Associations of folate/folic acid supplementation alone and in combination with other B vitamins on dementia risk and brain structure: evidence from 466,224 UK Biobank participants

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Abstract

Previous researchers have tried to explore the association between folate/folic acid intake and dementia incidence, but the results remain controversial. We evaluated the associations of folate/folic acid supplementation alone and in combination with other B vitamins on dementia risk and brain structure. A total of 466,224 UK Biobank participants were investigated. Cox proportional hazards models were used to assess the associations between folate/folic acid supplementation status and the risk of Alzheimer's disease (AD) and vascular dementia (VD). Multivariable linear regression models were employed to evaluate the association between folate/folic acid supplementation status and brain structure. In the final model, folate/folic acid supplementation alone was significantly associated with a higher risk of AD (hazard ratio [HR] =1.34, 95% confidence interval [CI] =1.06 to 1.69, p=0.015) and VD (HR=1.61, 95% CI=1.21 to 2.13, p=0.001). Folate/folic acid supplementation alone was associated with a reduction in the hippocampus (β= -95.25 mm^3, 95% CI= -165.31 to -25.19 mm^3, p=0.014) and amygdala (β= -51.85 mm^3, 95% CI= -88.02 to -15.68 mm^3, p=0.012). The risk of AD and VD, as well as brain structure, in the group with combined folate/folic acid supplementation and other B vitamins did not show a statistically significant difference compared to the reference group (all p>0.05). Folate/folic acid supplementation alone is significantly associated with a higher risk of AD and VD, as well as adverse alterations in brain structure. However, when combined with other B vitamins, these detrimental effects can be counteracted.

Keywords: folate, folic acid, Alzheimer’s disease, vascular dementia, brain structure

Introduction

The occurrence of dementia, which results in cognitive deterioration substantial enough to affect individuals, caregivers, families, and societies, represents a tremendous global issue(1). Currently, approximately 50 million individuals worldwide suffer from dementia, with over 9.9 million new cases diagnosed each year(1). The most prevalent subtypes of dementia, Alzheimer's disease (AD) and vascular dementia (VD), lack effective therapeutic approaches. Thus, there is an urgent need to identify individuals at high risk and implement interventions to prevent the onset of AD.

Nutrition is a crucial lifestyle factor that can modify the risk of dementia(2). Specifically, folate (also known as vitamin B9) is believed to influence brain functionality(3). Folate is a type of vitamin B found naturally in foods, although its levels can be reduced during food storage and cooking(4). Folic acid is an artificial form of folate with increased bioavailability, commonly used as a dietary supplement(5). Folate plays a significant role in processes involving deoxyribonucleic acid, including synthesis, repair, and methylation(6), thus impacting human health significantly. Previous studies have shown that administering folic acid to expectant mothers significantly reduces the likelihood of their newborns developing neural tube defects (NTDs)(7). Consequently, some nations have implemented compulsory folic acid fortification programs(8). However, several Western European countries have yet to enforce mandatory folic acid fortification, partly due to concerns about potential adverse effects on cancer incidence(9). Furthermore, a recent data analysis examining the correlation between national folic acid fortification and the risk of NTDs found no significant association between folic acid fortification and a decreased incidence of NTDs in the population(8). Therefore, the potential negative consequences of folic acid supplementation should be a focal point of attention.

Some previous studies have indicated that low serum folate concentrations are associated with reduced cognitive scores or an increased risk of dementia(10-11), and folate levels may also independently predict the development of dementia(12). These findings have been attributed to possible associated hyperhomocysteinemia(13). Based on this, some researchers believe that folic acid supplementation may slow the progression of cognitive impairment or dementia, although it has been demonstrated that excessively high folate levels can be detrimental to cognitive performance(14). Researchers have further explored the relationship between folate/folic acid supplementation and the incidence of dementia, but the results remain controversial(15-18). Many of the previous studies had small sample sizes, short follow-up times, and limited analysis of dementia subtypes, necessitating further evidence to investigate the associations between folate/folic acid supplementation status and dementia subtypes (including AD and VD). Additionally, Previous research has not explored the correlation between folate supplementation and brain volume; therefore, we were intrigued to explore whether folate supplementation influences changes in brain volume. Although it is widely accepted that both genetic and environmental factors contribute to the development of AD(19), the connections between genetic risk, folate/folic acid supplementation status, and AD risk are still unclear.

Therefore, we evaluated the association between folate/folic acid supplementation status and the risk of AD/VD, as well as brain structure, in a large cohort using data from a comprehensive national prospective study, the UK Biobank. We investigated potential interactions between folate/folic acid supplementation status and the AD genetic risk score (AD-GRS) in relation to AD risk. Additionally, subgroup analyses were conducted to assess whether there were potential modifications in the association between folate/folic acid supplementation status and the risks of AD and VD across different groups.

Methods

Study design and population

The UK Biobank is a substantial research cohort consisting of over 500,000 individuals enrolled in the United Kingdom between 2006 and 2010. These participants underwent baseline assessments, including physical, socio-demographic, and medical evaluations(20-21). Starting in 2014, the UK Biobank initiated magnetic resonance imaging (MRI), including brain imaging, with the goal of gathering imaging data from 100,000 participants. All participants provided informed consent for the UK Biobank project, which was authorized by the North West Multicentre Research Ethical Committee Study(22-23). This database contains comprehensive study data, including genetic information for the majority of participants. For our investigation, we accessed data from 502,411 individuals. Participants with dementia at baseline were excluded (n = 250). To account for potential confounding effects of long-term oral folic acid intake for therapeutic purposes, participants with folate deficiency anemia at baseline were also excluded (n = 937). Furthermore, individuals with insufficient information regarding folate/folic acid intake (n = 7,300) or lacking genetic data (n = 10,379) were excluded. Individuals who did not supplement with folate/folic acid but supplemented with other B vitamins were also excluded (n = 17,321). **Supplementary Figure 1** displays a research flow diagram outlining the analytical procedure.

