites, time, and APOEε4. False-discovery rate adjusted p-values (Q-values) are reported.

Results: Metabolite concentrations were not different in patients with dementia according to the presence of APOEε4. Overall, serum concentration of total homocysteine was inversely associated with MMSE performance, while betaine was positively associated with MMSE ($Q < 0.05$), but neither was associated with MMSE decline. Serum concentrations of betaine, dimethylglycine and sarcosine, however, were associated with slower MMSE

Abbreviations: Aβ, amyloid-β; $Aβ_{42}$, amyloid-β peptide, 42 amino acids long; AD, Alzheimer's disease; *APOE*, apolipoprotein E gene; APOEε4, apolipoprotein E *epsilon* 4 allele variant; BHMT, betaine-homocysteine methyltransferase; CSF, cerebrospinal fluid; DemVest, dementia study of Western Norway; GC–MS/MS, gas chromatography–tandem mass spectroscopy; GFR, glomerular filtration rate; LBD, Lewy body dementia (Dementia with Lewy bodies and Parkinson's disease dementia); LC–MS/MS, liquid chromatography-tandem mass spectroscopy; MDRD, modification of diet in renal disease; MMSE, mini-mental state examination; OCM, one carbon metabolism; PPAR, peroxisome proliferator-activated receptor; PLP, pyridoxal 5′phosphate; Q, Q-value (p-value adjusted for multiple comparisons); SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine

* Corresponding author at: Floenbakken 56, 5009 Bergen, Norway.
E-mail addresses: lassegiil@gmail.com, lasse.melver.giil@haraldsplass.no (L.M. Giil).

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decline in patients with APOEε4, but with faster MMSE decline in patients without the allele (all 3-way interactions: Q < 0.05).
Conclusion: Components of the choline oxidation pathway are associated with a better cognitive prognosis in APOEε4 carriers and a worse cognitive prognosis in non-carriers. Further research investigating targeted metabolic interventions according to APOE allele status is warranted.

1. Introduction

The apolipoprotein E epsilon 4 (APOEε4) allele variant of the *APOE* gene is the most important risk factor for dementia (Jansen, 2019). Although there is consensus on the key role of APOEε4 in disease initiation, there are conflicting findings regarding the relationship between APOEε4 and disease progression. Studies have identified both slower and more rapid cognitive decline in Alzheimer's disease (AD) patients with APOEε4, relative to APOEε4 non-carriers (Hoyt, 2005). There are well established modifiers of the APOEε4-associated risk of Alzheimer's disease, such as ethnicity and gender (Belloy et al., 2019). APOE in the brain is mainly expressed in astrocytes and microglia but can be induced in neurons under stress. APOEε4 is linked to increased amyloid-β aggregation (Aβ), reduced Aβ clearance, tau phosphorylation, mitochondrial dysfunction, reduced delivery of cholesterol to neurons and reduced synaptic plasticity (Liu, 2013). Most of these mechanisms are relevant to disease progression and potentially modifiable by other molecular pathways. Still, modification of APOE-related cognitive decline in patients with dementia has been relatively underexplored.

An important consideration for potential gene-environment interactions is epigenetic modification of gene expression. Indeed, increased DNA methylation of the promoter region of APOE is associated with an increased risk of incident dementia independent of the allele variant (Karlsson, 2018). One-carbon metabolism (OCM, see Fig. 1) can be modified by dietary intake and is linked to DNA-methylation. In the methionine cycle, 5-methyl-tetrahydrofolate serves as a methyl donor in the remethylation of homocysteine to methionine, catalyzed by methionine synthase with vitamin B12 as a cofactor. In the choline oxidation pathway, choline is oxidized to betaine, a methyl donor for betaine-homocysteine methyltransferase (BHMT) that remethylates homocysteine, forming methionine and dimethylglycine. Finally, methionine is converted to S-adenosylmethionine (SAM) which donates methyl groups to a variety of substrates, including DNA. S-adenosylhomocysteine (SAH), formed during SAM-dependent transmethylation reactions is hydrolysed to homocysteine, thereby completing the cycle. Of note, pan peroxisome proliferator-activated receptor (PPAR) agonists increases the level of most OCM metabolites (Lysne, 2016) and increases the expression of *APOE* by stimulating generation of liver X receptor (Yue and Mazzone, 2009).

Elevated plasma levels of total homocysteine have repeatedly been linked to incident dementia (Smith, 2018). Homocysteine has been related to oxidative stress, endothelial dysfunction and endoplasmic reticulum stress, potentially relevant mechanisms in neurodegeneration

