ARTICLE

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Health issues and nutrition in the elderly

Low folate predicts accelerated cognitive decline: 8-year follow-up of 3140 older adults in Ireland

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OBJECTIVE: To examine associations of plasma folate concentrations and risk of global and domain-specific cognitive decline in older people.
METHODS: Data of 3140 participants from The Irish Longitudinal Study on Ageing (TILDA), a nationally-representative cohort of adults aged ≥50 years were used over 8-year follow-up. Biannual cognitive assessments included the Mini-Mental State Examination (MMSE), verbal fluency and immediate and delayed word recall tests (Waves 1–5) and the Montreal Cognitive Assessment, (MoCA) (Waves 1 and 3). Plasma folate concentrations were measured in stored blood collected at baseline. Mixed effects Poisson and linear regression determined associations between baseline folate concentrations and cognition.
RESULTS: In multivariable-adjusted models of those aged ≥50 years at baseline, low folate at baseline (<11.2 nmol/L) was associated with higher proportions of MMSE errors (incidence rate ratio [IRR] = 1.10; 95% confidence interval [CI] (1.00, 1.21), lowest vs. highest quintile) over 8 years. Plasma folate <21.8 nmol/L predicted declines in episodic memory for immediate (beta [β] = −0.26; 95% CI (−0.48, −0.03), $\beta = -0.29$; 95% CI (−0.50, 0.08) and $\beta = -0.29$; (−0.50, −0.08), for lowest three vs. highest quintile) and delayed recall ($\beta = -0.20$; 95% CI (−0.38, −0.01), $\beta = -0.18$; 95% CI (−0.37, −0.01) and $\beta = -0.19$; (−0.36, −0.01) lowest three vs. highest quintile). There were no significant associations in a subsample aged ≥65 years.
CONCLUSION: In those aged ≥50 years, lower concentrations of folate may have differential relationships with cognitive domains. Folate <11.2 nmol/L predicted a decline in global cognitive function, while <21.8 nmol/L predicted poorer episodic memory. Low folate was associated with accelerated decline in cognitive function and is an important marker for cognitive decline among older people.

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IMPACT STATEMENT

We certify that this work is novel. This is the largest sample to date to investigate longitudinal associations of folate and cognitive function, using repeated assessments of cognitive tests in a deeply phenotyped cohort. These data pertain to community-dwelling individuals that were cognitively robust at baseline and demonstrate evidence of an accelerated decline in cognitive function over an 8-year follow-up period in those with lower folate levels at baseline. The results are more pronounced for tests of episodic memory—memory being a sensitive indicator for early cognitive decline and mild cognitive impairment, and this means that low folate may serve as a useful early marker of risk of accelerated cognitive decline. Low folate status is a modifiable risk factor for cognitive decline, but is an omni-present issue for older adults in Ireland and other countries where fortification with folic acid is not mandated.

WHY DOES THIS PAPER MATTER?

This paper matters as it demonstrates that even among a relatively health and cognitively robust community-dwelling sample, that low folate status predicts accelerated cognitive decline across several cognitive domains, over an 8-year time period.

INTRODUCTION

Approximately 7% of the world's population aged ≥65 years have dementia, a major cause of disability and mortality, that increases exponentially with age [1]. Cognitive impairment, a precursor to dementia, can range from mild to severe and is characterized by a deterioration in attention, executive function, learning and memory [2]. Maintenance of cognition is essential for functional independence in ageing. The optimization of nutritional status has been suggested to be a possible modifiable factor for prevention of cognitive impairment [3] and may be involved in the delay or prevention of chronic diseases including dementia [4, 5], cardiovascular disease [6] and structural changes in the brain [7–9].

Folate and vitamin B_{12} (B_{12}) are essential co-factors in one-carbon metabolism, DNA-methylation and nucleotide synthesis, processes that occur in all tissues in the body including the brain and have been linked to neurodegeneration [10]. Both vitamins

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Table 1. Details of cognitive tests.

