RESEARCH ARTICLE

Vitamin B12 and Homocysteine Levels Predict Different Outcomes in Early Parkinson's Disease

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ABSTRACT: **Background**: In moderately advanced Parkinson's disease (PD), low serum vitamin B12 levels are common and are associated with neuropathy and cognitive impairment. However, little is known about B12 in early PD.

Objective: To determine the prevalence of low vitamin B12 status in early PD and whether it is associated with clinical progression.

Methods: We measured vitamin B12 and other B12 status determinants (methylmalonic acid, homocysteine, and holotranscobalamin) in 680 baseline and 456 follow-up serum samples collected from DATATOP participants with early, untreated PD. Borderline low B12 status was defined as serum B12 <184 pmol/L (250 pg/mL), and elevated homocysteine was defined as >15 μmol/L. Outcomes included the UPDRS, ambulatory capacity score (sum of UPDRS items 13-15, 29&30), and MMSE, calculated as annualized rates of change.

Results: At baseline, 13% had borderline low B12 levels, 7% had elevated homocysteine, whereas 2% had both. Elevated homocysteine at baseline was associated with worse scores on the baseline MMSE. Analysis of study outcomes showed that compared with the other tertiles, participants in the low B12 tertile (<234 pmol/L; 317 pg/mL) developed greater morbidity as assessed by greater annualized worsening of the ambulatory capacity score. Elevated homocysteine was associated with greater annualized decline in MMSE (−1.96 vs. 0.06; $P = 0001$). Blood count indices were not associated with B12 or homocysteine status.

Conclusions: In this study of early PD, low B12 status was common. Low B12 at baseline predicted greater worsening of mobility whereas elevated homocysteine predicted greater cognitive decline. Given that low B12 and elevated homocysteine can improve with vitamin supplementation, future studies should test whether prevention or early correction of these nutritionally modifiable conditions slows development of disability.

Key Words: cyanocobalamin; gait instability; cognitive impairment; hyperhomocysteinemia

Progression of Parkinson's disease (PD) varies considerably among patients. The reasons for this variability are not well established, although later age of onset, initial symptoms of postural instability and gait disorder,[1] low serum urate,[2] and declining body mass index BMI[3] have been associated with more rapid decline. Identification of treatable conditions that contribute to PD morbidity might reduce the burden of this disease.

Although a large, population-based cohort study did not find an association between dietary B12 intake and development of PD,[4] vitamin B12 deficiency has many features that make it a candidate modifier of PD progression. First, a meta-analysis has shown that B12 is lower in those with PD than controls,[5] and low B12 is common, affecting 10% to 20% of people aged >60 years.[6,7] Moreover, B12 deficiency causes insidious,

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The list of DATATOP Investigators/coordinators can be found in reference 12 unless the author file will be part of the supplemental information.

Funding agencies: This study was supported by the Michael J. Fox Foundation for Parkinson's Research, Grant 8646, as well as unrestricted grants from the Ko and Tsu family and from William and Mary Ann S. Margaretten.

Relevant conflicts of interest/financial disclosures: Nothing to report.

Full financial disclosures and author roles may be found in the online version of this article.

Received: 29 September 2017; **Revised:** 12 December 2017; **Accepted:** 31 December 2017

Published online 00 Month 2018 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.27301

protean neurological features that are often expected as part of PD progression and therefore might prevent clinical recognition of the coexistent deficiency. These features include: cognitive and psychiatric disturbances (depression and paranoia), neuromuscular deficits (ataxia, neuropathy, and paresthesia), and autonomic dysfunction (anosmia, postural hypotension, incontinence, and impotence).[8,9]

A number of studies in treated PD patients have found correlations of low vitamin B12 levels with additional neurological features. For example, large fiber neuropathy is common in PD, affecting 16% in a systematic review, although estimates range from 6% to 58%.[10] In the majority of cases, B12 deficiency (or elevations of serum methylmalonic acid [MMA] or homocysteine [tHcy], consistent with B12 deficiency) was identified as the cause of the neuropathy.[10] Cognitive impairment is also common in PD and a frequent cause of disability later in the disease. One study found that B12 levels were lower in PD patients with cognitive impairment than those without cognitive impairment.[11]

The reports cited above show associations of low B12 levels with additional neurological features in small cross-sectional studies of moderately advanced PD patients. To determine whether low B12 status might be a target for disease-modifying therapy, we sought to determine the prevalence of low B12 status in early, untreated PD and whether it was associated with more rapid clinical decline using serum samples and the prospective, clinical outcomes collected as part of the DATATOP study.