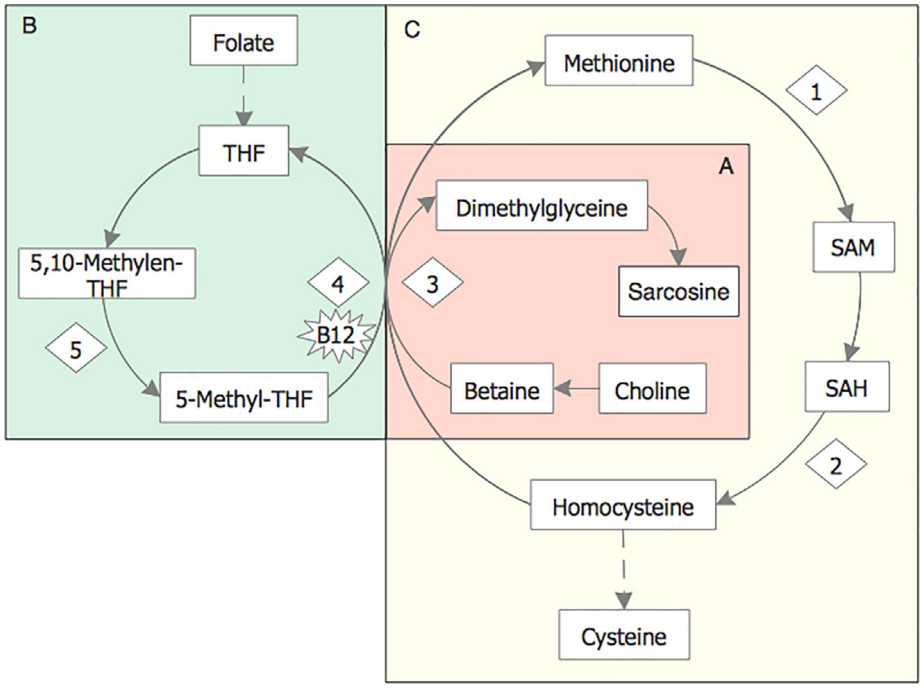

Fig. 1. Pathways of one-carbon Metabolism. One-carbon metabolism includes the choline oxidation pathway, the folate cycle and the methionine cycle. The essential nutrient folate is sequentially reduced (B) to tetrahydrofolate (THF), 5,10-methylene-tetrahydrofolate and 5-methyl-tetrahydrofolate by the rate-limiting enzyme methylenetetrahydrofolate reductase. Methionine synthase, with cobalamin (B12) as a cofactor, uses 5-methyl-THF as methyl donor to recycle homocysteine back to methionine. Methionine is also recycled in the choline oxidation pathway (A), where betaine-homocysteine methyltransferase remethylates homocysteine using betaine, the oxidized product of the essential nutrient choline. This pathway makes dimethylglycine, which is further oxidized to sarcosine. Methionine is catabolized (C) to S-adenosylmethionine (SAM), a central methyl donor in DNA-methylation. After methyl donation, S-adenosylhomocysteine (SAH) is formed, which in turn is hydrolized to homocysteine. Abbreviations: THF, tetrahydrofolate; B12, cobalamin; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine.

Enzymes

1: Methionine adenosyltransferase
2: Adenosylhomocysteinase
3: Betaine-homocysteine methyltransferase
4: Methionine synthase
5: Methylenetetrahydrofolate reductase

Cycles

A: Choline Oxidation Pathway
B: Folate Cycle
C: Methionine Cycle

(Moretti and Caruso, 2019). Yet, studies investigating whether homocysteine affects cognitive prognosis in patients with dementia have produced conflicting findings (Hoyt, 2005). The APOEε4 associated risk of dementia and cognitive decline is present in both sexes, some studies indicate that it is stronger in women (Beydoun, 2012; Mortensen and Hogh, 2001), while others did not find such differences (Hsiung et al., 2004). Higher levels of vitamin B12 and lower levels of methylmalonic acid were associated with better cognitive performance in APOEε4 carriers only (Vogiatzoglou, 2013), with similar findings described for homocysteine (Elias, 2008). Plasma homocysteine is more strongly associated with cerebrospinal fluid (CSF) phosphorylated tau in APOEε4 carriers compared to non-APOEε4 carriers (Dayon, 2017). In patients with alcoholism, high levels of homocysteine were associated with hippocampal atrophy only in APOEε4 carriers (Wilhelm, 2008). Although interactions between OCM and APOEε4 have been investigated, we could not identify studies examining OCM-APOEε4 interactions in relation to cognitive prognosis in patients with dementia. Furthermore, studies have tended to focus solely on a few metabolites within OCM, in particular homocysteine, perhaps overlooking other potentially important metabolites.

We aimed to investigate the association between one-carbon metabolites in serum at baseline and cognitive prognosis in patients with mild dementia who participated in a longitudinal cohort study. The main aim of the study was to assess whether the presence of at least one APOEε4 modified the relationship between OCM metabolites and cognitive prognosis. CSF $A\beta_{42}$, total tau and phosphorylated tau, were used to further explore any findings in APOEε4-defined subgroups of patients. Finally, positive findings were explored in analyses stratified by supplement use, gender and diagnosis.

2. Results

2.1. Subject demographics

The patients were well matched, comparing patients with and without APOEε4, although patients with APOEε4 were on average 3.6 years younger, reflecting the earlier age of onset among these patients. AD was most frequent (68%), whereas 42% of patients had Lewy body dementia (LBD, see section 4.1.). There were no significant differences in one-carbon metabolites according to the presence of APOEε4 allele (Table 1).

2.2. Cognitive decline and one-carbon metabolites

Whole cohort analyses demonstrated that a one standard deviation increase in log-transformed total homocysteine was associated with a −0.76 point lower Mini-Mental State Examination (MMSE) score (Q = 0.048), which did not change over time (Table 2). Conversely, a one standard deviation increase in betaine was associated with a 0.75 point higher MMSE test score (Q = 0.032), which also did not change over time.

2.3. Cognitive decline and one-carbon metabolites, according to APOEε4

In analysis of MMSE over time with time and APOEε4 status as predictors, the effect of time (years in study) was −2.87, (−3.43 to 2.31), p < 0.001, indicating the annual MMSE decline. Patients with at least one APOEε4 did not have more rapid cognitive decline compared to those who did not. At 2.5 years, the average MMSE score (centered intercept) was 17.1 (15.5–18.7), and this was −0.72 (−2.59 to 1.15), p = 0.45 lower in patients with at least one APOEε4 allele, who also had −0.20 (−0.93 to 0.51), p = 0.57 more decline per year (APOEε4*time) compared to patients without APOEε4. Higher serum concentrations of the choline oxidation pathway metabolites (betaine, dimethylglycine, and sarcosine) were associated with significantly less MMSE decline in patients with APOEε4, but greater MMSE decline in patients without APOEε4 (Q < 0.05 for all, see Table 3). The 3-way interaction between APOEε4, time in study, and dimethylglycine is illustrated in Fig. 2, showing the marginal difference from the overall MMSE decline. The 3-way interaction between APOEε4, time in study, and both betaine (p = 0.007) and dimethylglycine (p = 0.001) respectively, were also significant in the 120 patients not using vitamin supplements, and the effect sizes were comparable to the whole cohort (data not shown). We did not have power to assess these relationships in the 32 patients using vitamin supplements. Supplementary Table 1 shows adjustments for 3-way interactions between potential confounders, time and APOEε4. We did not identify a negative change-in-estimate above 10% or a change in the conclusion of the p-value for the identified associations with betaine, dimethylglycine and sarcosine for any of the tested confounders with the exception of diagnosis and sarcosine.

2.4. Post-hoc analyses stratified by gender and diagnosis

In analysis of MMSE over time with time and APOEε4, the MMSE score at 2.5 years (centered intercept) was 17.3 in men, 16.0 in women, 17.3 in AD, and 16.6 in LBD. The effect of time (annual MMSE decline) was −2.95 in men, −2.81 in women, −2.61 in AD and −3.45 in LBD, all p < 0.001. There was no significant associations between APOEε4 and MMSE scores at 2.5 years or decline in men (APOEε4 −1.73, p = 0.31, APOEε4*time −0.47, p = 0.40), women (APOEε4 −0.09, p = 0.94, APOEε4*time −0.04, p = 0.93), AD (APOEε4 −0.07,

Table 1
Study participant characteristics and serum concentrations of one-carbon metabolites.

