Predictive value of folate, vitamin B₁₂ and homocysteine levels in late-life depression

Jae-Min Kim, Robert Stewart, Sung-Wan Kim, Su-Jin Yang, Il-Seon Shin and Jin-Sang Yoon

Background
The role of folate, vitamin B₁₂ and homocysteine levels in depression is not clear.

Aims
To investigate cross-sectional and prospective associations between folate, vitamin B₁₂ and homocysteine levels and late-life depression.

Method
A total of 732 Korean people aged 65 years or over were evaluated at baseline. Of the 631 persons who were not depressed, 521 (83%) were followed over a period of 2–3 years and incident depression was ascertained with the Geriatric Mental State schedule. Serum folate, serum vitamin B₁₂ and plasma homocysteine levels were assayed at both baseline and follow-up.

Baseline sample and measurements
A cross-sectional survey of a geographically defined population was carried out in 2001. The sampling procedure and measurements have been described previously. In brief, 732 community residents aged 65 years or over were included in two catchment areas of Kwangju were recruited from national residents’ registration lists (5% refusal rate). Examinations included a fully structured diagnostic interview for depression; blood samples taken for folate, vitamin B₁₂, homocysteine and MTHFR genotype; and formal assessment of potential confounding factors.

Results
Lower levels of folate and vitamin B₁₂ and higher homocysteine levels at baseline were associated with a higher risk of incident depression at follow-up. Incident depression was associated with a decline in vitamin B₁₂ and an increase in homocysteine levels over the follow-up period.

Conclusions
Lower folate, lower vitamin B₁₂ and raised homocysteine levels may be risk factors for late-life depression.

Declaration of interest
None. Funding detailed in Acknowledgements

Folate, vitamin B₁₂, homocysteine and methylenetetrahydrofolate reductase (MTHFR) are involved in one-carbon transfer (methylation) reactions necessary for the production of monoamine neurotransmitters, phospholipids and nucleotides. Folate and vitamin B₁₂ deficiency, hyperhomocysteinaemia and the T677 allele of the MTHFR gene, which cause impaired methylation reactions in the central nervous system, have been associated with depressive disorders. However not all studies have found such associations. Discrepant findings in previous studies may relate to their cross-sectional design. In particular, changes in appetite and diet associated with depressive states may affect nutritional status, so that the direction of cause and effect remains unclear. To address this limitation, we analysed data from a 2-year longitudinal study to investigate both cross-sectional and prospective associations between these factors and depression in late life.

Method
A secondary analysis was made of data from a community-based prospective survey of late-life psychiatric morbidity conducted in Kwangju, Republic of Korea, from 2001 to 2003, in collaboration with the 10/66 International Dementia Research Program in Developing Countries. All participants gave written, formal informed consent at each examination. This study was approved by the Chonnam National University Hospital institutional review board.

Baseline sample and measurements
A cross-sectional survey of a geographically defined population was carried out in 2001. The sampling procedure and measurements have been described previously. In brief, 732 community residents aged 65 years or over were included in two catchment areas of Kwangju were recruited from national residents’ registration lists (5% refusal rate). Examinations included a fully structured diagnostic interview for depression; blood samples taken for...
Follow-up was carried out in 2003. The mean follow-up period was 2.4 years (s.d.=0.3). Attempts were made to follow up all previous participants. Identical procedures were used to identify depression (GMS–AGECAT) and further blood samples for folate, vitamin B12 and homocysteine were collected, centrifuged within 1 h and stored at −70°C. Assays were done after 1 year. Vitamin supplementation was investigated in the context of an inventory taken of all prescription and non-prescription medication taken in the past month.

Statistical analysis
Statistical analyses were carried out using SPSS version 12.0 for Windows. Associations between baseline depression and baseline quintiles of folate, vitamin B12 and homocysteine levels were assessed by χ²-tests (linear trend). Associations with demographic characteristics, assessment scales (MMSE and WHODAS-II), lifestyle characteristics (smoking, problem drinking and physical activity), vascular risk or disease and serum creatinine level were investigated using t-, χ²- or Mann–Whitney U-tests as appropriate. Odds ratios and their 95% confidence intervals were calculated for associations between baseline depression and baseline quintiles of folate, vitamin B12 and homocysteine, and for MTHFR genotype, in logistic regression models after adjustment for the other independent variables. For all analyses, quintiles of folate, vitamin B12 and homocysteine were entered as ordinal variables with one degree of freedom, in accordance with an a priori hypothesis that associations, if present, would show linearity across the distributions.