Domain	Test	Assessed in waves	Scoring	Administration	Effect measure	Interpretation of effects
Global cognition	MMSE	1, 2, 3, 4, 5	Total number of errors (0–30)	Participants were asked questions comprising attention, memory, orientation, language, and spatial ability	Rate ratio	>1 = worse cognition
	MoCA	1, 3		Participants were asked questions comprising attention, memory, orientation, language, executive function and spatial ability	Rate ratio	>1 = worse cognition
Semantic memory	Verbal fluency	1, 2, 3, 4, 5	Total number of words	Participants were asked to name as many animals as possible in 60 s	β coefficient	<0 = worse cognition
Episodic memory	Immediate recall	1, 2, 3, 4, 5	Total number of words (0–20)	Participants were read a list of ten words on two occasions and asked to recall as many words as possible from the list immediately after	β coefficient	<0 = worse cognition
	Delayed recall	1, 2, 3, 4, 5	Total number of words (0–10)	Participants were asked to recall as many words as possible from the list read previously (~15 min delay)	β coefficient	<0 = worse cognition

Higher scores indicate poorer cognitive performance on the MMSE and MoCA, while higher scores indicate better cognitive performance on verbal fluency and word recall.
CAPI computer-assisted personal interview, *MMSE* Mini-Mental State Examination, *MoCA* Montreal Cognitive Assessment.

are required for efficient metabolism of homocysteine (HCY), a cytotoxic intermediary amino acid produced by the methionine cycle [10] that has been associated with cardiovascular disease and stroke, as well as cognitive decline [11]. The importance of folate insufficiency in cognitive impairment and dementia has been extensively studied in recent years due to its key role in HCY regulation [12]. Several epidemiological studies have shown cross-sectional and prospective associations between low folate and the risk of cognitive impairment and dementia [13–21].

Overt folate deficiency (<7 nmol/L) has been associated with accelerated ageing processes in the brain [22]. While rarely observed in countries where mandatory folic acid fortification (MFAF) of staple grains is in place to reduce the incidence of neural tube defects [23], deficiency and low folate is frequently observed in countries without MFAF such as the UK [24] and Ireland [25]. The Irish Longitudinal Study on Ageing (TILDA) demonstrated a 15% prevalence of low-to-deficient folate status (<10 nmol/L) among older adults living in a setting of voluntary fortification, highlighting its lack of effectiveness for maintenance of folate status in older people [26].

Using TILDA data, we investigated the longitudinal associations of folate concentrations with changes in cognitive performance in community-dwelling people, including sequential assessments of cognitive function at 2, 4, 6- and 8 years follow-up. We hypothesized that low folate was associated with accelerated cognitive decline in older people living in Ireland.

METHODS

TILDA is a nationally-representative prospective cohort study of community-dwelling adults aged ≥50 years living in the Republic of Ireland. The study design and sampling procedure have been described previously [27]. Briefly, participants were asked to complete a structured interview at each wave, capturing information on their social, financial and health circumstances. At baseline (2009–2011), 8504 participants were recruited and data were collected every two years thereafter, with objective health assessments conducted at alternate waves. Data for this study were obtained from Waves 1 to 5 of TILDA, collected between 2009 and 2018. The median interval between interviews for participants between Wave 1 to Wave 2 was 2.0 years, Wave 2 to Wave 3 was 2.3 years, Wave 3 to Wave 4 was 1.7 years and Wave 4 to Wave 5 was 2.0 years. Ethical approval was obtained at each data collection from the Faculty of Health Sciences Research Committee, Trinity College Dublin. Written consent was obtained from all participants.

Assessment of cognitive function

Participants underwent a battery of cognitive assessments at each wave which included the Mini-Mental State Examination [28] (MMSE; Global Cognition), a verbal fluency test (Semantic Memory) and immediate and delayed word recall tests (Episodic Memory). The Montreal Cognitive Assessment, (MoCA; Global Cognition) was measured at Waves 1 and 3 [29]. Table 1 describes the details of the cognitive tests. Data are presented by the cognitive domains for each test. Both the MMSE and MoCA scores are subject to ceiling effects, therefore the numbers of errors were calculated with the outcome representing the count of errors made during the test (30 minus the raw score) and used in analyses rather than total score.

Measurement of B vitamins

In Wave 1 during the health assessment, a non-fasting blood sample was collected by venipuncture into one 10 ml K2EDTA tube (BD, Becton, Dickinson Limited, Oxford, UK) for immediate analysis and two 10 ml ethylene diamine tetra-acetic acid (EDTA; BD, Becton, Dickinson Limited) tubes for long term storage (Sarstedt; Numbrecht, Germany). Samples were kept chilled and were centrifuged (3000 rpm for 15 min). Aliquots of plasma were labeled and stored at −80 °C within 48 h of blood collection and stored until required for analysis. The protocol for blood sample collection, processing, and storage used is detailed elsewhere [30]. Blood samples were analysed for plasma folate and B_{12} determined by microbiological assay; Lactobacillus casei *(L. rhamnosis (NCIB 10463; ATCC 27773))*, limit of detection 0.6 nmol/L [31] and Lactobacillus leichmannii

(*L. leichmanii (delbrueckii) (NCIB 12519, ATCC 43787)), limit of detection 3.7 pmol/L* [32], respectively. Inter-assay coefficients of variability (CV) for both methods were <10.9%.