	Dementia	APOEε4[c]		
	N = 152	Yes, N = 90	No N = 62	p-value [d]
Age, years[a]	75.1 [7.3]	73.6 [0.8]	77.2 [0.9]	0.002* [e]
Female, %	57	58	55	.72[f]
Education, years[b]	9 [4]	9 [4]	9 [4]	0.23[g]
GFR, ml/min/1.72 m^{2a}	79.2 [26.7]	79.7 [20.8]	78.5 [20.5]	0.73 [e]
Current smokers, %	20	20	21	.88[f]
MMSE, score[a]	23.7 [2.7]	23.6 [3.0]	23.8 [2.4]	0.65 [e]
LBD, % (AD as reference)	42 [68]	42.2	37.1	.53[f]
Any B12 or folate supplement, %	21.1	23.3	17.7	.41[f]
Vitamin B12, %	8.6	10.0	6.5	.44[f]
Folate, %	4.6	4.4	4.8	.91[f]
Multivitamin, %	8.6	10.0	6.5	.44[f]
Total homocysteine, μmol/L[b]	12.5 [4.6]	13.6 [5.9]	13.7 [6.6]	0.59[g]
Methionine, μmol/L[b]	27.1 [8.0]	27.0 [8.4]	27.4 [7.6]	0.31[g]
Choline, μmol/L[b]	12.9 [4.8]	13.0 [4.8]	13.2 [4.8]	0.95[g]
Betaine, μmol/L[b]	36.6 [13.7]	36.5 [12.9]	36.3 [15.7]	0.69[g]
Dimethylglycine, μmol/L[b]	4.46 [2.81]	4.62 [2.61]	4.27 [3.16]	0.67[g]
Sarcosine, μmol/L [b]	1.26 [0.68	1.28 [0.71]	1.26 [0.68]	0.90[g]
Folate, nmol/L [b]	13.4 [33.6]	13.1 [33.5]	13.7 [45.8]	0.62[g]
Cobalamin, pmol/L[b]	499 [3 3 2]	482 [2 9 3]	503 y	0.79[g]
Pyridoxal 5′-phosphate, nmol/L[b]	31.6 [33.9]	30.3 [33.8]	31.9 [35.9]	0.86[g]

Abbreviations: AD, Alzheimer's disease; APOE, apolipoprotein E; ε, episolon; GFR, glomerular filtration rate; IQR, interquartile range; LBD, Lewy Body Dementia; N, number of participants.

[a] Mean and standard deviation.

[b] Median and interquartile range.

[c] Of the 90 patients with at least one APOEε4 gene, 72 had one ε4 (with either ε2 or ε3) and 18 had two (ε4ε4).

[d] All p-values refer to univariate differences between patients with at least one APOEε4 allele versus none.

[e] Student's t-test.

[f] Pearson's X^2 test.

[g] Mann-Whitney U test.

* p < 0.05.

Table 2
MMSE over 5 Years and One-Carbon Metabolites.

	Metabolite	Estimate	LCI	UCI	p	Q
Methionine	Total homocysteine	−0.76	−1.37	0.15	0.02*	0.048*
Cycle	Methionine	0.40	−0.11	0.91	0.12	0.283
Choline	Choline	0.11	−0.50	0.70	0.74	0.984
Oxidation	Betaine	0.75	0.21	1.30	0.01*	0.037*
Pathway	Dimethylglycine	0.21	−0.35	0.77	0.46	0.741
	Sarcosine	0.11	−0.41	0.63	0.68	0.982
Folate Cycle	Folate	0.03	−0.57	0.64	0.91	0.987
	Cobalamin	−0.01	−0.53	0.52	0.99	0.991

Note. A linear mixed effects model with MMSE as the outcome was used. Covariates were age and Lewy Body Dementia, both interacting with time, and gender, pyridoxal 5′phosphate (log transformed) and the metabolite (no significant interactions between metabolites and time).
Abbreviations: LCI, lower level 95% confidence interval; MMSE, Mini-Mental State Examination; UCI, upper level 95% confidence interval; Q, p-value adjusted for multiple comparisons, see statistics.
* $p < 0.05$, $Q < 0.05$.

p = 0.95, APOEε4*time −0.20, p = 0.62) or LBD (APOEε4 −1.62, p = 0.42, APOEε4*time −0.08, p = 0.90). The three-way interactions between APOEε4, time and gender (p = 0.60), and APOEε4, time and diagnosis (p = 0.82) were both insignificant. Table 4 summarizes the associations between betaine, dimethylglycine and sarcosine with MMSE scores over time according to APOEε4. The 3-way interactions are significant in both males and females for betaine and dimethylglycine, although stronger in men. Sarcosine was only significant in men. Betaine and dimethylglycine were significant in patients with AD, and dimethylglycine was also significant in LBD. Sarcosine did not reach significance in either diagnostic category. Notably, the directions of the effect sizes (i.e. positive for APOEε4 interaction on both intercept and slopes) of the interactions indicate that the relationships are not fundamentally altered in either subgroup.

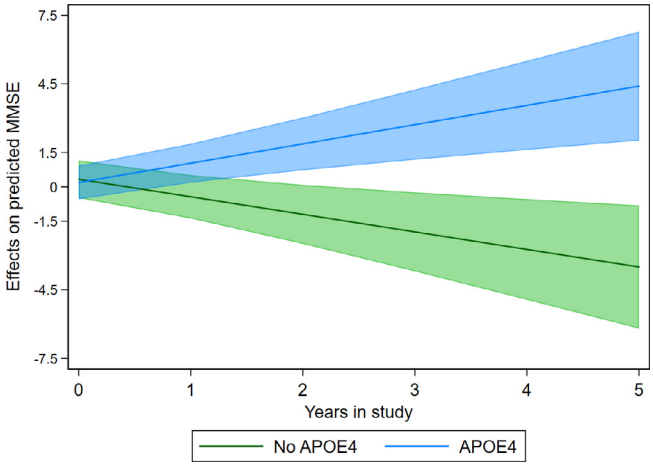

Fig. 2. Average marginal effect of dimethylglycine on MMSE over 5 years according to APOEε4. The predicted marginal fixed effects of dimethylglycine on MMSE over 5-years, adjusted for age and LBD interacting with time, and gender, glomerular filtration rate, and pyridoxal 5′-phosphate. Abbreviations: APOEε4, apolipoprotein E epsilon 4; LBD, Lewy Body Dementia; MMSE, Mini-Mental State Examination.

2.5. One-carbon metabolites, cerebrospinal fluid biomarkers and APOEε4

Post-hoc exploration of baseline CSF biomarkers revealed that serum choline, betaine and dimethylglycine concentrations were significantly correlated with total and phosphorylated tau in the CSF of patients without APOEε4, but not in patients with the allele. Similarly, serum dimethylglycine was inversely correlated with CSF $A\beta_{42}$ concentrations in patients without APOEε4, but not in those with APOEε4. Further analyses indicated that there was significant inequality between the above spearman correlations, comparing APOEε4 and non-APOEε4

Table 3
Cognitive decline and the one-carbon metabolites according to APOEε4.