Exposure

Information regarding the supplementation or non-supplementation of folate/folic acid and other B vitamins was gathered using a touchscreen questionnaire during baseline assessments. Participants were asked the question, "Do you regularly take any of the following?" and were provided with options such as "Folate or folic acid" and "Vitamin B" to indicate their response. Using these data, we categorized our participants into the following groups: folate/folic acid non-supplementation, folate/folic acid supplementation alone, and folate/folic acid supplementation with other B vitamins.

Outcomes: dementia and brain structure

In our study, the primary outcomes were AD and VD, which were determined through health-related outcomes from UK Biobank, combining algorithmically defined outcomes with previously validated ICD-10 codes of mental and behavioral disorders. The algorithmically defined outcomes were established by incorporating participants' self-reported medical conditions, medications, and linked data from hospital admission and death registries. Field ID used for the diagnosis of AD and VD was provided in **Supplementary Table 1**. Death events were defined based on death registration information in the database. The follow-up period was defined as the time between the assessment center visit and the diagnosis of dementia, death, or the last data collection date (March 10, 2022), whichever occurred first.

Since 2014, the UK Biobank has utilized a 3-Tesla Siemens Skyra scanner for brain MRI on a subset of participants, consistently using the same hardware and software for acquiring and assessing imaging data(24). For our investigation, the selection of brain regions evaluated is based on those associated with dementia in prior studies(25-28). We obtained T1 brain structural

data through brain MRI, which included normalized measurements of grey and white matter volumes, as well as white matter hyperintensities and subcortical volume data derived from T1 brain images. Specifically, subcortical volumes of the hippocampus, thalamus, caudate, putamen, pallidum, amygdala, and accumbens.

Definition of AD-GRS

The study employed genotyping and imputation techniques that were previously described in UK Biobank studies(29). To avoid false positives, any newly discovered genes were excluded. Researchers selected 29 specific single nucleotide polymorphisms (SNPs) associated with AD from previous genome-wide association studies (GWAS)(19, 30-31). The detailed calculation method can be found in **Supplementary Method 1**. The selected SNPs are listed in **Supplementary Table 2**. Beta coefficients for each SNP were calculated using previous GWAS data(31), and participants were categorized based on their AD-GRS scores. The first quintile was classified as the low scores ($\geq$ -0.00743 and $\leq$ -0.00321), the second to fourth quintiles represented the intermediate scores (> -0.00321 and $\leq$ -0.00112), and the fifth quintile was categorized as the high scores (> -0.00112 and $\leq$ 0.0179).

Covariates

A set of independent variables was selected for this investigation based on clinical expertise and consensus among the authors. These variables included sociodemographic characteristics (age, sex, body mass index (BMI), Townsend deprivation index (TDI), assessment center, education, ethnicity), lifestyle behaviors (healthy diet, smoking, alcohol consumption, and iron supplementation), disease history (hypertension, cardiovascular disease, diabetes, stroke),

triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and C-reactive protein (CRP). Assessment centers are classified as England, Wales, and Scotland. Education was categorized as either college/university degree or other, based on a questionnaire. The healthy diet score was determined using factors such as beef/mutton intake, vegetable intake, fruit intake, fish intake, cereal intake, and sodium concentration in urine. The detailed calculation method for the healthy diet score has been described in other literature(32). All factors were obtained from the UK Biobank database. Each participant was assigned one point for each favorable dietary factor, resulting in a total dietary score ranging from 0 to 6. A diet score of at least 3 was considered to indicate a healthy diet(32).

Statistical analyses

Baseline characteristics were presented for participants categorized by folate/folic acid intake status. Continuous variables were reported as mean and standard deviation (SD) or median and interquartile range, while categorical variables were reported as number and percentage. All missing variables were subjected to multiple imputation utilizing the mice function from the R "mice" package, which conducted 5 imputations over 5 iterations. The details regarding the missing covariates can be found in **Supplementary Table 3**. Total AD and VD cases were recorded during the observation period.

Cox proportional hazard regression modeling using the function "coxph" (R package "survival") was employed to determine hazard ratio (HR) and 95% confidence interval (CI) for

the probability of developing AD and VD, with the follow-up period as the time scale. The Schoenfeld residual test verified the proportional hazard assumption; variables with a P-value > 0.05 satisfied this assumption. If unmet, time-dependent covariates were incorporated in all models. To control potential confounders, potential dementia risk factors were incorporated as covariates based on prior research(33), and these covariates were sequentially adjusted in the models. Increased age and being female were significantly linked to an elevated risk of dementia, with Model 1 aiming to determine if folate/folic acid supplementation was associated with this risk, independently of age and sex. Considering other sociodemographic characteristics and lifestyle behaviors may influence dementia risk, Model 2 additionally adjusts for BMI, TDI, assessment center, educational level, ethnicity, healthy diet, iron supplementation, smoking, and alcohol consumption. Recognizing that diabetes, hypertension, myocardial infarction, and stroke are factors elevating dementia risk, and that certain biochemical markers are also associated with this risk, Model 3 further adjusts for hypertension, cardiovascular disease, diabetes, stroke, TG, HDL-C, LDL-C, and CRP.