Materials and Methods

Study Design

DATATOP was a double-blind, randomized trial designed to test whether treatment with deprenyl (selegiline hydrochloride) and/or the antioxidant, α-tocopherol, slowed PD progression. The 800 participants were enrolled between 1987 and 1988.[12] Eligible subjects had early PD and were excluded if they had begun PD medications or had severe tremor or dementia (Mini-Mental State Examination [MMSE] score of $\leq$22). Subjects were randomized to one of four treatment assignments: deprenyl (10 mg/d) and α-tocopherol-placebo; α-tocopherol (2,000 IU/D) and deprenyl-placebo; active deprenyl and active α-tocopherol; or double placebo. Eligible subjects were not allowed to take vitamin supplements whose contents exceeded amounts found in a "standard multivitamin" (MVI; B12 = 6 mcg, B6 = 2 mg, folate = 0.4mg, and D = 400 IU) within a month before enrollment. After the baseline visit, subjects were dispensed an optional standard MVI. Subjects were considered to have PD if the investigator maintained $\geq$60% confidence in the diagnosis at every visit.[13] These criteria excluded 57 subjects. Baseline serum specimens from 680 of the remaining 743 subjects were available for this study. Subjects provided written informed consent for DATATOP according to the regulations of their local institutional review board (IRB). The UCSF IRB deemed this study exempt.

Serum B12 Status Measurements

Because serum B12 alone has limited sensitivity to detect B12 deficiency,[14,15] we also measured serum MMA, tHcy, and holotranscobalamin (holoTC)—which measures the amount of B12 bound to transcobalamin.[8,16] We prespecified two thresholds for B12 status using the UC Davis clinical laboratory cutoff of $<$157 pmol/L (212 pg/mL) as deficient and $<$184 pmol/L (250 pg/mL) as "borderline low." For MMA, we prespecified $>$0.4 μmol/L as elevated using the UC Davis and ARUP guidelines.[16] For tHcy, we prespecified $>$15 pmol/L to indicate moderately elevated levels.[16] For holoTC, we defined $<$ 35 pmol/L as deficient.[16] We also measured B12 determinants in these same subjects who had serum samples available at study endpoint and continued in the study for 9 to 24 months in order to assess changes from baseline.

DATATOP specified a standard protocol for blood collection and processing of serum: Blood samples were allowed to clot for 30 minutes at room temperature, then immediately centrifuged, the serum removed, and aliquots frozen immediately and stored at −70°C. At UC Davis, 680 baseline and 456 follow-up DATATOP serum samples were thawed and separated for blinded analysis. B12 was measured using the Siemens Centaur Vitamin B12 Chemiluminescent Assay. HoloTC was measured using a kit assay (Axis Shield, Dundee, Scotland). Because tHcy levels may be spuriously elevated when measured in serum prepared from clotted blood kept at room temperature for prolonged periods, it is commonly performed on plasma. However, for serum centrifuged within 1 hour of collection, there is an approximate increase of only 1 μM/h, which is not dependent on the initial tHcy concentration.[17] tHcy was measured by high-performance liquid chromatography using postcolumn fluorescence detection.[18] MMA was determined on an Agilent liquid chromatography/mass spectrometry system (Agilent Technologies, Santa Clara, CA).

Clinical Outcomes

Following the baseline visit, subjects were evaluated every 3 months up to 24 months. At each visit, subjects were assessed for disability sufficient to require levodopa therapy (the primary endpoint) and for secondary outcomes including the total UPDRS, and its subscores including mental (Part 1), activities of daily living (ADL; Part 2), and motor (Part 3). The ambulatory capacity score was determined by adding individual items of the UPDRS falling (Item 13), freezing when walking (Item 14), walking (Item 15), gait (Item

29), and postural stability (Item 30). This score has been validated as a global measure of ambulatory function in PD.[19] Cognitive tests were also performed including the MMSE, Symbol Digit Modalities Test, Selective Reminding Test, and New Dot Test. Demographic characteristics included age, sex, BMI, time postdiagnosis of PD, Hamilton Depression Score, Schwab & England score, and Hoehn and Yahr score (H & Y). Mean corpuscular volume (MCV), hematocrit, and uric acid levels were also assessed.

The annualized change in PD related scores, cognitive scores, and BMI were determined based on the change from baseline to the primary endpoint or the final visit if the primary endpoint was not reached. Rate was calculated as the change in score from baseline divided by the number of days between the two assessments multiplied by 365.