For investigating prospective associations, participants with case-level depression at baseline were excluded, and case-level depression at follow-up (incident depression) was treated as the dependent variable. Associations between incident depression and baseline quintiles of folate, vitamin B12 and homocysteine were estimated in logistic regression models both before and after adjustment for relevant factors including vitamin supplementation. In a secondary analysis, associations between baseline folate, vitamin B12 and homocysteine levels and depression at follow-up were calculated and re-categorised by quintiles. Associations between changes in these levels and incident depression were calculated and investigated further in identical logistic regression models to the analyses of baseline levels as predictors.

Results
Participants’ characteristics at baseline
Of 732 participants at baseline, case-level depression was present in 101 (13.8%). Mean levels of folate, vitamin B12 and homocysteine for the total sample were 24.4 nmol/l (s.d.=12.5), 385.6 pmol/l (s.d.=168.3) and 12.8 μmol/l (s.d.=5.7) respectively. Frequencies of the MTHFR allele were C 0.45 and T 0.55, and the genotype distribution was C/C 18.7%, C/T 52.7% and T/T 28.6% (test for Hardy–Weinberg equilibrium: χ²=2.091, P<0.05). Folate level was correlated positively with vitamin B12 level (r=0.112, P=0.002) and negatively with homocysteine level (r=−0.310, P<0.001). Vitamin B12 level was negatively correlated with homocysteine level (r=−0.289, P<0.001). Homocysteine levels were significantly associated with MTHFR genotype, with mean levels of 12.1 μmol/l (s.d.=5.6), 12.4 μmol/l (s.d.=4.6) and 13.8 μmol/l (s.d.=7.2) for the C/C, C/T and T/T genotypes respectively (F=5.301, P=0.003). There was no association between MTHFR genotype and folate or vitamin B12 levels (all P values >0.1). Other characteristics of the sample and unadjusted associations with depression at baseline are summarised in Table 1.

Of 631 participants without depression at baseline, 521 (85%) completed all evaluations at follow-up and formed the study sample. Of the remaining 110, contact could not be established with 58 (52%), 23 (21%) had died, 21 (19%) refused to participate and 8 (7%) were too unwell. Baseline characteristics of participants from the baseline non-depressed group who were followed up are displayed in the last column of Table 1. Between the participants and non-participants at follow-up, there was no substantial difference in any independent variable (all P values >0.06). Mean changes in levels from baseline to follow-up were as follows: folate −4.9 nmol/l (s.d.=12.1), vitamin B12 +48.0 pmol/l (s.d.=139.7) and homocysteine +1.6 μmol/l (s.d.=5.0). Figure 1 summarises the prevalence and incidence of depression according to baseline levels of folate, vitamin B12 and homocysteine, and change in these levels over the follow-up period.
Baseline folate, vitamin B₁₂ and homocysteine levels, and baseline depression

Depression at baseline was associated with lower levels of vitamin B₁₂ (χ²=4.190, P=0.041) and higher levels of homocysteine (χ²=4.901, P=0.027), but was not significantly associated with folate levels (χ²=1.445, P=0.250) (Fig. 1). These findings persisted after adjustment for potential confounders (Table 2). Prevalence of depression by MTHFR genotype was 14.6% for C/C, 15.0% for C/T and 11.0% for T/T (Fig. 1). Adjusted associations between these factors are displayed in Table 3. In summary, incident depression was associated with lower baseline levels of folate and vitamin B₁₂ and homocysteine, and depression at follow-up was not substantially changed when adjusted for baseline depression scale score (unadjusted odds ratios 1.24, 1.28 and 1.18 respectively, adjusted odds ratios 1.23, 1.27 and 1.18 respectively). In further exploratory stratified analyses, the association between descending baseline homocysteine level and incident depression was significantly modified by MTHFR genotype: odds ratios for decreasing folate quintiles were 1.18 (95% CI 0.79–1.76), 1.22 (95% CI 0.86–1.73) and 1.85 (95% CI 1.14–3.00) within CC, CT and TT genotypes respectively (P=0.021 for statistical interaction). No significant interaction was found between MTHFR genotype and vitamin B₁₂ or homocysteine as exposures, no significant gender interaction was found for any exposure and no significant two-way or three-way interactions were found between the three exposures of interest in predicting incident depression (data not shown). Among the 732 participants at baseline, a previous history of depression prior to age 60 years was reported by 16 (16%) of the 101 participants with current depression and by 17 (3%) of the remaining 631 participants. The findings of interest were not materially altered following restriction to those without a history of depression.