To further investigate the association between folate/folic acid supplementation and brain structure, we employed multivariate linear regression models. When assessing gray matter, white matter, and white matter hyperintensities, all covariates mentioned above were adjusted. When assessing white matter hyperintensities and subcortical volumes, we additionally adjusted for the total intracranial volume (the sum of gray matter, white matter, and cerebrospinal fluid). We employed the false discovery rate (FDR) method and adjusted P-values using the "p.adjust" function in R.

To explore the potential modification of the relationship between folate/folic acid supplementation status and AD risk by genetic susceptibility, the association between folate/folic acid supplementation status and AD-GRS was evaluated using a final multivariate Cox proportional hazards model.

Subgroup analyses were conducted to assess potential modifying effects of sex (male or female), age (years, < 60 or ≥ 60), BMI (kg/m2, < 30 or ≥ 30), TDI (≤ median or > median), education (college/university degree or others), ethnicity (White or others), healthy diet (yes or no), iron supplementation (yes or no), smoking (never/previous or current), and alcohol (never/previous or current). Subgroup interactions were assessed for statistical significance using the likelihood ratio test.

To ensure the robustness of the results, several sensitivity analyses were performed. Firstly, participants with a follow-up period of less than 2 years were excluded to minimize the influence of reverse causation. Secondly, participants with missing values for variables were eliminated. Thirdly, participants concurrently taking other vitamins (A, C, D, or E) were excluded. Fourthly, using pairwise propensity score matching (PSM) based on all the relevant covariates included in our study, we investigated the associations between folate/folic acid supplementation status and the risk of AD and VD (**Supplementary Method 2**). Finally, E-values were calculated to evaluate the resilience of the association against possible unmeasured confounding factors (**Supplementary Method 3**). The analysis was conducted using R version 4.2.3, and statistical significance was considered as $P<0.05$ using two-sided tests.

Results

Participant characteristics

Of the 466,224 study participants, 10,916 (2.3%) reported regular supplementation of folate/folic acid in the touchscreen questionnaire (**Table 1**). Participants who reported folate/folic acid supplementation, regardless of whether they consumed other B vitamins, tended to be female, non-white, have a high TDI, follow a healthy diet, and abstain from alcohol. In terms of disease history, those who consumed folate/folic acid alone also had higher rates of hypertension, cardiovascular disease, diabetes, and stroke. Additionally, participants who reported intake of folate/folic acid (regardless of other B vitamins consumption) were more likely to take iron supplements. Specifically, among participants who consumed both folate/folic acid and other B vitamins, there was a higher proportion of individuals concurrently supplementing with iron. In participants who supplemented with folate/folic acid alone, the concentration of CRP was the highest.

Folate/folic acid supplementation status and the risk of AD and VD

During the median follow-up period of 12.7 years, we identified 2,949 new cases of AD and 1,603 new cases of VD. **Table 2** presents the association between folate/folic acid supplementation status and incident AD, as evaluated by three different models. In Model 1, a statistically significant association was observed between supplementation of folate/folic acid

alone and AD risk (HR = 1.44, 95% CI = 1.14 to 1.81, p = 0.002). After adjusting for additional factors including BMI, TDI, assessment center, education, ethnicity, healthy diet, iron supplementation, smoking, and alcohol (Model 2), the HR associated with folate/folic acid supplementation alone was 1.35 (95% CI = 1.07 to 1.70, p = 0.012). The final adjusted multivariate analysis (Model 3), which incorporated disease history and biochemical markers as well, revealed a persistent statistically significant association between folate/folic acid supplementation alone and increased AD risk (HR = 1.34, 95% CI = 1.06 to 1.69, p = 0.015). However, the risk of AD in the folate/folic acid supplementation with other B vitamins group did not show a statistically significant difference compared to the reference group (p > 0.05). The association between folate/folic acid supplementation status and the incidence of VD followed a similar pattern as AD. The risk of VD events was associated with folate/folic acid supplementation alone, with respective HRs in the three models of 1.96 (95% CI = 1.48 to 2.59, p < 0.001), 1.78 (95% CI = 1.34 to 2.36, p < 0.001), and 1.61 (95% CI = 1.21 to 2.13, p = 0.001). Similarly, similar to the findings in AD, the risk of VD associated with folate/folic acid supplementation with other B vitamins group did not show a statistically significant difference.

Folate/folic acid supplementation status and brain structure

Brain MRI was completed for 40,142 participants, with a median follow-up of 9.2 years between folate supplementation assessment and brain MRI. The relationship between folate/folic acid supplementation status and brain structure was investigated in this study. According to **Table 3**, folate/folic acid supplementation alone showed a correlation with changes in the volume of

certain brain structures. Specifically, in the final model, the group with folate/folic acid supplementation alone was associated with a reduction in the volumes of hippocampus (β = -95.25 mm^3, 95% CI = -165.31 to -25.19 mm^3, p = 0.014) and amygdala (β = -51.85 mm^3, 95% CI = -88.02 to -15.68 mm^3, p = 0.012). The associations with the volumes of the white matter, white matter hyperintensities, thalamus, pallidum, and accumbens were statistically significant only in partially adjusted models. On the other hand, the group with folate/folic acid supplementation along with other B vitamins did not show any significant correlation with brain structure (all p > 0.05).