Statistical Analysis

Baseline B12, MMA, tHcy, and holoTC levels for the 680 PD subjects were summarized descriptively with frequency distributions and geometric means and 95% confidence intervals (95% CI). Means for baseline characteristics were reported by baseline B12 tertiles and statistically compared using Tukey's studentized range tests. Least square means for baseline B12, MMA, holoTC, and tHcy thresholds were compared using linear models that adjusted for sex, age, and treatment group. Analysis of adjusted mean annualized change in scores (including MMSE and ambulatory capacity) by baseline B12 tertiles and B12, MMA, holoTC, and tHcy thresholds included adjustments for baseline values. Mean annualized change in scores were also reported by follow-up B12 thresholds. Baseline H & Y scores are reported as medians with ranges. Multiple comparisons were accounted for by applying the Bonferroni correction in which P values < 0.004 were considered statistically significant.

Results

We measured serum B12, tHcy, holoTC, and MMA levels collected at baseline from 680 DATATOP subjects who maintained a diagnosis of PD throughout the study and for whom serum was available. Histograms show the baseline distributions (Fig. 1, upper

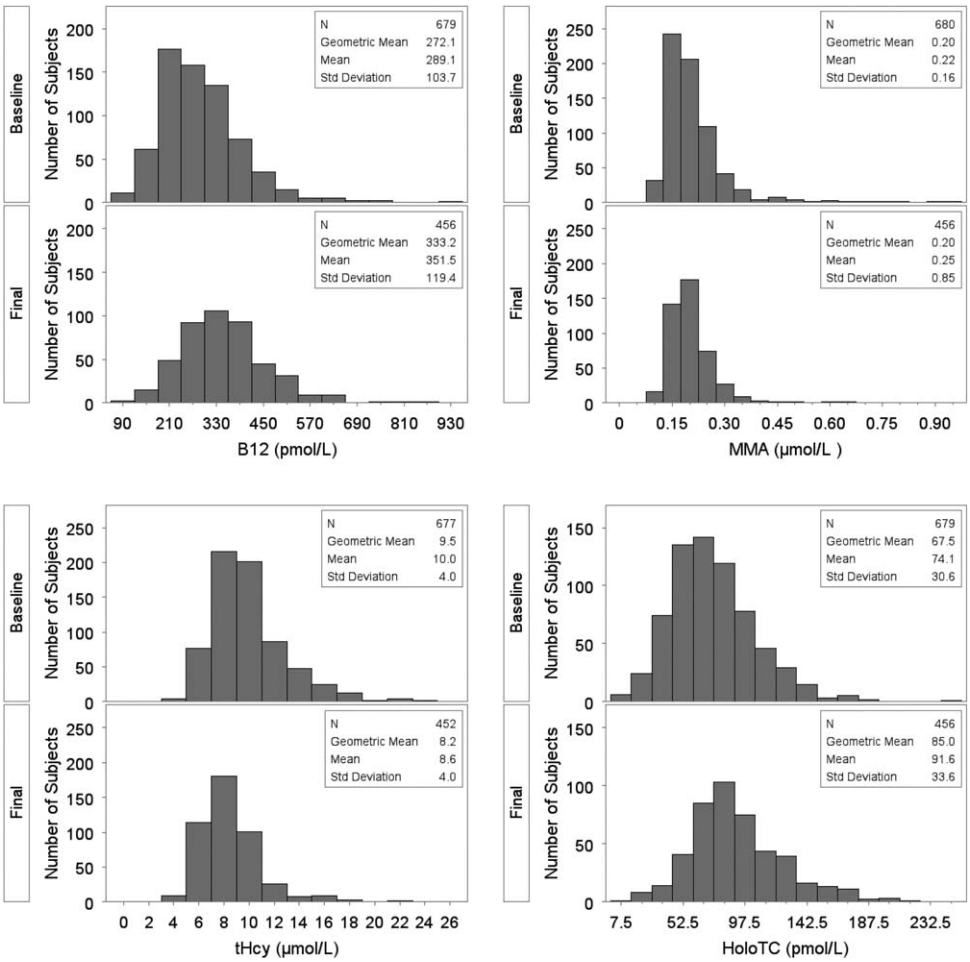

FIG. 1. Distributions of vitamin B12, MMA, tHCY, and HoloTC at baseline (upper panel) and final time points (lower panel).