### Table 1: Baseline characteristics of the study sample

<table>
<thead>
<tr>
<th>Participants at baseline</th>
<th>Total sample (n=732)</th>
<th>No depression (n=631)</th>
<th>Depression (n=101)</th>
<th>P*</th>
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</thead>
<tbody>
<tr>
<td>Demographic characteristics</td>
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</tr>
<tr>
<td>Age, years: mean (s.d.)</td>
<td>72.8 (5.9)</td>
<td>72.7 (5.8)</td>
<td>73.7 (6.3)</td>
<td>0.095</td>
</tr>
<tr>
<td>Female gender, n (%)</td>
<td>432 (59.0)</td>
<td>359 (56.9)</td>
<td>73 (72.3)</td>
<td>0.004</td>
</tr>
<tr>
<td>Education, years: median (IQR)</td>
<td>1 (0–6)</td>
<td>1 (0–6)</td>
<td>0 (0–6)</td>
<td>0.321</td>
</tr>
<tr>
<td>Assessment scales</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>MMSE score: mean (s.d.)</td>
<td>23.3 (5.0)</td>
<td>23.3 (4.9)</td>
<td>22.1 (5.5)</td>
<td>0.006</td>
</tr>
<tr>
<td>WHODAS-II score: median (IQR)</td>
<td>3.3 (0–9)</td>
<td>2.8 (0–7)</td>
<td>8.7 (2–20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lifestyle characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>294 (40.2)</td>
<td>250 (39.6)</td>
<td>44 (43.6)</td>
<td>0.453</td>
</tr>
<tr>
<td>Current problem drinking, n (%)</td>
<td>213 (29.1)</td>
<td>185 (29.3)</td>
<td>26 (27.7)</td>
<td>0.743</td>
</tr>
<tr>
<td>Low physical activity, n (%)</td>
<td>229 (31.3)</td>
<td>176 (27.9)</td>
<td>53 (52.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vascular risk score: median (IQR)</td>
<td>1 (0–2)</td>
<td>1 (0–2)</td>
<td>2 (1–3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum creatinine, μmol/l: mean (s.d.)</td>
<td>70.7 (26.5)</td>
<td>70.7 (17.7)</td>
<td>79.6 (61.9)</td>
<td>0.075</td>
</tr>
</tbody>
</table>

IQR, interquartile range; MMSE, Mini-Mental State Examination; WHODAS-II, World Health Organization Disability Assessment Schedule II.