Folate/folic acid supplementation status and AD-GRS for AD risk

In this study, we investigated the potential relationship between genetic susceptibility and folate/folic acid supplementation status in relation to AD risk (**Figure 1**). Our findings demonstrate a significant interaction between folate/folic acid supplementation status and AD-GRS (p for interaction < 0.001). Specifically, we observed that the supplementation of folate/folic acid alone was associated with an increased risk of AD in individuals with intermediate or high AD-GRS. Furthermore, those with high AD-GRS who also took folic acid supplementation with other B vitamins demonstrated an increased risk of developing AD. Notably, participants with a high AD-GRS and folate/folic acid supplementation alone exhibited the highest risk of AD (HR = 2.31, 95% CI = 1.47 to 3.62, p < 0.001). Furthermore, there was no statistically significant association between the risk of AD and the group with folate/folic acid supplementation along with other B vitamins, even among participants with the highest AD-GRS (HR = 2.00, 95% CI = 0.99 to 4.05, p = 0.0554).

Subgroup analyses

To investigate the potential impact of subgroups, we conducted several subgroup analyses using the final model. Our findings indicate that the association between folate/folic acid supplementation status and AD risk is not influenced by sex, age, BMI, TDI, education, ethnicity, healthy diet, iron supplementation, smoking, and alcohol (all p for interactions > 0.05) (**Supplementary Figure 2**). However, we observed a stronger interaction between iron supplementation and folate/folic acid supplementation status in relation to VD risk (p for interaction = 0.006) (**Figure 2**). Specifically, participants who did not consume iron but had folate/folic acid supplementation alone exhibited an increased risk of VD (HR = 1.66, 95% CI = 1.23 to 2.23, p = 0.001), while the risk was even higher in the group with folate/folic acid supplementation along with other B vitamins (HR = 2.16, 95% CI = 1.32 to 3.54, p = 0.002). Furthermore, within the subgroups of females, or individuals with low BMI, or those with low TDI, we observed a stronger association between folate/folic acid supplementation alone and the risk of AD or VD, as indicated by higher HR values.

Sensitivity analyses

Sensitivity analyses were conducted to assess the robustness of our study. We excluded participants with less than 2 years of follow-up, and the results remained consistent (**Supplementary Table 4**). Furthermore, when individuals with missing covariate values were excluded, the associations between folate/folic acid intake status and the risk of AD and VD did not change significantly (**Supplementary Table 5**). After removing participants taking other

vitamins (A, C, D, or E), the associations remained consistent (**Supplementary Table 6**). Following pairwise PSM, the observed associations between folate/folic acid supplementation and risks of AD and VD aligned with our primary analysis (**Supplementary Tables 7-10**). The agreement between these sensitivity analyses and our main analysis confirms the stability of our findings. Additionally, we calculated E-values and found that the point estimate and lower confidence bound for AD events were 2.01 and 1.31, respectively. This means that unmeasured confounding factors would need to have a risk ratio of at least 2.01, independent of other confounding factors, with folate/folic acid intake alone and AD in order to eliminate the observed association. For VD events, the E-value of the point estimate was 2.60, and the E-value of the lower confidence bound was 1.71. These results can be interpreted similarly to the description provided above for AD.

Discussion

By capitalizing on the ample sample size and prolonged longitudinal tracking provided by the UK Biobank, our study provides compelling evidence linking folate/folic acid supplementation alone to a significantly increased risk of incident AD and VD. These associations remained independent of traditional risk factors, including sociodemographic characteristics, lifestyle behaviors, disease history, and biochemical markers. Furthermore, folate/folic acid supplementation alone was significantly associated with a reduction in the volume of hippocampus and amygdala. The risk of AD and VD, as well as brain structure, in the group with combined folate/folic acid supplementation and other B vitamins did not show a statistically

significant difference compared to the reference group. We also observed that the supplementation of folate/folic acid alone was associated with an increased risk of AD in individuals with intermediate or high AD-GRS, and those with high AD-GRS who also took folic acid supplementation with other B vitamins demonstrated an increased risk of developing AD. In subgroups of females, or individuals with low BMI, or low TDI, those with folate/folic acid supplementation alone exhibited a higher risk of AD and VD. Notably, among participants without iron intake, the risk of VD was significantly elevated in those who consumed folate/folic acid, regardless of whether they also consumed other B vitamins.

Currently, studies on the impact of folate/folic acid supplementation on cognitive function and dementia yield conflicting results. A Chinese study involving individuals with mild cognitive impairment showed that oral administration of folic acid improved cognitive function after 6 and 24 months of follow-up(15-16). However, other studies have reported inconsistent conclusions. A study of community-based populations in the United States found that high folic acid supplementation is associated with accelerated rates of cognitive decline, and this rapid decline is linked to higher dietary folic acid consumption(17). A combined analysis of data from three cohort studies indicated that high folate/folic acid supplementation may have cognitive harm in older adults with low vitamin B12 levels(14)(15). These disparate findings may result from limited sample sizes and short tracking durations in earlier research studies. Our study leverages the UK Biobank's advantages in terms of sample size and prolonged tracking period to provide more robust evidence that folate/folic acid supplementation alone can increase the risk of developing AD and VD in generally healthy individuals. It is important to note that previous

studies examining the association between folate/folic acid consumption and dementia did not specifically investigate subtypes of dementia, including AD and VD.

Although the exact mechanisms underlying the relationship between folate/folic acid intake and dementia are unknown, several scenarios could explain these results. Firstly, long-term folic acid intake can lead to high serum folate levels(34), causing an imbalance between vitamin B12 and folate(35). This imbalance may exacerbate the consequences of vitamin B12 insufficiency, which has been linked to cognitive impairment(36) and increased dementia risk. Secondly, high levels of folic acid can result in elevated circulating unmetabolized folic acid(37), which can reduce the activity of natural killer cells(38). The detrimental effects of increased plasma concentration of unmetabolized folic acid may contribute to the higher risk of dementia. Another possible mechanism is that high folic acid levels induce oxidative stress(39), which is known to be associated with the pathophysiology of dementia(40). Lastly, gut microbiota may also play a role. Evidence suggests that excessive folic acid intake can alter the composition of intestinal microbiota(41). Considering the potential involvement of gut microbiota in central metabolic processes, it is tempting to hypothesize a link to dementia(42). Further studies are needed to better understand the precise roles of folate/folic acid intake in dementia and to identify additional mechanisms that may be involved.