	APOEε4 [a]	Intercept (centered)[b]				Slope[c]				
		Est	LCI	UCI	p	Est	LCI	UCI	p	Q
HCY	Yes	0.83	−1.28	2.94	0.44	0.33	−0.43	1.08	0.39	0.699
	No	−0.97	−2.63	0.69	0.25	−0.08	−0.66	0.50	0.78	
MET	Yes	1.23	−0.95	3.42	0.27	0.70	−0.09	1.48	0.08	0.219
	No	−0.41	−2.16	1.35	0.65	−0.46	−1.09	0.17	0.15	
CHOL	Yes	0.23	−1.88	2.35	0.83	0.42	−0.32	1.17	0.27	0.532
	No	0.47	−1.19	2.12	0.58	−0.06	−0.62	0.51	0.84	
BET	Yes	1.84	−0.16	3.84	0.07	1.14	0.44	1.83	0.001*	0.008*
	No	−0.01	−1.50	1.48	0.99	−0.53	−1.04	−0.01	0.047*	
DMG	Yes	3.90	1.93	5.87	< 0.001**	1.62	0.93	2.31	< 0.001**	0.002*
	No	−1.61	−3.11	−0.11	0.04*	−0.78	−1.30	−0.26	0.003*	
SARC	Yes	2.52	0.42	4.62	0.02*	0.94	0.18	1.70	0.02*	0.048*
	No	−1.50	−3.14	0.14	0.07	−0.60	−1.19	−0.02	0.04*	
FOL	Yes	−0.38	−2.46	1.70	0.72	−0.02	−0.76	0.72	0.95	0.991
	No	0.29	−1.22	1.80	0.71	0.03	−0.50	0.56	0.91	
COB	Yes	0.07	−2.02	2.17	0.95	0.02	−0.72	0.77	0.96	0.991
	No	−0.39	−2.04	1.28	0.65	−0.15	0.74	0.45	0.63	

Note. Linear mixed model as described in Table 2 and statistics with a 3-way interaction between at least one APOEε4, time and the log-transformed metabolite. Continuous predictors were standardized.
Abbreviations: APOEε4, apolipoprotein E *epsilon* 4; BET, betaine, CHOL, choline; COB, cobalamin; DMG, dimethylglycine; Est, estimate; FOL, folate; HCY, homocysteine; LBD, Lewy Body Dementia (vs. Alzheimer's disease); LCI, lower level 95% confidence interval; MET, methionine; MMSE; Mini-Mental State Examination; SARC, sarcosine; UCI, upper level 95% confidence interval; Q, p-value adjusted for multiple comparisons, see statistics.
[a] 90 patients had APOEε4, combined with any other ε, 62 had no APOEε4.
[b] The intercepts of the metabolite in the absence of APOEε4 (reference group) reflects the difference from the mean in points on the MMSE at year 2.5 for a 1 standard deviation change in the log-transformed metabolite. The intercepts of patients with APOEε4 represents the difference in effect size from no APOEε4.
[c] The slope indicates the point change in MMSE per year for a 1 standard deviation change in the log-transformed metabolite for patients with no APOEε4 (metabolite*time) and the relative difference from this for patients with APOEε4 (metabolite*time*APOEε4).
* p and Q < 0.05.
** p and Q < 0.001.

Table 4
Post-hoc analysis: Cognitive decline and selected metabolites according to APOEε4 in males and females and according to diagnosis.

	APOEε4 [a]	Male patients (n = 66)				Female patients (n = 86)			
		Intercept [b]		Slope [c]		Intercept [b]		Slope [c]	
		Est	p	Est	p	Est	p	Est	p
BET	Yes	3.70	0.005*	1.56	< 0.001**	1.05	0.20	0.82	0.04*
	No	0.01	0.98	−0.36	0.24	0.67	0.41	−0.42	0.11
DMG	Yes	4.48	0.002*	1.92	< 0.001**	2.24	0.03*	1.20	0.004*
	No	−2.21	0.04*	−0.94	0.014*	−0.79	0.31	−0.59	0.07
SARC	Yes	4.52	0.02*	1.81	0.003*	0.86	0.47	0.31	0.49
	No	−2.02	0.17	−0.96	0.04*	−0.87	0.36	−0.30	0.39
		Alzheimer's disease (n = 91)				Lewy body dementia (n = 61)			
BET	Yes	1.41	0.11	1.21	< 0.001**	0.69	0.73	0.57	0.45
	No	0.23	0.73	−0.49	0.03*	0.55	0.67	−0.19	0.73
DMG	Yes	2.71	0.003*	1.44	< 0.001**	3.64	0.03*	1.31	0.02*
	No	−0.79	0.30	−0.62	0.009*	−2.04	0.12	−0.70	0.04*
SARC	Yes	1.60	0.11	0.62	0.08	3.35	0.08	1.18	0.09
	No	−0.83	0.28	−0.28	0.32	−2.55	0.12	−1.06	0.10

Note. Linear mixed model with MMSE as the outcome, adjusted for age, time in study and their interaction stratified by gender with a 3-way interaction between at least one APOEε4 (no APOEε4 as the reference group), time and the log-transformed metabolite. Continuous predictors were standardized.
Abbreviations: AD, Alzheimer's disease; APOEε4, apolipoprotein E *epsilon* 4; BET, betaine, DMG, dimethylglycine; Est, estimate; LBD, Lewy body dementia; MMSE; Mini-Mental State Examination; SARC, sarcosine.

[a] Of 66 males, 38 (58%) had APOEε4 combined with any other ε, 28 (42%) had no APOEε4. Of 86 females (60%), 52 had at least one APOEε4 allele while 34 (40%) did not. Of 91 patients with AD, 52 (57%) had at least one APOEε4 allele, while 39 (43%) did not. Of 61 patients with LBD, 38 (62%) had at least one APOEε4 allele, while 23 (38%) did not.

[b] The intercepts of the metabolite in the absence of APOEε4 (reference group) reflects the difference from the mean in points on the MMSE at year 2.5 for a 1 standard deviation change in the log-transformed metabolite. The intercepts of patients with APOEε4 represents the difference in effect size from no APOEε4.

[c] The slope indicates the point change in MMSE per year for a 1 standard deviation change in the log-transformed metabolite for patients with no APOEε4 (metabolite*time) and the relative difference from this for patients with APOEε4 (metabolite*time*APOEε4).

* p and Q < 0.05.

** p and Q < 0.001.

carriers, suggesting the presence of interactions (Fig. 3).