Covariates

Socioeconomic covariates included were age, sex and educational attainment (primary, secondary or tertiary/higher); lifestyle characteristics including physical activity (Short-Form International Physical Activity Questionnaire) [33], smoking status, alcohol use and use of folic acid (FA) supplements; and health characteristics included weight and height (Body Mass Index (BMI) derived), (kg/m^2), depressive symptoms (Center for Epidemiologic Studies Depression Scale (CES-D) scale [34], hypertension (doctor diagnosis or prescribed medications) and diabetes (doctor diagnosis or prescribed medications). Plasma B_{12}, (categorized as < or ≥258 pmol/L, vitamin D (plasma 25-hydroxyvitamin D) and lutein concentrations were also included. Medications (up to 20 prescription and non-prescription medications, including food supplements) were classified by World Health Organization, Anatomical Therapeutic Chemical (ATC) classification codes. These covariates were chosen as they have previously been or were expected to be associated with cognitive performance or folate metabolism.

Analytical sample

Of the 8504 participants interviewed in Wave 1, 3871 were eligible at baseline after exclusion criteria (Supplementary Fig. S1). Individuals aged <50 years ($n = 330$), without health assessment data ($n = 3140$) and blood samples ($n = 108$), in receipt of B_{12} injections ($n = 5$), and for whom folate and/or B_{12} measurements exceeded the 99th percentiles ($n = 74$ and $n = 371$, respectively) were excluded from the analysis. Individuals with MMSE scores of <24 [35] and MoCA below established age and education normative cut-offs [36] at baseline were also excluded ($n = 605$) to remove participants with possible mild cognitive impairment (MCI). Prospective analyses used eligible baseline participants with two or more follow-up cognitive measurements and those who did not receive B_{12} injections in subsequent waves, yielding a final analytical sample of 3140 participants. Data on missing samples are presented in Supplementary Table S1.

Statistical analysis

Baseline characteristics of the sample were summarized by quintiles of plasma folate. Categorical variables were examined using percentages and continuous variables were explored using means and standard deviations, or medians and interquartile ranges, where appropriate. Performance scores for all cognitive tests were obtained for each wave. Histograms and Q–Q plots were used to assess marginal distributions of cognitive variables. Mixed effects models were used to examine the longitudinal associations between baseline folate status and cognitive function and take account for clustering of repeated measures within the same individual [37]. Further details of the methodology used are presented in Supplementary Appendix S1. Cognitive function scores for MMSE and MoCA errors were right-skewed and Poisson models were used. Verbal fluency, immediate and delayed recall were modeled using linear regression models. Separate models were run for each cognitive outcome with the theoretically most favorable folate quintile (highest) used as the reference category. The analysis allowed us to examine if the effect of the association across quintiles of folate was apparent over the full exposure distribution (or was limited to the extremes). Model 1 are unadjusted regression models. Model 2 adjusts for age, age squared, sex, sociodemographic, behavioral, lifestyle and medical covariates. Statistical significance was defined as $P < 0.05$ and were performed using Stata 15.1 (StataCorp, College Station, TX).

RESULTS

Baseline characteristics

The mean (standard deviation) age of the baseline sample was 62.1 years (7.2), 52.7% were female. Cognitive scores for participants at each wave show small differences between waves, with the most notable decline evident with verbal fluency (Table 2). Sample characteristics were compared by quintiles of folate concentrations at Wave 1 and are summarized in Table 3. Relative to those in the highest quintile, participants in the lowest quintile of folate at baseline (<11.2 nmol/L) were more likely to be male, smoke, classified as obese (BMI ≥ 30), have higher levels of creatinine, report depressive symptoms and have lower mean B_{12} and vitamin D. Furthermore, they were less likely to use a FA supplement. Cognitive function scores did not differ across quintile groups at Wave 1. Supplementary Table S2 summarizes the sample characteristics that were compared by B_{12} groups (Deficient < 148 pmol/L, Low 148 –<258 pmol/L and Normal ≥258 pmol/L).