Interestingly, we observed a trend of increased risk of AD and VD in the group receiving pure folate/folic acid supplementation, but not in the group combining folate/folic acid with other B vitamins. In other words, combining folate/folic acid supplementation with other B vitamins may

help counteract the elevated risk of AD and VD associated with folate/folic acid supplementation alone. This finding is not coincidental; a previous study demonstrated that high plasma folate levels and folate supplementation are associated with rapid cognitive decline in individuals with plasma vitamin B12 levels below 258 pmol/L, while folate-containing vitamin B12 supplements have no impact on cognitive function decline in individuals with plasma vitamin B12 levels ≥ 258 pmol/L(43). Therefore, concurrent vitamin B12 supplementation may potentially mitigate the risk of cognitive decline induced by folate by increasing plasma vitamin B12 concentration. This may be due to the complex interactions between B vitamins. The inclusion of other B vitamins, such as vitamin B12 and vitamin B6, along with folate, may reduce the risk of AD and VD(44). Therefore, comprehensive supplementation with other vitamins may potentially alleviate the increased risk of AD and VD associated with folate supplementation.

As far as we know, this study represents the first investigation of the interplay between folate/folic acid supplementation and genetic risk on incident AD. Based on previous research findings, it has been observed that genetic factors may be associated with nutrient intake, including folic acid. Therefore, we assessed the potential interaction between AD-GRS and folic acid supplementation, a correlation that could hold significant implications for understanding the pathophysiological mechanisms of the disease. Our findings revealed a significant interaction between folate/folic acid intake and AD-GRS, indicating that the effect of folate/folic acid supplementation on AD risk is influenced by genetic factors. Specifically, we observed that the supplementation of folate/folic acid alone was associated with an increased risk of AD in individuals with intermediate or high AD-GRS, and those with high AD-GRS who also took folic acid supplementation with other B vitamins demonstrated an increased risk of developing

AD. The highest risk was observed in participants with a high AD-GRS and folate/folic acid supplementation alone. Interestingly, there was no significant association between AD risk and the group of participants who consumed folate/folic acid along with other B vitamins, even among those with the highest AD-GRS. These findings underscore the complex interplay between genetic susceptibility, folate/folic acid supplementation, and AD risk, highlighting the importance of personalized approaches in understanding and managing AD.

Our study examined the association between folate/folic acid supplementation and brain structure in a sample of participants, providing preliminary evidence of the potential impact of folate/folic acid supplementation on brain structure. Specifically, independent intake of folate/folic acid was correlated with volume changes in some brain regions, particularly showing significant reductions in hippocampus and amygdala. These findings may reflect damage or degeneration in these regions, which are known to play crucial roles in cognitive functions, particularly memory, and alterations in their structure may influence cognitive functioning(45). A plausible explanation for the observed correlation between folate/folic acid intake and brain structure is that excessive folic acid supplementation, leading to a high folate status, might diminish the availability of methyl donors or impede the activity of the methylene-THF reductase enzyme, which plays a pivotal role in the methylation of homocysteine(46). This interference could result in an accumulation of homocysteine. There is evidence that elevated homocysteine levels possess neurotoxic properties, potentially leading to brain atrophy(47). Furthermore, increased homocysteine might enhance the presence of phosphorylated tau proteins, which have been linked to atrophy in some brain regions(48). In contrast, we found no correlation between brain structure and the group of participants who consumed folate/folic acid

along with other B vitamins, suggesting potential interactions or offsetting effects between folate/folic acid and other B vitamins in influencing brain structure, similar to their impact on the risks of AD and VD. These findings emphasize the potential impact of folate/folic acid supplementation alone on brain structure and provide a basis for further research into its underlying mechanisms.

Based on subgroup analysis, our study identified a significant interaction between iron supplementation and folate/folic acid supplementation status in relation to VD risk. Specifically, individuals who did not consume iron but had folate/folic acid supplementation alone exhibited an increased risk of VD. Moreover, the risk was even higher in individuals who had folate/folic acid supplementation along with other B vitamins. Notably, no similar interactions were found between iron supplementation and folate/folic acid supplementation in relation to the incidences of AD. This discrepancy may be attributed to the distinct pathogenesis of VD. Previous studies have demonstrated that serum iron can stimulate the production of nitric oxide through iron-containing hemoglobin(49). Nitric oxide acts as a potent vasodilator, leading to increased cerebral blood flow(49). Iron supplementation has been shown to reduce the occurrence of VD, which may partly explain our findings. Furthermore, in specific subgroups of females, or in individuals with low BMI or low TDI, those with folate/folic acid supplementation alone exhibited a higher risk of AD and VD. These findings enhance our understanding of the complex relationship between nutrient supplementation and dementia risk.