Baseline folate, vitamin B₁₂ and homocysteine levels, and incident depression

Incident depression was associated with lower baseline levels of folate (χ²=6.701, P=0.010) and vitamin B₁₂ (χ²=6.317, P=0.012) and higher baseline levels of homocysteine (χ²=3.335, P=0.021) (Fig. 1). Adjusted associations between these factors are displayed in Table 3. In summary, incident depression was associated with all three factors in the directions anticipated, with associations remaining significant after adjustment for other covariates (Table 3, model 6). When the three blood levels of interest were entered in combination (Table 3, models 7–9) the associations with lower folate and vitamin B₁₂ were reduced only marginally when adjusted for each other, with larger reductions when adjusted for homocysteine. On the other hand, the association between raised baseline homocysteine level and incident depression was reduced substantially when adjusted for individual vitamin levels. Incident depression was not associated with MTHFR genotype (χ²=2.346, P=0.167). In a secondary analysis of the whole follow-up sample, the associations between folate, vitamin B₁₂ and homocysteine, and depression at follow-up were not substantially changed when adjusted for baseline depression scale score (unadjusted odds ratios 1.24, 1.28 and 1.18 respectively, adjusted odds ratios 1.23, 1.27 and 1.18 respectively). In further exploratory stratified analyses, the association between descending folate and incident depression was significantly modified by MTHFR genotype: odds ratios for decreasing folate quintiles were 1.18 (95% CI 0.79–1.76), 1.22 (95% CI 0.86–1.73) and 1.85 (95% CI 1.14–3.00) within CC, CT and TT genotypes respectively (P=0.021 for statistical interaction). No significant interaction was found between MTHFR genotype and vitamin B₁₂ or homocysteine as exposures, no significant gender interaction was found for any exposure and no significant two-way or three-way interactions were found between the three exposures of interest in predicting incident depression (data not shown). Among the 732 participants at baseline, a previous history of depression prior to age 60 years was reported by 16 (16%) of the 101 participants with current depression and by 17 (3%) of the remaining 631 participants. The findings of interest were not materially altered following restriction to those without a history of depression.

### Table 2: Logistic regression models for the association between baseline folate, vitamin B₁₂ and homocysteine levels and baseline depression (n=732)

<table>
<thead>
<tr>
<th>Odds ratio (95% CI) for depression per quintile change</th>
<th>Folate (decrease)</th>
<th>Vitamin B₁₂ (decrease)</th>
<th>Homocysteine (increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>1.10 (0.95–1.27)</td>
<td>1.17 (1.01–1.36)</td>
<td>1.19 (1.02–1.38)</td>
</tr>
<tr>
<td>Model 1: adjusted for age, gender and education</td>
<td>1.16 (0.99–1.37)</td>
<td>1.21 (1.03–1.41)</td>
<td>1.30 (1.10–1.53)</td>
</tr>
<tr>
<td>Model 2: model 1 plus MMSE and WHODAS-II</td>
<td>1.18 (1.00–1.40)</td>
<td>1.22 (1.04–1.44)</td>
<td>1.32 (1.12–1.58)</td>
</tr>
<tr>
<td>Model 3: model 2 plus smoking, alcohol and activity</td>
<td>1.15 (0.97–1.36)</td>
<td>1.20 (1.02–1.42)</td>
<td>1.31 (1.10–1.56)</td>
</tr>
<tr>
<td>Model 4: model 3 plus vascular risk score</td>
<td>1.13 (0.95–1.34)</td>
<td>1.21 (1.03–1.43)</td>
<td>1.28 (1.07–1.52)</td>
</tr>
<tr>
<td>Model 5: model 4 plus serum creatinine</td>
<td>1.12 (0.94–1.33)</td>
<td>1.23 (1.04–1.45)</td>
<td>1.25 (1.04–1.49)</td>
</tr>
</tbody>
</table>

MMSE: Mini-Mental State Examination; WHODAS-II: World Health Organization Disability Assessment Schedule II.
Incident depression and co-occurring changes in folate, vitamin B\textsubscript{12} and homocysteine levels

Incident depression was more frequent in people with a relative decline in vitamin B\textsubscript{12} levels ($\chi^2=5.735$, $P=0.017$) and with a relative increase in homocysteine ($\chi^2=6.594$, $P=0.010$) (Fig. 1), whereas no association was found with change in folate levels ($\chi^2=0.971$, $P=0.324$). Adjusted associations between these factors are displayed in Table 4. The association between decline in vitamin B\textsubscript{12} levels and incident depression remained strong after adjustment for other covariates, was increased in strength after adjustment for vitamin supplementation at follow-up, and was decreased in strength following adjustment for homocysteine change. The association between an increase in homocysteine levels and incident depression changed little following adjustment for all other covariates.