3. Discussion

Investigating cognitive decline in patients with dementia, we observed that circulating total homocysteine was inversely associated with MMSE performance, while serum betaine was positively associated with MMSE, but neither were associated with more rapid decline. However, subgroup analyses based on the presence of the APOEε4 allele variant demonstrated that higher serum concentrations of betaine, dimethylglycine, and sarcosine, downstream metabolites in the choline oxidation pathway, were associated with attenuated MMSE decline in patients with APOEε4. In contrast, high concentrations of the same metabolites were associated with greater MMSE decline in patients without APOEε4. Spearman correlations on a small subgroup with CSF biomarkers indicated differential correlations according to APOEε4 allele status between metabolites of the choline oxidation pathway and CSF biomarkers of dementia that were in line with the overall findings.

Consistent with our whole cohort findings, elevated homocysteine has been shown to be inversely associated with cognitive performance in AD (Smith, 2018; Farina, 2017). Betaine, a methyl group donor, was significantly associated with a better MMSE performance. Neither

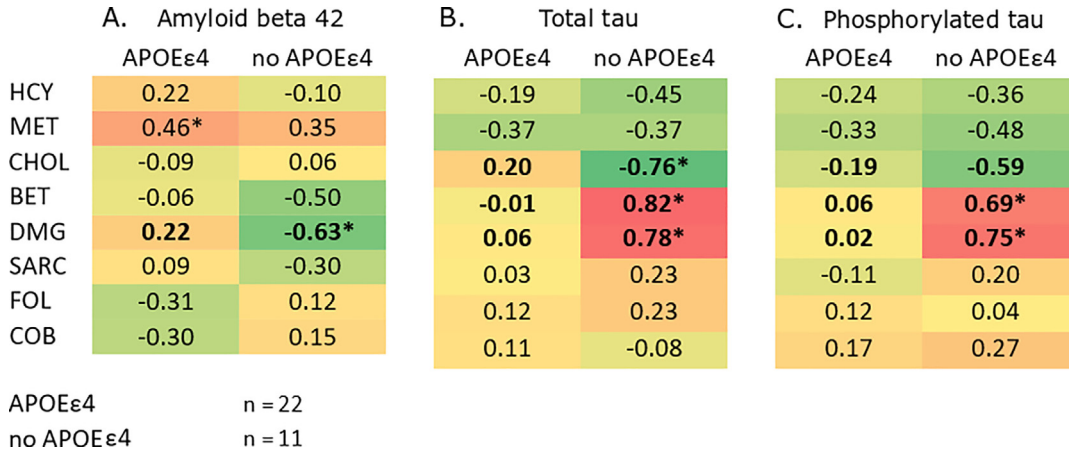

Fig. 3. CSF biomarkers of dementia and serum one-carbon metabolites according to APOEε4. Spearman correlations between **(A)** amyloid-β_{42}, **(B)** total tau, and **(C)** phosphorylated tau in the cerebrospinal fluid (CSF) and serum metabolites. The strength is indicated by colour (heatmap) from negative (green) to neutral (yellow) to positive (red). Bold indicates that the difference in Spearman correlations between the two groups was significant, suggesting interaction. *Indicates that the correlation (not the difference) was significant. Abbreviations: APOEε4, at least one apolipoprotein epsilon 4 allele; BET, betaine; CHOL, choline; COB, cobalamin; DMG, dimethylglycine; FOL, folate; HCY, total homocysteine; MET, methionine.

homocysteine nor betaine were associated with a more rapid MMSE decline. Interestingly however, our data suggests that higher concentrations of components of the choline oxidation pathway may be of benefit to patients with APOEε4, but potentially detrimental to patients without this allele. In the present study, serum metabolite concentrations did not appear to be affected by APOEε4 allele status. Similarly, betaine concentrations in serum and CSF have previously been shown to be comparable in healthy persons, mild cognitive impairment, and AD patients. The investigators also noted that serum and CSF concentrations of choline and betaine were correlated (van Wijk, 2017). The interactions between APOEε4 with homocysteine covered in the introduction might be of relevance to these related metabolites. However, we did not identify convincing interactions between homocysteine and APOEε4 with regards to cognitive prognosis in this study. The possible mechanisms underlying our observations are not clear, yet there are potentially relevant observations in the literature. Differential cognitive effects of dietary manipulation according to APOEε4 has been shown in experimental animals on a high fat diet (Johnson, 2017) and mid-life blood glucose is more related to post-mortem AD neuropathology in APOEε4 carriers (Bangen, 2016). In principle, these findings support the notion that metabolic factors may affect cognitive function and neuropathology differently in APOEε4 carriers compared to non-carriers, although the studies did not specifically assess the choline oxidation pathway. The expression of genes involved in cholesterol metabolism and transport, and brain-derived neurotrophic factor (BDNF) are induced in the hypothalamus of hens following betaine supplementation (Idriss, 2018). It is plausible that a betaine-induced upregulation of cholesterol transport could benefit APOEε4 carriers, if betaine also has such an effect in humans (Holtzman et al., 2012). BDNF is downregulated in AD and secretion is reduced with APOEε4 (Sen et al., 2017), in theory supporting our finding of protective associations with higher concentrations of betaine and downstream metabolites in APOEε4 carriers. Betaine is concentrated in hippocampal neurons relative to the interstitial fluid and is considered to be neuroprotective under conditions of hyperexcitability and osmotic stress (van Wijk, 2017; Knight, 2017). It has been suggested that GABAergic inhibitory networks are vulnerable to APOEε4-mediated neurotoxicity, leading to hyperexcitability in the hippocampus and cortex (Najm et al., 2019). The functions of dimethylglycine, which showed the strongest differential association with cognitive decline according to APOEε4 in our study, and sarcosine, are not well characterized in the brain nor are their links to APOEε4. Among the one-carbon metabolites, alternations in plasma concentrations of dimethylglycine were the most pronounced following pan-PPAR activation (Lysne, 2016), which is also known to increase *APOE* expression (Yue and Mazzone, 2009). Several lines of evidence suggest a loss-of-function hypothesis regarding APOEε4, and APOE levels are lower in APOEε4 carriers (Belloy et al., 2019). Further, PPAR-γ gene variations may modify the risk of AD in an APOEε4 dependent manner (Combarros, 2011), suggesting that underlying activity in PPAR pathways, unmeasured in our study, could be of relevance to the observed interactions. Finally, the results regarding sarcosine were confounded by including a 3-way interaction with diagnosis, APOEε4 and time.