This study possesses several major strengths. Firstly, one of the standout strengths of our study lies in its focus on the associations between folate/folic acid supplementation and risks associated with AD and VD. By doing so, we offer a more nuanced understanding of how folate supplementation may be linked to specific forms of dementia. Secondly, our study is the first to delve into the relationship between folate/folic acid supplementation and brain volume, which can provide valuable insights into its broader impact on cognitive health and brain aging. Thirdly, our study included 466,224 participants and abundant data on social characteristics, diet, health status, and other covariates, which ensure comprehensive subgroup and sensitivity analyses to ensure the robustness of the results. Lastly, our study represents the first endeavor to investigate the potential relationships between genetic risk and folate/folic acid supplementation in relation to AD risk. The results of our analysis support the notion that folate/folic acid supplementation alone may be a potential risk factor for dementia and that when combined with other B vitamins, it could help mitigate this risk. These findings have significant clinical and practical implications for current dietary guidelines and public health policies, emphasizing the importance of considering the overall balance of B vitamins when consuming folate, particularly in countries where folate fortification is widespread, to avoid potential adverse effects.

This study has several potential limitations that warrant careful consideration. First, information regarding folate/folic acid supplementation was obtained through a self-reported baseline questionnaire that lacked comprehensive data on the dose, formulation, or duration of folate/folic acid supplementation. This limitation makes it impossible to assess any dose-response relationships for folate/folic acid supplements and potentially weakens our findings. Additionally, the UK Biobank did not collect data on serum folate and homocysteine levels, nor

did it include data on categorized vitamin B supplementation. As a result, we were unable to further analyze the effects of dementia in relation to differences in folate or homocysteine levels, as well as the specific distinctions among various types of vitamin B. Second, despite the large overall sample size, there is a significant imbalance between the numbers in the three groups. Although we implemented paired PSM to address and compensate for this imbalance, it remains an inevitable limitation of our study. Third, regular supplementation of folate/folic acid may be indicative of a healthy lifestyle. Despite meticulous adjustment for potential confounders related to lifestyle factors and the computation of E values, there may still be residual confounders stemming from unmeasured or unknown lifestyle factors that could have influenced the observed associations. Four, most participants in the UK Biobank may not have reached the age of dementia onset by the end of our follow-up period. The use of relevant hospitalization and death data to identify dementia and its subtypes is another potential limitation. However, previous validation studies have reported an 84.5% positive predictive value for dementia in the UK Biobank, indicating a relatively reliable dataset(50). Finally, the UK Biobank participants primarily consist of individuals of white ethnicity, and the limited representation of other racial groups may limit the generalizability of our findings to some extent. Therefore, caution should be exercised when interpreting our findings, and further evidence will be necessary to firmly establish the link between folate/folic acid supplementation and an increased risk of AD and VD.

Conclusion

In summary, our results indicate that folate/folic acid supplementation alone may have unfavorable implications for cognitive health, while combining it with other B vitamins can counteract these adverse impacts. These findings have significant public health implications for dementia prevention, emphasizing the importance of careful consideration when formulating dietary guidelines and public health policies. It is crucial to weigh the advantages and disadvantages of folate/folic acid supplementation more judiciously in order to make informed decisions.

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Conflict of Interest

The authors report no competing interests.

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Authors' contributions

JL and AX conceived and designed the research objectives. YL, SY, XH, ST, and HC participated in data acquisition and analysis. YL interpreted the results and drafted the manuscript. SY and XH revised the manuscript. All authors have approved the final version of the manuscript.

Data availability

The authors can obtain data upon reasonable request, subject to the consent of UK Biobank. The application number 76636 for the UK Biobank Resource was used to conduct this investigation.

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Table 1. UK Biobank participants' characteristics (n=466,224)

Characteristics	Folate/folic acid non-supplementation	Folate/folic acid supplementation alone	Folate/folic acid supplementation with other B vitamins
N	455308	7425	3491
Sex, N (%)			
Female	243679 (53.5)	5188 (69.9)	2289 (65.6)
Male	211629 (46.5)	2237 (30.1)	1202 (34.4)
Age, Median (IQR), years	58.00 (50.00, 63.00)	59.00 (50.00, 64.00)	58.00 (50.00, 63.00)
BMI, Median (IQR), kg/m^2	26.75 (24.16, 29.90)	26.68 (23.92, 30.19)	26.04 (23.37, 29.26)
TDI, Median (IQR)	-2.18 (-3.67, 0.46)	-1.58 (-3.42, 1.53)	-1.49 (-3.34, 1.73)
Assessment center, N (%)			
England	403620 (88.6)	6504 (87.6)	3089 (88.5)
Wales	19074 (4.2)	315 (4.2)	151 (4.3)
Scotland	32614 (7.2)	606 (8.2)	251 (7.2)
Education, N (%)			

College/University degree	148800 (32.7)	2427 (32.7)	1532 (43.9)
Others	306508 (67.3)	4998 (67.3)	1959 (56.1)
Ethnic, N (%)			
White	432327 (95.0)	6724 (90.6)	3156 (90.4)
Others	22981 (5.0)	701 (9.4)	335 (9.6)
Healthy diet, N (%)	298858 (65.6)	4911 (66.1)	2400 (68.7)
Iron supplementation, N (%)	11769 (2.6)	878 (11.8)	1391(39.8)
Smoking, N (%)			
Never	250312 (55.0)	3858 (52.0)	1784 (51.1)
Previous	157550 (34.6)	2748 (37.0)	1320 (37.8)
Current	47446 (10.4)	819 (11.0)	387 (11.1)
Alcohol, N (%)			
Never	19709 (4.3)	521 (7.0)	195 (5.6)
Previous	15620 (3.4)	519 (7.0)	237 (6.8)
Current	419979 (92.2)	6385 (86.0)	3059 (87.6)
Hypertension, N (%)	121548 (26.7)	2317 (31.2)	850 (24.3)
Cardiovascular disease, N (%)	20198 (4.4)	528 (7.1)	172 (4.9)

Diabetes, N (%)	22688 (5.0)	508 (6.8)	199 (5.7)
Stroke, N (%)	5786 (1.3)	172 (2.3)	66 (1.9)
TG, Median (IQR), mmol/L	1.49 (1.05, 2.15)	1.42 (1.01, 2.04)	1.36 (0.96, 1.98)
HDL-C, Median (IQR), mmol/L	1.40 (1.17, 1.67)	1.42 (1.19, 1.71)	1.48 (1.22, 1.77)
LDL-C, Median (IQR), mmol/L	3.52 (2.95, 4.12)	3.36 (2.80, 4.01)	3.42 (2.84, 4.00)
CRP, Median (IQR), mg/L	1.32 (0.65, 2.73)	1.79 (0.77, 4.33)	1.23 (0.58, 2.71)

Notes: BMI = body mass index; TDI = Townsend deprivation index; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; CRP, C-reactive protein.