Associations with clinical categories of folate and vitamin B\textsubscript{12} deficiency and hyperhomocysteaemia

The prevalence of baseline folate deficiency was 4.0%, vitamin B\textsubscript{12} deficiency 16.8% and hyperhomocysteaemia 22.1%. Odds ratios for associations with baseline depression were 1.32 (95% CI 0.49–3.54) for folate deficiency, 1.57 (95% CI 0.94–2.61) for vitamin B\textsubscript{12} deficiency and 1.78 (95% CI 1.13–2.84) for hyperhomocysteaemia. After adjustment for the other factors listed in Table 2, respective odds ratios were 1.86 (95% CI 0.59–5.80), 1.91 (95% CI 1.08–3.39) and 1.78 (95% CI 1.03–3.08). Respective odds ratios for incident depression adjusted for other covariates listed in Table 3 (model 6) were 1.94 (95% CI 0.58–6.47), 1.78 (95% CI 0.90–3.51) and 1.69 (95% CI 0.88–3.26).

Discussion

To our knowledge, this study is the first community-based, prospective investigation of associations between folate, vitamin B\textsubscript{12}, homocysteine and late-life depression. Principal findings were that incident depression was predicted by lower folate and vitamin B\textsubscript{12} levels and higher homocysteine levels 2 years previously, and was associated with a decline in vitamin B\textsubscript{12} levels and an increase in homocysteine levels over the intervening period. No direct association with MTHFR genotype was found, although the associations between folate levels and incident depression were modified by this factor. Associations between higher baseline homocysteine levels and incident depression were partly accounted for by vitamin B\textsubscript{12} and folate levels.

Methodological issues

Previous community studies investigating the association between these factors and depression have been cross-sectional in design.\textsuperscript{1,4,15–19} This limits the extent to which causal relationships can be clarified, since measures of nutritional status such as vitamin levels may be affected by the emergence of depressed states and associated alterations in appetite and food intake. Relative deficiency may, in turn, account for associations with raised homocysteine levels. Most studies have also been limited in the use of brief screening instruments to define depression,\textsuperscript{1,15–19} and in numbers of potential confounding factors considered, or in the specific nature of the cohorts analysed. Strengths of our study were that prospective data on both depression and the blood assays of interest were obtained from a community population, that depression was ascertained using a widely validated diagnostic schedule, and that a large number of potential confounding factors were considered in the analyses. The follow-up rate was reasonable and not apparently differential with respect to risk factors of interest. The study sample was restricted to older age ranges, but it is this group who are likely to be most vulnerable to nutritional deficiency. Limitations of the study were that data on vitamin supplementation were not available at the baseline evaluation, and that at the follow-up evaluation the information on mental health was restricted to the previous month. Detailed constituents of vitamin preparations were also not available. The statistical models were constructed...
 Previous case–control studies using clinical samples have reported associations between folate deficiency and depression at baseline in this particular population. The prospective association between lower folate levels and incident depression was not explained by other potential confounding characteristics, depression ascertainment or blood assays. In our study folate deficiency was not associated with depression in cross-sectional analyses, but lower folate levels were associated with a higher likelihood of incident depression 2 years later. The cross-sectional association between depression and folate deficiency might be obscured by selection bias if people with both depression and nutritional deficiency were less likely to participate, or if they were more prone to be hospitalised and therefore underrepresented in community samples. It is also possible that people with longer-lasting depressive states, who are over-represented in cross-sectional surveys, may regulate their diet in a way that might compensate for earlier deficiencies. The prevalence of folate deficiency at baseline was relatively low, but this is likely to be explained by the relatively high intake of folate-containing green vegetables in Korean populations, which has been previously recognised. Nevertheless, folate level remained negatively correlated with homocysteine level in our sample, as has been reported elsewhere. The lower prevalence of folate deficiency might have obscured the association between the folate deficiency and depression at baseline in this particular population. The prospective association between lower folate levels and incident depression was not explained by other potential confounding factors.