Regression analyses

Regression analyses examining associations between baseline folate and cognitive function are presented in Fig. 1. Multivariable-adjusted models demonstrated that folate levels of <11.2 nmol/L (lowest quintile) at baseline were associated with increases of MMSE errors (Incidence Rate Ratio [IRR] = 1.10; 95% confidence interval [95% CI] 1.00, 1.21), over eight years when compared to the reference category, the highest quintile (32.6–81.3 nmol/L). Baseline folate levels <21.8 nmol/L (quintiles 1–3) predicted an overall decline in episodic memory for both immediate (beta [β] = −0.26; (95% CI −0.48, −0.03), $\beta = -0.29$; (−0.50, −0.08) and $\beta = -0.29$; (−0.50, −0.08), for quintiles 1–3 vs. highest quintile) and delayed recall ($\beta = -0.20$; 95% CI = −0.38 to −0.01, $\beta = -0.18$; 95% CI −0.37, 0.01 and $\beta = -0.19$; (−0.36, −0.01) for

Table 2. Unadjusted cognitive function scores for all eligible and available participants at each wave.

	Wave 1	Wave 2	Wave 3	Wave 4	Wave 5
Global cognition					
MMSE errors[a]	1 (0–2)	0 (0–1)	1 (0–1)	1 (0–1)	1 (0–1)
MoCA errors[a]	4 (2–5)	–	3 (2–5)	–	–
Semantic memory					
Verbal fluency[b]	22.4 ± 6.9	20.5 ± 5.9	19.9 ± 5.7	19.6 ± 5.7	19.5 ± 5.7
Memory					
Immediate recall[b]	14.1 ± 2.8	14.5 ± 2.8	14.3 ± 2.9	14.2 ± 3.0	14.1 ± 3.0
Delayed recall[b]	6.6 ± 2.1	6.6 ± 2.3	6.5 ± 2.3	6.4 ± 2.4	6.4 ± 2.4

Available cognitive data varies for each test depending on whether it was included in the interview or health assessment; data from Wave 1 (collected October 2009 to July 2011; $N = 3123–3140$), Wave 2 (collected February to December 2012; $N = 3077–3095$), Wave 3 (collected March 2014 to December 2015; $N = 2962–3136$), Wave 4 (collected January to December 2016; $N = 2919–2939$) and Wave 5 (collected January 2018 to December 2018; $N = 2664–2691$). Higher scores indicate poorer cognitive performance on the MMSE and MoCA, while higher scores indicate better cognitive performance on verbal fluency and word recall.

MMSE Mini-Mental State Examination, *MoCA* Montreal Cognitive Assessment.

[a]Median (IQR) (*IQR* interquartile range).

[b]Mean ± SD (*SD* Standard deviation).

Table 3. Characteristics of sample at baseline (2009–2011), according to plasma folate concentrations in quintiles.