The percentages may not add up to 100% due to rounding.

Table 2. Folate/folic acid supplementation status and the risk of AD and VD in the UK Biobank (n=466,224)

	Folate/folic acid status				
	Folate/folic acid non-supplementation	**Folate/folic acid supplementation alone**		**Folate/folic acid supplementation with other B vitamins**	
AD					
No. of events/ Total cases	2846/455308	73/7425		30/3491	
	HR	**HR (95%CI)**	***P* value**	**HR (95%CI)**	***P* value**
Model 1	1 (ref)	1.44 (1.14, 1.81)	0.002*	1.37 (0.96, 1.97)	0.084
Model 2	1 (ref)	1.35 (1.07, 1.70)	0.012*	1.22 (0.84, 1.77)	0.290
Model 3	1 (ref)	1.34 (1.06, 1.69)	0.015*	1.23 (0.85, 1.79)	0.266
VD					
No. of events/ Total cases	1534/455308	51/742		18/3491	
	HR	**HR (95%CI)**	***P* value**	**HR (95%CI)**	***P* value**
Model 1	1 (ref)	1.96 (1.48, 2.59)	<0.001*	1.59 (1.00, 2.53)	0.050*
Model 2	1 (ref)	1.78 (1.34, 2.36)	<0.001*	1.36 (0.84, 2.20)	0.205

Model 3	1 (ref)	1.61 (1.21, 2.13)	0.001*	1.38 (0.86, 2.23)	0.185

Notes: AD = Alzheimer's disease; VD = vascular dementia; HR = hazard ratio; CI = confidence interval.

*Represent significant associations ($p < 0.05$).

Model 1 was adjusted for age and sex. Model 2 additionally adjusted for BMI, TDI, education, ethnicity, assessment center, healthy diet, iron supplementation, smoking, and alcohol. Model 3 was based on model 2 and additionally adjusted for the history of hypertension, cardiovascular disease, diabetes, stroke, and the levels of TG, HDL-C, LDL-C, and CRP.

Table 3. Associations between folate/folic acid supplementation status and brain structure (mm^3)

	Folate/folic acid status				
	Folate/folic acid non-supplementation	Folate/folic acid supplementation alone		Folate/folic acid supplementation with other B vitamins	
	Beta	Beta (95%CI)	*P* value	Beta (95%CI)	*P* value
Volume of grey matter (n=40,142)					
Model 1	1 (ref)	-1496.90 (-4649.78, 1655.98)	0.440	721.31 (-3795.50, 5238.12)	0.754
Model 2	1 (ref)	-1113.77 (-4234.00, 2006.45)	0.549	1044.90 (-3471.96, 5561.76)	0.650
Model 3	1 (ref)	-691.80 (-3803.26, 2419.57)	0.721	1272.00 (-3230.47, 5774.15)	0.659
Volume of white matter (n=40,142)					
Model 1	1 (ref)	-3657.83 (-7050.18, -265.47)	0.043 *	-2378.72 (-7238.60, 2481.17)	0.337
Model 2	1 (ref)	-3472.41 (-6866.02, -78.80)	0.095	-1572.53 (-6485.14, 3340.09)	0.751

Model 3	1 (ref)	-3491.51 (-6886.44, -96.58)	0.122	-1543.16 (-6455.72, 3369.39)	0.708
Volume of white matter hyperintensities (n=38,856)					
Model 1	1 (ref)	658.24 (102.38, 1214.09)	0.025 *	-265.18 (-1068.35, 537.98)	0.518
Model 2	1 (ref)	669.83 (115.76, 1223.90)	0.038 *	-222.85 (-1031.98, 586.28)	0.668
Model 3	1 (ref)	548.10 (-2.36, 1098.57)	0.097	-277.00 (-1080.52, 526.52)	0.564
Volume of hippocampus (n=40,126)					
Model 1	1 (ref)	-123.71 (-195.58, -51.83)	0.001 *	52.60 (-50.37, 155.56)	0.317
Model 2	1 (ref)	-117.92 (-189.70, -46.14)	0.002 *	45.00 (-58.91, 148.90)	0.481
Model 3	1 (ref)	-95.25 (-165.31, -25.19)	0.014 *	48.67 (-52.70, 150.04)	0.429
Volume of thalamus (n=40,126)					
Model 1	1 (ref)	-114.05 (-224.84, -3.26)	0.055 *	-12.71 (-171.43, 146.01)	0.875