TABLE 1. Mean baseline characteristics of participants according to tertiles of baseline B12 levels

	Baseline B12 Tertile			
	First (<234 pmol/L)	Second (234-321 pmol/L)	Third (>321 pmol/L)	Overall
No. of subjects	227	228	224	679
Study follow-up, months	13.9	14.9	14.9	14.6
Age, years	61.9	59.9	60.7	60.8
Time postdiagnosis, years	1.2	1.0	1.2	1.1
H & Y, median (range)	1.8 (1.0-2.5)	1.5 (1.0-2.5)	1.5 (1.0-4.0)	1.5 (1.0-4.0)
Schwab & England by rater	90.2	90.9	91.3	90.8
Female, %	32.6	30.3	38.8	33.9
Gastrointestinal comorbidity, %	22.0	21.9	27.2	23.7
Total UPDRS	24.7	25.2	25.5	25.1
UPDRS, Part 1 (Mental subscore)	1.0	1.1	1.1	1.1
UPDRS, Part 2 (ADL subscore)	7.0	7.8	7.4	7.4
UPDRS, Part 3 (Motor subscore)	16.8	16.3	17.1	16.7
Ambulatory capacity	1.4	1.6	1.5	1.5
MMSE	28.8	28.9	29.0	28.9
Symbol Digit Modalities Test	38.6	39.3	39.4	39.1
Selective Reminding Test total recall	43.5	45.2	44.5	44.4
New Dot Test	12.6	13.0	12.9	12.8
Hamilton Depression Rating Scale	2.6	2.8	2.4	2.6
MCV, fL	91.0	90.2	90.1	90.4
Hematocrit, %	0.43	0.43	0.43	0.43
Uric acid, mg/dL	302.4	317.4*	289.5*	303.0
BMI	26.2	26.3	25.9	26.1
Creatinine, μmol/L	95.1	96.2	97.7	96.3
tHcy, μmol/L	11.3*†	9.7*‡	8.9†‡	10.0
MMA, μmol/L	0.27*†	0.20*	0.18†	0.22
HoloTC, pmol/L	55.9*†	72.4*‡	93.3†‡	73.8

Results are mean values unless otherwise indicated. BMI was calculated as weight in kilograms divided by height in meters squared. Uric acid was no longer significantly different after correction for sex.
*†‡Matching symbols indicate statistically significant differences ($P < 0.05$) using Tukey's studentized range test based on one-way analysis of variance with $P < 0.004$.

panels). For B12, the geometric mean (95% CI) was 272 pmol/L (265, 279), for MMA 0.199 μmol/L (0.193, 0.205), tHcy 9.5 μmol/L (9.3, 9.7) and holoTC 68 pmol/L (65, 70).

Prevalence of Low B12 or Elevated tHcy Status

At baseline, 13% of subjects had borderline low B12 levels (<184 pmol/L or 250 pg/mL) and 5% had deficient B12 levels (<157 pmol/L or 212 pg/mL). tHcy was moderately elevated (>15 μmol/L) in 7% of subjects. Only 14% of those with borderline low B12 also had elevated tHcy.

B12 Status Determinants Over Time

In order to characterize B12 status over time, we performed a second B12 status measurement in those subjects who continued in the study for 9 to 24 months and had available serum samples from those later visits: mean (standard deviation) = 17.7 (3.3) months. Comparison of follow-up frequency histograms in lower panels of Figure 1 shows increases in B12 status with rightward shifts in the distributions of B12 and holoTC and a leftward shift of tHcy, whereas MMA remained relatively unchanged. Of the 456 subjects who underwent both measurements, 226 had an increase of >20% in B12 levels, 210 stayed within 20% of the original B12 measurement, whereas only 19 had a decrease >20%. There was a mean annualized increase in B12 of 52.6 pmol/L, a mean annualized decrease of tHcy of 0.83 μmol/L, and a mean annualized increase of holoTC of 14.7 pmol/L.

Associations of B12 Status With Baseline Features

Table 1 shows the baseline characteristics according to baseline serum B12 tertiles. Demographics and baseline clinical features, including age, UPDRS, MMSE, MCV, and Hct, were similar. Mean uric acid was slightly higher in the middle B12 tertile than in the high B12 tertile. After adjustment for sex, uric acid levels were not significantly different. Further analysis of baseline features was performed according to preselected cut-off values (Supporting Table 1). There was no association between baseline features and B12 threshold <157 pmol/L, MMA >0.4 μmol/L, or HoloTC level <35. However, compared to subjects with baseline tHcy ≤15 μmol/L, those with tHcy >15 μmol/L had lower MMSE scores (MMSE 28.26 vs. 28.9; $P = 0.003$).