		Plasma folate level, nmol/L					
Characteristic	All participants	Quintile 1 0.9–11.2 nmol/L	Quintile 2 11.2–15.6 nmol/L	Quintile 3 15.7–21.8 nmol/L	Quintile 4 21.8–32.5 nmol/L	Quintile 5 32.6–81.3 nmol/L	*p* value[a]
n	3140	573	638	629	646	654	
Age, mean ± SD	61.4 ± 8.0	61.6 ± 8.4	60.7 ± 8.0	60.6 ± 7.7	61.7 ± 8.0	62.3 ± 7.9	<0.001
Female, *N* (%)	1661 (52.9)	290 (50.6)	303 (48.4)	330 (52.5)	345 (53.4)	387 (59.2)	<0.01
Educational attainment, *N* (%)							
Primary	639 (20.4)	121 (21.1)	139 (21.8)	132 (21.0)	131 (20.3)	116 (17.7)	0.418
Secondary	1377 (43.9)	264 (46.1)	272 (42.6)	277 (44.0)	284 (44.0)	280 (42.8)	
Tertiary	1124 (35.8)	188 (32.8)	227 (35.6)	220 (35.0)	231 (35.8)	258 (39.5)	
Lives with someone, *N* (%)	2639 (83.7)	94 (16.4)	106 (16.6)	106 (16.9)	91 (14.1)	114 (17.4)	0.537
Smoke, *N* (%)	426 (13.6)	91 (15.9)	113 (17.7)	75 (11.9)	75 (11.6)	72 (11.0)	<0.01
Alcohol use, *N* (%)	2379 (80.4)	415 (78.6)	474 (79.8)	486 (81.5)	495 (80.1)	509 (81.7)	0.658
BMI, mean ± SD	27.5 (4.7)	27.8 (4.8)	27.9 (5.2)	27.6 (4.4)	27.6 (4.4)	27.0 (4.5)	<0.05
Obese, *N* (%)	1013 (32.4)	191 (33.5)	218 (34.3)	219 (35.0)	215 (33.5)	170 (26.1)	<0.001
Exercise group, *N* (%)							
Low	836 (26.6)	158 (27.6)	170 (26.7)	164 (26.1)	174 (26.9)	170 (26.0)	0.855
Moderate	1116 (35.5)	207 (36.1)	209 (32.8)	227 (36.1)	232 (35.9)	241 (36.9)	
High	1188 (37.8)	208 (36.3)	259 (40.6)	238 (37.8)	240 (37.2)	243 (37.2)	
Creatinine, mean (95% CI)*	78.9 [78.4, 79.6]	81.4 [79.9, 82.9]	79.1 [77.7 80.4]	79.1 [78.1, 81.1]	78.2 [76.9, 79.6]	76.9 [75.6, 78.1]	<0.01
Women, mean (95% CI)*	70.4 [69.8, 71.1]	71.8 [70.3, 73.3]	70.4 [68.8, 71.9]	70.5 [69.1, 72.0]	69.8 [68.4, 71.2]	69.9 [68.5, 71.3]	0.883
Men, mean (95% CI)*	88.8 [87.9, 89.7]	91.6 [89.4, 93.8]	87.4 [85.6, 89.1]	89.5 [87.2, 91.8]	87.9 [86.0, 89.9]	87.5 [85.8, 89.3]	<0.01
Folic acid supplement use, *N* (%)	107 (3.4)	13 (2.3)	8 (1.3)	20 (3.2)	22 (3.4)	44 (6.7)	<0.001
Number of medications, mean ± SD	2.3 ± 2.4	2.3 ± 2.6	2.2 ± 2.6	2.0 ± 2.1	2.4 ± 2.4	2.4 ± 2.4	0.061
High blood pressure, *N* (%)	1259 (40.3)	245 (43.0)	259 (40.7)	224 (35.8)	257 (40.1)	274 (42.0)	0.102
Diabetes, *N* (%)	192 (6.2)	32 (5.6)	36 (5.7)	36 (5.8)	48 (7.6)	40 (6.2)	0.588
History of stroke, *N* (%)	30 (1.0)	6 (1.1)	5 (0.8)	9 (1.4)	2 (0.3)	8 (1.2)	0.278
CES-D, mean ± SD	5.2 ± 6.5	5.3 ± 7.0	5.2 ± 6.7	5.4 ± 6.5	5.3 ± 6.5	4.8 ± 6.0	0.561
Folate nmol/L, mean (95% CI)*	23.1 [22.6, 23.6]	8.8 [8.7, 9.0]	13.4 [13.2, 13.5]	18.6 [18.5, 18.7]	26.4 [26.2, 26.6]	46.5 [45.6, 47.3]	<0.001
B_{12} pmol/L, mean (95% CI)*	333.5 [330.6, 337.5]	290.8 [282.2, 299.5]	300.2 [292.3, 308.2]	335.6 [327.2, 344.0]	349.2 [340.8, 357.6]	386.5 [377.2, 395.0]	<0.001
Vitamin D nmol/L, mean (95% CI)*	53.4 [52.5, 54.2]	46.4 [44.6, 48.3]	49.6 [47.9, 51.5]	51.7 [50.0, 53.5]	56.8 [55.0, 58.7]	62.8 [61.0, 64.6]	<0.001
Lutein μmol/L, mean (95% CI)*	0.2 [0.2, 0.2]	0.2 [0.2, 0.2]	0.2 [0.2, 0.2]	0.2 [0.2, 0.2]	0.2 [0.2, 0.2]	0.2 [0.2, 0.2]	<0.001
MMSE errors, median (IQR)	1 (0–2)	1 (0–2)	1 (0–2)	1 (0–2)	1 (0–2)	1 (0–1)	0.094
MoCA errors, median (IQR)	4 (2–5)	4 (2–6)	4 (2–5)	4 (2–5)	4 (2–5)	3.5 (2–5)	<0.05
Verbal fluency, mean ± SD	22.4 ± 6.9	22.3 ± 6.8	22.4 ± 7.2	22.5 ± 6.7	22.3 ± 6.8	22.5 ± 6.8	0.979
Immediate recall, mean ± SD	14.1 ± 2.8	14.0 ± 2.9	14.0 ± 2.9	14.1 ± 0.8	14.3 ± 2.8	14.3 ± 2.8	0.102
Delayed recall, mean ± SD	6.6 ± 2.1	6.4 ± 2.2	6.5 ± 2.1	6.5 ± 2.1	6.5 ± 2.2	6.7 ± 2.1	0.289