Model 2	1 (ref)	-101.50 (-211.52, 8.52)	0.100	-42.59 (-201.85, 116.67)	0.680
Model 3	1 (ref)	-56.14 (-160.66, 48.37)	0.362	-33.85 (-185.09, 117.38)	0.687
Volume of caudate (n=40,126)					
Model 1	1 (ref)	9.81 (-59.74, 79.35)	0.782	63.71 (-35.93, 163.34)	0.263
Model 2	1 (ref)	12.48 (-56.88, 81.83)	0.724	45.85 (-54.55, 146.25)	0.573
Model 3	1 (ref)	19.79 (-49.24, 88.81)	0.679	48.49 (-51.38, 148.37)	0.522
Volume of putamen (n=40,126)					
Model 1	1 (ref)	-90.12 (-176.46, -3.78)	0.051	-22.56 (-146.25, 101.13)	0.721
Model 2	1 (ref)	-83.25 (-169.32, 2.83)	0.090	-36.97 (-161.57, 87.63)	0.596
Model 3	1 (ref)	-59.93 (-143.44, 23.58)	0.255	-32.68 (-153.51, 88.16)	0.704
Volume of pallidum (n=40,126)					

Model 1	1 (ref)	-41.17 (-79.07, -3.28)	0.041*	17.65 (-36.63, 71.94)	0.524
Model 2	1 (ref)	-39.85 (-77.67, -2.02)	0.066	9.83 (-44.92, 64.58)	0.796
Model 3	1 (ref)	-29.90 (-67.25, 7.46)	0.190	12.68 (-41.37, 66.73)	0.699
Volume of amygdala (n=40,126)					
Model 1	1 (ref)	-57.11 (-93.34, -20.88)	0.003*	-17.05 (-68.95, 34.86)	0.520
Model 2	1 (ref)	-55.48 (-91.69, -19.27)	0.005*	-19.54 (-71.96, 32.87)	0.608
Model 3	1 (ref)	-51.85 (-88.02, -15.68)	0.012*	-19.41 (-71.75, 32.92)	0.578
Volume of accumbens (n=40,126)					
Model 1	1 (ref)	-24.77 (-41.11, -8.43)	0.004*	-4.71 (-28.11, 18.70)	0.694
Model 2	1 (ref)	-22.35 (-38.67, -6.03)	0.012*	-2.21 (-25.83, 21.42)	0.885
Model 3	1 (ref)	-15.78 (-31.33, -0.23)	0.087	-0.98 (-23.48, 21.52)	0.932

Notes: CI = confidence interval.

*Represent significant associations ($p < 0.05$).

Model 1 was adjusted for age and sex. Model 2 additionally adjusted for BMI, TDI, education, ethnicity, assessment center, healthy diet, iron supplementation, smoking, and alcohol. Model 3 was based on model 2 and additionally adjusted for the history of hypertension, cardiovascular disease, diabetes, stroke, and the levels of TG, HDL-C, LDL-C, and CRP. When assessing white matter hyperintensities and subcortical volumes, Model 3 additionally adjusted for the total intracranial volume (the sum of gray matter, white matter, and cerebrospinal fluid)

Figure 1. Association of folate/folic acid supplementation status and AD-GRS for AD risk. The multivariable model was adjusted for age, sex, BMI, TDI, education, ethnicity, assessment center, healthy diet, iron supplementation, smoking, alcohol, history of hypertension, cardiovascular disease, diabetes, stroke, and the levels of TG, HDL-C, LDL-C, and CRP. The vertical line indicates a reference value of 1.

Figure 2. Folate/folic acid supplementation status and VD risk among different subgroups. Associations of folate/folic acid supplementation status and VD risk were grouped by sex, age, BMI, TDI, education, ethnicity, healthy diet, iron supplementation, smoking, and alcohol. The multivariable model was adjusted for age, sex, BMI, TDI, education, ethnicity, assessment center, healthy diet, iron supplementation, smoking, alcohol, history of hypertension, cardiovascular disease, diabetes, stroke, and the levels of TG, HDL-C, LDL-C, and CRP. The vertical line indicates a reference value of 1.

Figure 1

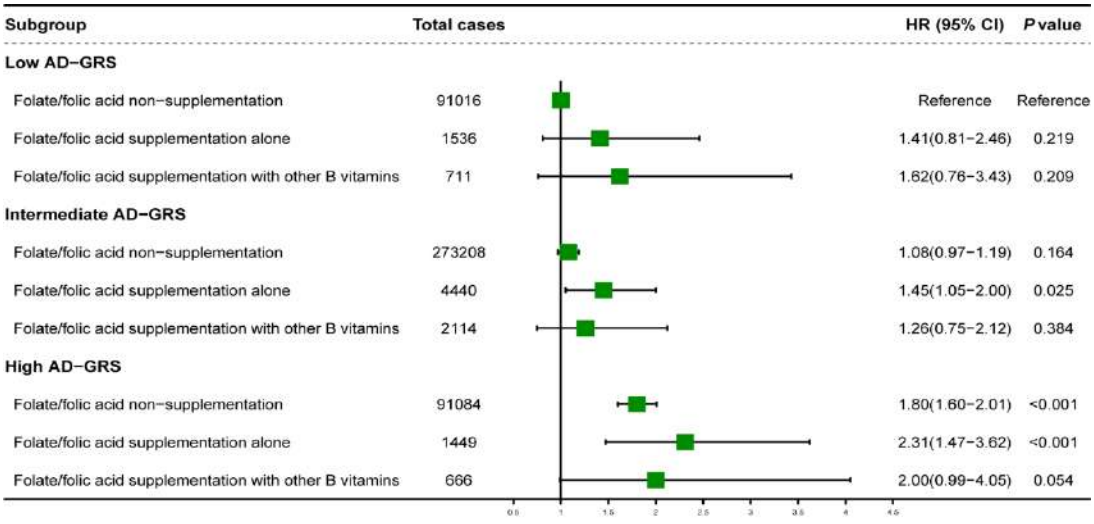

Figure 2