Stratified analyses suggested that the interactions involving the choline oxidation metabolites were stronger in men and in patients with AD, compared to women and patients with LBD. Such analyses might be of importance due to possible gender differences in cognitive decline (Beydoun, 2012; Mortensen and Hogh, 2001). A recent study identified that metabolic biomarkers had differential associations with AD biomarkers strata defined by gender and APOEε4 (Arnold, 2019:). Further, a small study on the treatment of cognitive impairment in AD with intranasal insulin identified differences in the therapeutic response according to gender and APOEε4 carrier status (Claxton, 2013). From a neuropathological and genetic stand point, LBD seems to be a mixture of AD and Parkinson pathologies, and stratified analyses could thus be informative (Guerreiro, 2016). However, our study did not have the statistical power to investigate whether these differences were statistically significant, and the stratified analyses should indeed be interpreted with caution. Notwithstanding the problems with statistical power, the interactions involving dimethylglycine were significant in all subgroups.

Post-hoc analyses of CSF biomarkers, though limited by the small sample size, indicated that serum choline was inversely associated, while serum betaine and dimethylglycine were both positively associated with CSF total and phosphorylated tau in APOEε4 non-carriers. Serum dimethylglycine was inversely correlated with CSF $A\beta_{42}$ (low $A\beta_{42}$ in the CSF is related to higher parenchymal Aβ) in the APOEε4 non-carriers. CSF tau and $A\beta_{42}$ predict cognitive decline in AD and LBD (Rolstad, 2013; Abdelnour, 2016). Neurofibrillary tangle pathology and synaptic loss at autopsy correlate most strongly with cognitive performance (Nelson et al., 2009) (Yue and Mazzone, 2009). Betaine supplementation, typically inducing BHMT, reduces Aβ formation by stimulating α-secretase in cell culture (Liu, 2014). In line with a beneficial effect, betaine supplements reduced the hyperphosphorylation of tau in animal models with hyperhomocysteinemia and diabetes (Chai, 2013; Tseng and Graves, 1998). In our study, associations suggesting benefit of betaine and downstream metabolites were only observed in APOEε4 carriers.

Our study raises the question as to whether components of the choline oxidation pathway are differentially related to cognition and AD pathology according to the presence of APOEε4. Supplemental choline, betaine and dimethylglycine are available and betaine supplementation has shown promise in rodent models. A critical issue is to clarify if these findings are related to the metabolites themselves, underlying metabolic regulators or downstream mechanisms such as DNA methylation. In principle, our findings support personalizing metabolic interventions for dementia according to *APOE* status, but further studies are necessary to support that hypothesis.

The main limitation in our study is the use of a relatively small sample size to assess complex interactions, and their confounders. Our findings should be confirmed and experimental studies are required to assess the mechanisms. The circulating metabolites mainly reflect enzyme activity in the liver and kidneys. Furthermore, nutrient intake is an unmeasured confounder. Accordingly, future studies also measuring one-carbon metabolites in the CSF would be of major interest. Finally, we did not measure APOE levels in either serum or CSF, which could have further strengthened the methodology.

In conclusion, our study of cognitive decline in patients with mild dementia suggests that components of the choline oxidation pathway appear to be beneficial among individuals with the APOEε4 allele. In contrast, in non-carriers of the allele, choline oxidation metabolites are associated with increased cognitive decline, which may be related to detrimental association between choline oxidation metabolites and CSF concentrations of $A\beta_{42}$ and tau.

4. Material and methods

4.1. Participants

One-hundred and fifty-two patients with mild dementia due to AD and LBD who had performed *APOE* gene sequencing, and had available sera for profiling of metabolites, were recruited from the Dementia Study of Western Norway (DemVest). DemVest is a longitudinal cohort study with annual follow-up examination that includes assessment with the MMSE. At baseline, clinical assessments, an extensive neuropsychological test battery and structural neuroimaging was performed, as described previously (Aarsland, 2008). Patients with AD or LBD were recruited from 2005 to 2012. An MMSE score of 20 or higher or Clinical Dementia Rating Scale global score of at least one were used as criteria for mild dementia. Potential participants were excluded if they had acute delirium, previous bipolar or psychotic disorder, terminal illness, or a major somatic illness which according to a clinician

could impact on study participation or cognition. LBD encompassed both Dementia with Lewy Bodies and Parkinson's Disease with Dementia, as the pathology is similar. LBD patients were selectively recruited from 2007 onwards and are therefore overrepresented. The patients were evaluated annually until death or withdrawal, but to avoid floor effects, we only considered the first five years of follow-up in this study. The participants were classified as current smokers and current non-smokers according to self-reported smoking. Vitamin supplement use was identified by self-report and a review of medical records. APOE genotyping was performed as described previously (Berge, 2014). The Regional Committee for Medical and Health Research Ethics approved the study protocol, and a notification of change related to biomarkers (REC number: 2010/633). All participants provided a signed informed consent after a detailed explanation of the procedures.

4.2. Biomarker analyses

At baseline non-fasting blood samples were collected and processed. Aliquots of serum were stored at −80 °C until analysis. Measurement of one-carbon and related metabolites was performed at BEVITAL (Bergen, Norway, www.bevital.no), which performed targeted metabolic profiling of biomarkers across seven complementary analytical platforms. Serum concentrations of folate (Molloy and Scott, 1997) and vitamin B12 (Kelleher and Broin, 1991), were measured by microbiological assay. Vitamin B6 (Midttun et al., 2009), choline and its metabolites (Midttun et al., 2013) were analyzed by liquid chromatography-tandem mass spectroscopy (LC–MS/MS). Total homocysteine, total cysteine and methylmalonic acid were analyzed by gas chromatography-tandem mass spectroscopy (GC–MS/MS) (Midttun, 2016). Lumbar puncture with collection of CSF was performed in 33 out of 152 patients. Baseline CSF concentrations of $A\beta_{42}$, total tau and phosphorylated tau were measured by ultra-sensitive multiarray assay and enzyme-linked immunosorbent assays, as described previously (Mulugeta, 2011). The glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease (MDRD) study equation (Levey, 2009). All laboratory staff were blinded to all participant data.

4.3. Cognitive assessment

The MMSE is a 30-point questionnaire performed by patients that takes between 5 and 10 min to perform. Questions are simple and assess orientation, registration, attention, calculation, recall, language and the ability to follow simple commands. The reliability, construct validity and criterion validity of the MMSE for detecting cognitive impairment is satisfactory (Tombaugh and McIntyre, 1992). We used repeated MMSE scores to assess disease progression. The annual rate of MMSE decline in AD patients has been estimated at 3.3 points per year in a meta-analysis (Han, 2000). A change of 2–4 points is considered a reliable change in MMSE (Hensel et al., 2007). With 5 years of annual MMSE assessments, our data provides valid estimates of cognitive deterioration. In healthy persons, MMSE performance is lower in older persons and in persons with lower educational attainment (Crum, 1993). The situation is different in patients with AD, where the rate of MMSE decline often decreases with age (Holland, 2012) and can also be slower in patients with lower educational attainment (Roselli, 2009). However, these findings on MMSE decline in AD are not consistent across studies (Clark, 1999).