SD standard deviation, *CI* confidence interval.
*Geometric mean.
[a]Significance testing used one-way ANOVAs for normally distributed continuous characteristics, Kruskal–Wallis for non-normally distributed continuous characteristics and chi-square tests for categorical characteristics.

quintiles 1–3 vs. highest quintile) over eight years. In Model 1, significant increases in the number of MoCA errors amongst those in the lowest quintile of folate (<11.2 nmol/L) were observed (IRR = 1.10; 95% CI 1.03 to 1.18) over four years, however, this effect was attenuated with adjustment for additional covariates (Model 2). Results for Models 1 and 2 are presented in Supplementary Tables S3, S4, respectively.

Sensitivity analysis

Regression estimates of the sensitivity analysis conducted on subsamples of participants are presented in Supplementary Table S5. Among the subsample aged ≥65 years, there were no significant associations between baseline folate and changes in cognitive function.

DISCUSSION

We investigated the hypothesis that low folate status was associated with accelerated cognitive decline by examining the longitudinal associations between baseline folate status and repeated measures of five tests of cognitive function. To our knowledge, this is the largest longitudinal study to examine this issue using repeated measurements of tests exploring multiple cognitive domains. The results indicate that over an 8-year follow-up period, low folate (<11.2 nmol/L) was associated with decline in global cognitive function and a modest insufficiency (<21.8 nmol/L) predicted declines in both immediate and delayed recall.

Folate insufficiency is often observed among older Europeans and increases in prevalence with age [24]. Causes of deficiency include decreased dietary intake and malabsorption, age-related impairments in folate transport and metabolism, use of anti-folate medications, genetic factors and excessive alcohol intake [38]. Our findings are consistent with other studies showing low folate status was associated with higher risks of cognitive impairment or dementia [13–21]. In one study of older adults (≥60 years), those with MCI or dementia had significantly lower serum folate levels than healthy controls. Subjects in the lowest tertile (<13.5 nmol/L) had an adjusted Odds Ratio (OR) of 3.8 (95% CI: 1.3, 11.2; $P = 0.018$) for dementia [13]. Further, a meta-analysis examining thirteen studies (≥60 years) demonstrated low folate was associated with general and specific impairments in cognition such as attention, episodic and visuospatial memory or abstract reasoning (OR = 1.66, 95% CI: 1.40, 1.96) [19]. In the current study, the results for episodic memory add to emerging evidence that folate may have differential relationships with specific domains of cognition. This may be due to its role in hippocampal neurogenesis, as shown in animal models [39, 40]. The hippocampus is among the most important structures in the brain involved in memory [41] and is critical in the pathogenesis of diseases such as Alzheimer's and dementia. High concentrations of HCY have been associated with hippocampal atrophy [42]. Adequate folate is essential for balanced HCY levels and normal protein methylation processes in the brain, needed for cell signaling pathways, therefore it is conceivable that B-vitamin–related memory deficits could be due to changes of hippocampal neuronal processes linked to encoding information in episodic memory. Our results also suggest that there may be a continuum of folate levels related to performance even within the 'low' and 'low-normal' ranges established in literature [26]. Over eight years, low folate was associated with declining MMSE, a routine screening test often regarded as a crude indicator of cognitive performance. While MMSE was a key test in many studies assessing B vitamins and cognitive function, we included additional tests to add granularity to our study and to explore subtle changes across separate domains of cognition. Using folate quintiles, a modest insufficiency at baseline predicted a decline in episodic memory, suggesting it may be subtle enough to predict deleterious effects among more sensitive cognitive tests.