### Table 3

<table>
<thead>
<tr>
<th>Odds ratio (95% CI) for depression per quintile change</th>
<th>Folate (decrease)</th>
<th>Vitamin B₁₂ (increase)</th>
<th>Homocysteine (increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>1.28 (1.06–1.56)</td>
<td>1.27 (1.05–1.54)</td>
<td>1.25 (1.03–1.52)</td>
</tr>
<tr>
<td>Model 1: adjusted for age, gender and education</td>
<td>1.28 (1.05–1.57)</td>
<td>1.28 (1.05–1.55)</td>
<td>1.26 (1.03–1.56)</td>
</tr>
<tr>
<td>Model 2: model 1 plus MMSE and WHODAS-II</td>
<td>1.30 (1.06–1.59)</td>
<td>1.29 (1.06–1.56)</td>
<td>1.30 (1.05–1.61)</td>
</tr>
<tr>
<td>Model 3: model 2 plus smoking, alcohol and activity</td>
<td>1.31 (1.07–1.61)</td>
<td>1.28 (1.05–1.56)</td>
<td>1.29 (1.04–1.60)</td>
</tr>
<tr>
<td>Model 4: model 3 plus vascular risk score</td>
<td>1.32 (1.07–1.61)</td>
<td>1.30 (1.07–1.58)</td>
<td>1.27 (1.02–1.58)</td>
</tr>
<tr>
<td>Model 5: model 4 plus serum creatinine</td>
<td>1.31 (1.07–1.61)</td>
<td>1.31 (1.07–1.59)</td>
<td>1.26 (1.01–1.57)</td>
</tr>
<tr>
<td>Model 6: model 5 plus vitamin supplementation²</td>
<td>1.30 (1.06–1.60)</td>
<td>1.31 (1.08–1.59)</td>
<td>1.25 (1.01–1.56)</td>
</tr>
<tr>
<td>Model 7: model 6 plus folate level</td>
<td>1.28 (1.05–1.56)</td>
<td>1.17 (0.93–1.47)</td>
<td></td>
</tr>
<tr>
<td>Model 8: model 6 plus vitamin B₁₂ level</td>
<td>1.27 (1.03–1.59)</td>
<td>1.17 (0.94–1.47)</td>
<td></td>
</tr>
<tr>
<td>Model 9: model 6 plus homocysteine level</td>
<td>1.25 (1.01–1.54)</td>
<td>1.26 (1.03–1.54)</td>
<td></td>
</tr>
</tbody>
</table>

MMSE, Mini-Mental State Examination; WHODAS-II, World Health Organization Disability Assessment Schedule II.

² Vitamin supplementation ascertained at follow-up.
factors (Table 3), and homocysteine, as a potential mediating factor, explained only a small proportion of this association. It is of interest that the association between lower folate and incident depression was significantly modified by MTHFR genotype, with strongest associations in those with the T/T genotype. A recent study suggested that, since the MTHFR gene influences the functioning of the folate metabolic pathway, folate or its derivatives might be causally related to risk of depression.14

Vitamin B12 deficiency and depression

The cross-sectional significant association observed between lower vitamin B12 levels and depression is consistent with previous findings from both clinical samples22 and community populations,6,15 although not all studies have found this.1 In prospective analyses, incident depression was associated both with lower baseline vitamin B12 levels and with a previous decline in vitamin B12 levels from baseline to follow-up. Vitamin B12 is required for the synthesis of S-adenosylmethionine, which is an important methyl donor in many reactions. Methylmalonic acid is a more specific marker of inhibition of function at a cellular level. Methylmalonic acid is a more specific marker of function at a cellular level and confounding effects may be underestimated.

Implications for public health and future research

Our findings in this prospective community study support roles for folate, vitamin B12, and homocysteine levels in the aetiology of late-life depression. From a public health perspective, there may be good arguments for focusing interventions for the prevention of depression on nutritionally deficient, frail populations. Although the use of vitamin supplements did not substantially modify the observed associations, further research is likely to be required as the ascertainment in this study might have been incomplete and obscured by dietary habits. Relationships with the dose, duration and (particularly) constituents of vitamin supplements should be investigated. However, it should be borne in mind that the results of observational research are often not confirmed by interventional studies. For example, a recent study reported that homocysteine reduction with B vitamins did not reduce the risk of recurrent cardiovascular disease after acute myocardial infarction,30 despite the fact that raised homocysteine levels had repeatedly been found to be associated with increased risk of cardiovascular disease in observational studies. In addition, although a role of MTHFR genotype was not supported in our study, gene–environment and gene–gene interactions require further evaluation.
Acknowledgements
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References