The CDR is a semi-structured interview of patients and informants concerning six domains: memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. Each domain is rated on a 5-point Likert scale, and a global CDR score is then calculated classifying patients a normal (score = 0), very mild dementia (0.5), mild dementia (1), moderate dementia (2) and severe dementia (3). It is considered a reliable and valid tool to stage dementia (Morris, 1997). Studies indicate that it measures both cognitive and functional status in patients with dementia (Coley, 2011).

4.4. Statistics

Prior to multivariate analysis, all metabolites were log-transformed, as they followed a log-normal distribution, and continuous predictors standardized. The outcome variable (MMSE) and time (in years) were not standardized, as the purpose was only to compare effect sizes of the metabolites. Linear mixed effects models with random intercepts and slopes were used to assess associations between baseline variables and MMSE over 5 years. Interactions with time were included. Due to the relatively small sample size, the number of variables in each model was kept low. The base model for MMSE over 5 years included age, age*time, gender, LBD, LBD*time, PLP, and GFR. Each metabolite was included separately to the base model, followed by the introduction of a 3-way interaction between APOEε4, time and the metabolite. Following this, 3-way interactions between potential confounders, time and APOEε4 were introduced. Age, gender, diagnosis, GFR, and PLP were introduced from the base model. Additionally, current smoking and years of educational attainment were introduced in 3-way interactions. The change-in-estimate was calculated from the effect size of the metabolite*time*APOEε4 interaction before and after introducing the confounder*time*APOEε4 interaction as: ((adjusted effect size – base model effect size)/base model effect size)*100 (Tong and Lu, 2001). An attenuation in effect size of 10% or a change in the conclusion of the p-value was considered as evidence of minor and major confounding, respectively. P-values adjusted for multiple comparisons (Q-values) were calculated according to the Benjamini-Hochberg procedure, with a false discovery rate of 0.05 and alpha at 0.05. Post-hoc analyses were performed to assess whether supplements affected the results by stratifying the cohort into patients who either used or did not use relevant supplements containing vitamin B6, B12 or folate. Additional post-hoc stratification included gender and diagnosis (AD vs. LBD). Due to the small size of the subgroups based on diagnosis and gender, only age and gender were included as covariates. Further, the equality of Spearman correlations between serum metabolites and CSF biomarkers were tested according to APOEε4 as a non-parametric test of interaction (Caci, 2000). Analyses were conducted in Stata 15 (https://www.stata.com). The analytic syntax for Stata 15 is included in the supplementary material, showing the main analyses for dimethylglycine. Q-values were estimated using the R-package p-adjust (R version 3.5.2, https://www.R-project.org).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brainres.2019.146519.

References

Aarsland, D., et al., 2008. Frequency and case identification of dementia with Lewy bodies using the revised consensus criteria. Dement. Geriatr. Cogn. Disord. 26 (5), 445–452.

Abdelnour, C., et al., 2016. Alzheimer's disease cerebrospinal fluid biomarkers predict cognitive decline in lewy body dementia. Mov. Disord. 31 (8), 1203–1208.

Arnold, M., et al., The Alzheimer's Disease Metabolome: Effects of Sex and APOE ε4 genotype. bioRxiv, 2019: p. 585455.

Bangen, K.J., et al., 2016. Interaction between midlife blood glucose and APOE genotype predicts later Alzheimer's disease pathology. J. Alzheimers Dis. 53 (4), 1553–1562.

Belloy, M.E., Napolioni, V., Greicius, M.D., 2019. A quarter century of APOE and Alzheimer's disease: progress to date and the path forward. Neuron 101 (5), 820–838.

Berge, G., et al., 2014. Apolipoprotein E epsilon2 genotype delays onset of dementia with Lewy bodies in a Norwegian cohort. J. Neurol. Neurosurg. Psychiatry 85 (11), 1227–1231.

Beydoun, M.A., et al., 2012. Sex differences in the association of the apolipoprotein E epsilon 4 allele with incidence of dementia, cognitive impairment, and decline. Neurobiol. Aging 33 (4), 720–731.e4.

Caci, H.M., CORTESTI: stata module to test equality of two correlation coefficients. 2000.

Chai, G.S., et al., 2013. Betaine attenuates Alzheimer-like pathological changes and memory deficits induced by homocysteine. J. Neurochem. 124 (3), 388–396.

Clark, C.M., et al., 1999. Variability in annual mini-mental state examination score in patients with probable alzheimer disease: a clinical perspective of data from the consortium to establish a registry for Alzheimer's disease. JAMA Neurol. 56 (7), 857–862.

Claxton, A., et al., 2013. Sex and ApoE genotype differences in treatment response to two doses of intranasal insulin in adults with mild cognitive impairment or Alzheimer's disease. J. Alzheimers Dis. 35 (4), 789–797.

Coley, N., et al., 2011. Suitability of the Clinical Dementia Rating-Sum of Boxes as a single primary endpoint for Alzheimer's disease trials. Alzheimers Dement. 7 (6), 602–610 e2.

Combarros, O., et al., 2011. APOE dependent-association of PPAR-gamma genetic variants with Alzheimer's disease risk. Neurobiol. Aging 32 (3), 547.e1-6.

Crum, R.M., et al., 1993. Population-based norms for the Mini-Mental State Examination by age and educational level. JAMA 269 (18), 2386–2391.

Dayon, L., et al., 2017. One-carbon metabolism, cognitive impairment and CSF measures of Alzheimer pathology: homocysteine and beyond. Alzheimers Res. Ther. 9 (1), 43.

Elias, M.F., et al., 2008. Homocysteine and cognitive performance: modification by the ApoE genotype. Neurosci. Lett. 430 (1), 64–69.

Farina, N., et al., 2017. Homocysteine concentrations in the cognitive progression of Alzheimer's disease. Exp. Gerontol. 99, 146–150.

Guerreiro, R., et al., 2016. Genome-wide analysis of genetic correlation in dementia with Lewy bodies, Parkinson's and Alzheimer's diseases. Neurobiol. Aging 38, 214.e7–214.e10.

Han, L., et al., 2000. Tracking cognitive decline in Alzheimer's disease using the mini-mental state examination: a meta-analysis. Int. Psychogeriatr. 12 (2), 231–247.

Hensel, A., Angermeyer, M.C., Riedel-Heller, S.G., 2007. Measuring cognitive change in older adults: reliable change indices for the Mini-Mental State Examination. J. Neurol. Neurosurg. Psychiatry 78 (12), 1298–1303.