Evidence from randomized controlled trials (RCTs) using FA and other B vitamins to lower HCY have shown no consistent benefit of supplementation over approximately five years on cognitive outcomes. FA supplementation was associated with improved domain-specific cognitive performance in RCTs with relatively large samples and ≥2 years follow-up [43–45]. Another trial examined individuals with high HCY to exclude causes other than low folate concentrations and FA supplementation (0.8 mg oral FA per day) was associated with improved memory, processing speed and sensorimotor speed after 3 years [44]. Furthermore, supplementation of daily oral 0.4 mg FA and 100 µg B_{12} promoted improvement in cognitive function after 2 years, particularly in immediate and delayed recall [45]. While FA supplementation (0.4 mg per day for 2 years) for individuals with MCI ($n = 180$) was associated with better performance in tests of both memory and attention [20]. However, a meta-analysis of 11 RCTs involving 37,485 individuals by Clarke et al. concluded that HCY-lowering by supplementation with B vitamins for 5 years had no significant effect on individual domains or global cognitive function [46]. Further, a recent systematic review and meta-analysis of 31 RCTs of B-vitamin supplementation in individuals with and without cognitive impairment also concluded that B vitamins did not generally improve cognitive performance or slow cognitive decline compared to placebo [47]. The interpretation of such findings should however consider the limitations of available data and lack of an obvious benefit of lowering HCY using B vitamins, rather than evidence of no effect. Inconsistencies among RCT results may be explained by chance, bias, power issues or possible methodological differences between the studies, including FA fortification and trial duration, thereby making it difficult to draw firm conclusions.

It is plausible that biological mechanisms other than hyperhomocysteinemia may underlie the associations between low folate and cognitive impairment, with proposed mechanisms including impaired methylation and mis-incorporation of uracil into DNA. The complex synergistic interactions between nutrients are also important to consider. Vitamins and nutrients often function as a collection of co-factors, therefore interventions using singular or closely-related compounds may have too-narrow a focus. This is illustrated by another trial using FA, B6 and B_{12}, showing treatment was effective only in those with high baseline omega-3 (n-3) fatty acid concentrations. In fact, n-3 fatty acid status was protective against brain atrophy only in the presence of B-vitamin supplementation, suggesting that both are needed for effectiveness [48].

The major dietary source of folate is green leafy vegetables and lower incidence of Alzheimer's disease or dementia has been associated with higher dietary folate intake [49]. Thus, even if low folate status is a surrogate for a less healthy diet and consequently is associated with poorer cognitive function, it is important nonetheless to consider what it tells us about individuals with lower folate levels. The present study shows that those with lower baseline folate were more likely to be male, smokers and obese, to have lower mean B_{12} and vitamin D levels, but also had higher creatinine levels. They were less likely to use a FA supplement, which may be an indicator of lower socioeconomic status. While it is possible that those taking dietary supplements engage in healthier behaviors which may positively impact cognitive performance, FA-containing supplement use was low, ranging from 3 to 6% in the whole sample (Table 3).

Limitations were present. The analysis was limited to the use of non-fasting plasma folate and tHcy and B_{12}-biomarkers such as methyl-malonic acid and holotranscobalamin were not measured. The B-vitamin analysis was conducted at one time point, thus there is a possibility of regression dilution. Longitudinal studies are subject to attrition, therefore we cannot rule out selection bias leading to under- or over-estimation of observed associations. This, and the likelihood