Holland, D., et al., 2012. Rates of decline in Alzheimer disease decrease with age. PLoS One 7 (8), e42325.

Holtzman, D.M., Herz, J., Bu, G., 2012. Apolipoprotein E and apolipoprotein E receptors: normal biology and roles in Alzheimer disease. Cold Spring Harb. Perspect. Med. 2 (3), a006312.

Hoyt, B.D., et al., 2005. Individual growth curve analysis of APOE epsilon 4-associated cognitive decline in Alzheimer disease. Arch. Neurol. 62 (3), 454–459.

Hsiung, G.Y., Sadovnick, A.D., Feldman, H., 2004. Apolipoprotein E epsilon4 genotype as a risk factor for cognitive decline and dementia: data from the Canadian Study of Health and Aging. CMAJ 171 (8), 863–867.

Idriss, A.A., et al., 2018. Dietary betaine supplementation in hens modulates hypothalamic expression of cholesterol metabolic genes in F1 cockerels through modification of DNA methylation. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 217, 14–20.

Jansen, I.E., et al., 2019. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. Nat. Genet. 51 (3), 404–413.

Johnson, L.A., et al., 2017. Apolipoprotein E4 and insulin resistance interact to impair cognition and alter the epigenome and metabolome. Sci. Rep. 7, 43701.

Karlsson, I.K., et al., 2018. Apolipoprotein E DNA methylation and late-life disease. Int. J. Epidemiol.

Kelleher, B.P., Broin, S.D., 1991. Microbiological assay for vitamin B12 performed in 96-well microtitre plates. J. Clin. Pathol. 44 (7), 592–595.

Knight, L.S., et al., 2017. Betaine in the brain: characterization of betaine uptake, its influence on other osmolytes and its potential role in neuroprotection from osmotic stress. Neurochem. Res. 42 (12), 3490–3503.

Levey, A.S., et al., 2009. A new equation to estimate glomerular filtration rate. Ann. Intern. Med. 150 (9), 604–612.

Liu, C.-C., et al., 2013. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. Nat. Rev. Neurol. 9 (2), 106–118.

Liu, X.P., et al., 2014. Betaine suppressed Abeta generation by altering amyloid precursor protein processing. Neurol. Sci. 35 (7), 1009–1013.

Lysne, V., et al., 2016. Peroxisome proliferator-activated receptor activation is associated with altered plasma one-carbon metabolites and B-vitamin status in rats. Nutrients 8 (1).

Midttun, O., et al., 2016. Combined measurement of 6 fat-soluble vitamins and 26 water-soluble functional vitamin markers and amino acids in 50 μL of serum or plasma by high-throughput mass spectrometry. Anal. Chem. 88 (21), 10427–10436.

Midttun, O., Hustad, S., Ueland, P.M., 2009. 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. 23 (9), 1371–1379.

Midttun, O., Kvalheim, G., Ueland, P.M., 2013. High-throughput, low-volume, multi-analyte quantification of plasma metabolites related to one-carbon metabolism using HPLC-MS/MS. Anal. Bioanal. Chem. 405 (6), 2009–2017.

Molloy, A.M., Scott, J.M., 1997. Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. Methods Enzymol. 281, 43–53.

Moretti, R., Caruso, P., 2019. The controversial role of homocysteine in neurology: from labs to clinical practice. Int. J. Mol. Sci. 20 (1), 231.

Morris, J.C., 1997. Clinical dementia rating: a reliable and valid diagnostic and staging measure for dementia of the Alzheimer type. Int. Psychogeriatr. 9 (Suppl. 1), 173–176 discussion 177–8.

Mortensen, E.L., Hogh, P., 2001. A gender difference in the association between APOE genotype and age-related cognitive decline. Neurology 57 (1), 89–95.

Mulugeta, E., et al., 2011. CSF amyloid beta38 as a novel diagnostic marker for dementia with Lewy bodies. J. Neurol. Neurosurg. Psychiatry 82 (2), 160–164.

Najm, R., Jones, E.A., Huang, Y., 2019. Apolipoprotein E4, inhibitory network dysfunction, and Alzheimer's disease. Mol. Neurodegener. 14 (1), 24.

Nelson, P.T., Braak, H., Markesbery, W.R., 2009. Neuropathology and cognitive impairment in Alzheimer disease: a complex but coherent relationship. J. Neuropathol. Exp. Neurol. 68 (1), 1–14.

Rolstad, S., et al., 2013. Cerebrospinal fluid biomarkers mirror rate of cognitive decline. J. Alzheimers Dis. 34 (4), 949–956.

Roselli, F., et al., 2009. Rate of MMSE score change in Alzheimer's disease: influence of education and vascular risk factors. Clin. Neurol. Neurosurg. 111 (4), 327–330.

Sen, A., Nelson, T.J., Alkon, D.L., 2017. ApoE isoforms differentially regulates cleavage and secretion of BDNF. Mol. Brain 10 (1), 19.

Smith, A.D., et al., 2018. Homocysteine and dementia: an international consensus statement. J. Alzheimers Dis. 62 (2), 561–570.

Tombaugh, T.N., McIntyre, N.J., 1992. The mini-mental state examination: a comprehensive review. J. Am. Geriatr. Soc. 40 (9), 922–935.

Tong, I.S., Lu, Y., 2001. Identification of confounders in the assessment of the relationship between lead exposure and child development. Ann. Epidemiol. 11 (1), 38–45.

Tseng, H.C., Graves, D.J., 1998. Natural methylamine osmolytes, trimethylamine N-oxide and betaine, increase tau-induced polymerization of microtubules. Biochem. Biophys. Res. Commun. 250 (3), 726–730.

van Wijk, N., et al., 2017. Nutrients required for phospholipid synthesis are lower in blood and cerebrospinal fluid in mild cognitive impairment and Alzheimer's disease dementia. Alzheimers Dement. (Amst) 8, 139–146.

Vogiatzoglou, A., et al., 2013. Cognitive function in an elderly population: interaction between vitamin B12 status, depression, and apolipoprotein E epsilon4: the Hordaland Homocysteine Study. Psychosom. Med. 75 (1), 20–29.

Wilhelm, J., et al., 2008. Apolipoprotein E polymorphism, homocysteine serum levels and hippocampal volume in patients with alcoholism: an investigation of a gene-environment interaction. Pharmacogenom. J. 8 (2), 117–121.

Yue, L., Mazzone, T., 2009. Peroxisome proliferator-activated receptor gamma stimulation of adipocyte ApoE gene transcription mediated by the liver receptor X pathway. J. Biol. Chem. 284 (16), 10453–10461.