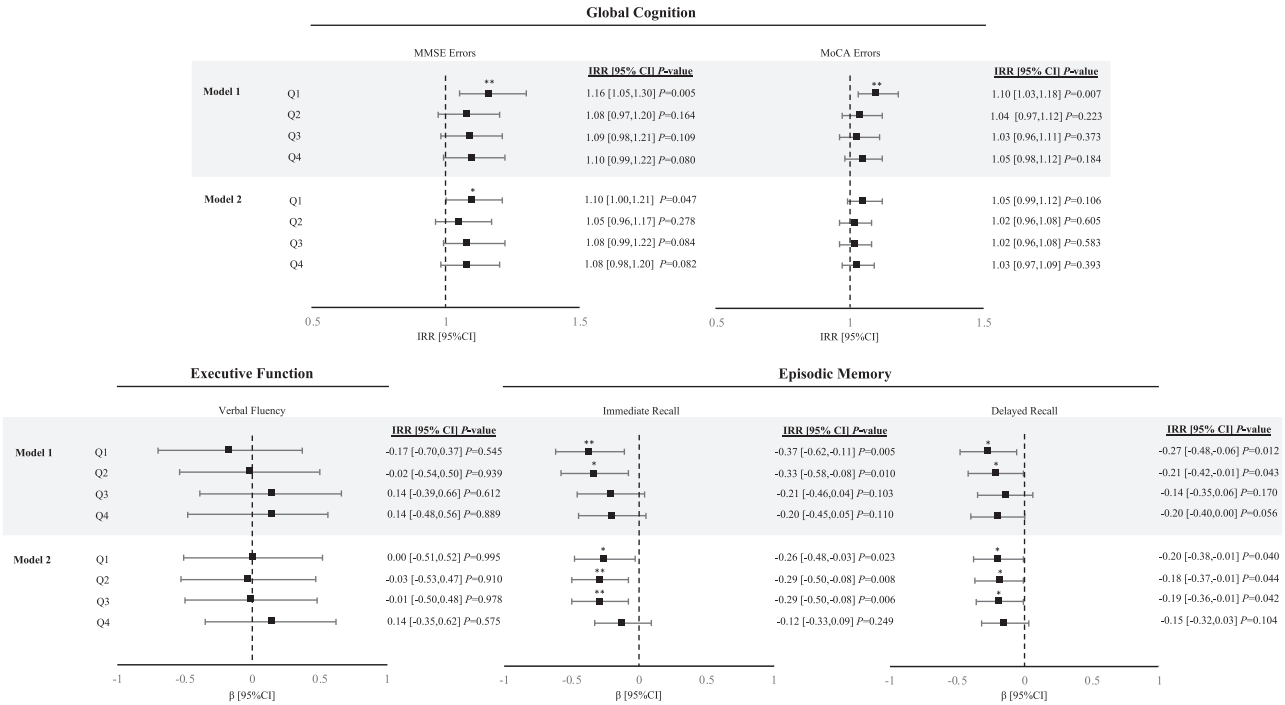

Fig. 1 Univariate and multivariable longitudinal associations between baseline folate and cognitive assessments (MMSE, MoCA, verbal fluency, immediate recall, and delayed recall). Reference category: Q5 Quintile 5 (Plasma folate 32.6–81.3 nmol/L). Q1 Quintile 1 (Plasma folate 0.9–11.2 nmol/L); Q2 Quintile 2 (Plasma folate 11.2–15.6 nmol/L); Q3 Quintile 3 (Plasma folate 15.7–21.8 nmol/L); Q4 Quintile 4 (Plasma folate 21.8–32.5 nmol/L). [a]IRR Incident Rate Ratio. [b]β Beta Coefficient. $^{**}P < 0.01$; $^{*}P < 0.05$. Model 1 = Basic model. Longitudinal cognitive variable and quintile of baseline folate controlling for time (wave). Model 2 = Sociodemographic, Health Indicators, Lifestyle, and Medical model. Model 1+ additional adjustment for age, age^2, sex, educational attainment, baseline vitamin B_{12} status (< or ≥258 pmol/L), baseline vitamin D, baseline lutein, smoking status, alcohol consumption, exercise levels (IPAQ), BMI category, depressive symptoms (CES-D), diabetes, use of folic acid supplements, antihypertensive medications.

that cognitive decline commenced earlier than age 65, may explain the lack of associations observed in the subsample aged ≥65 years. While our analysis controlled for a wide range of known covariates it is impossible to eliminate the risk of residual confounding. The factors controlled for in the statistical analyses were chosen a priori to be consistent with previous publications using folate and cognitive function in this population [50]. Finally, the sample consisted of high-functioning, largely healthy and younger community-dwelling participants and may not represent the older population living in residential care.

Our study has several strengths. The five cognitive tests examined encompassed three domains, global cognition, semantic and episodic memory. The longitudinal design included multiple assessments over 8 years, objective assessments of vitamin biomarkers using gold standard methods and detailed phenotypic data pertaining to sociodemographic and lifestyle factors, diseases and medication use in a large, well-characterized sample from one population. The use of mixed effects models in addition to the consideration of relevant covariates and interactions in the analyses, adds to the rigor of the current study. This study was performed in Ireland, a country that does not have MFAF, and we consider this to be a strength. With a high prevalence of folate insufficiency observed in the population, it allows reliable assessment of the associations with cognitive function over time.

In summary, we have shown that a decline in global cognitive performance and episodic memory among older adults that were cognitively normal can be predicted by low baseline folate levels and therefore it may be an important indicator for risk of early decline. This could have implications for clinical and public health policy recommendations for prevention of cognitive decline in older people.

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AUTHOR CONTRIBUTIONS

DMAO'C, CAMcG, RAK: Conception of research and study design. DMAO'C, SS, CDL, CAMcG: Analysis and interpretation of data. DMAO'C, SS, CAMcG: Preparation of paper DMAO'C, SS, CDL, AMO'H, EL, AMM, RC, CAMcG, RAK: Critical revision of paper. DMAO'C, CAMcG, RAK: Final approval for publication.

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COMPETING INTERESTS

The authors declare no competing interests.

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