TABLE 2. Adjusted mean annualized change in outcomes according to tertiles of baseline B12 levels

Least Squares Mean Annualized Change Outcome	Baseline B12 Tertile		
	First (<234 pmol/L)	Second (234.1-321 pmol/L)	Third (>321 pmol/L)
UPDRS, Total	14.03	11.50	11.03
UPDRS, Part 1 (Mental subscore)	0.75	0.43	0.43
UPDRS, Part 2 (ADL subscore)	4.37	3.48	3.48
UPDRS, Part 3 (Motor subscore)	8.64	7.58	7.04
Ambulatory capacity	1.53[a,b]	0.83	0.77
Falling	0.14	0.07	−0.01
Freezing when walking	0.16	0.12	0.09
Walking	0.47[a,b]	0.28	0.23
Gait	0.36	0.29	0.33
Postural stability	0.38	0.14	0.15
MMSE	−0.13	−0.22	0.14
Symbol Digit Modalities Test	−0.33	0.11	0.89
Selective Reminding Test total recall	0.65	1.56	2.38
New Dot Test	−0.12	−0.07	−0.08
Hamilton Depression Rating Scale	−0.18[b]	1.74	1.33
Schwab & England rater	−7.07	−8.58	−7.53
BMI	−0.08	−0.19	1.11

Models are adjusted for baseline value of the outcome, age, sex, and treatment group. BMI was calculated as weight in kilograms divided by height in meters squared).
[a]$P < 0.004$ compared to third tertile.
[b]$P < 0.004$ compared to combined second and third tertiles.

Associations of Baseline B12 Status With Study Outcomes

Analysis of outcomes according to baseline B12 tertiles showed greater annualized changes in ambulatory capacity scores in the low B12 tertile (Table 2). The greater deterioration in ambulatory capacity in the low B12 tertile, as reflected in higher scores, was attributed to greater increases in each of its UPDRS components, particularly in walking and postural instability items of the UPDRS. Trends for greater increases in total UPDRS for those in the lowest B12 tertile were observed, but were not statistically significant.

Table 3 displays adjusted annualized outcomes according to baseline B12 status determinant thresholds. Although there were greater mean annualized changes in total UPDRS scores for those with deficient B12 (<157 pmol/L), low holoTC, and elevated MMA (Table 3), these differences were not significant. However, elevated baseline tHcy did correlate with greater declines in MMSE; those with tHcy >15 μmol/L had an adjusted mean annualized decline in MMSE of 1.96 points compared to a slight increase of 0.06 points, for those with baseline tHcy ≤15 ($P = 0.001$).

Outcomes According to B12 Status at Baseline and Later Measurement

Because measurements of the second serum sample revealed improvement in B12 status for nearly half the subjects who participated >9 months, we also performed an exploratory analysis of outcomes according to classification by both the initial and follow-up B12 levels (Table 4). For those subjects with B12 <184 pmol/L at both time points, the annualized change in UPDRS was nearly twice those with B12 ≥184 pmol/L at baseline and follow-up (14.38 vs. 7.85), but after adjustment for multiple comparisons, this difference was not significant. Similar findings were observed using a more stringent B12 threshold of <157 pmol/L (Supporting Table 2).

Discussion

We explored the relationship of serum B12 status determinants with baseline features and outcomes in DATATOP, a study of early PD. At baseline, 13% of subjects had borderline low B12 levels (<184 pmol/L) and 7% had moderately elevated tHcy (>15 μmol/L). Baseline elevated tHcy was associated with lower baseline MMSE as well as greater declines in MMSE. Analysis according to baseline B12 tertile showed that those in the low tertile developed greater impairment of gait and stability according to the ambulatory capacity score. Those in the low B12 tertile had similar hematological parameters (MCV and hematocrit), which clinicians may rely upon to consider B12 deficiency and only then order serum B12 status indicator tests to confirm or reject this possibility.

For those participants who continued the study ≥9 months and had a second blood sample available, we found increases in serum B12, holoTC, and a decline in tHcy during the study. These findings are consistent with improved nutritional status during the course of the study, likely attributed to subjects starting the

TABLE 3. Adjusted mean annualized change in outcomes according to baseline B12, MMA, Holo TC, and tHCY levels

Least Squares Mean Annualized Change Outcome	Baseline B12 <157 pmol/L N = 33	Baseline B12 ≥157 pmol/L N = 646	P Value	Baseline MMA >0.4 μmol/L N = 28	Baseline MMA ≤0.4 μmol/L N = 652	P Value	Baseline HoloTC <35 pmol/L N = 44	Baseline HoloTC ≥35 pmol/L N = 636	P Value	Baseline tHcy >15 μmol/L N = 46	Baseline tHcy ≤15 μmol/L N = 631	P Value
Total UPDRS	13.07	12.16	0.76	15.60	12.05	0.28	15.88	11.95	0.14	11.26	12.30	0.71
UPDRS, Part 3 (Motor subscore)	8.31	7.73	0.78	10.31	7.65	0.24	9.80	7.62	0.24	7.41	7.81	0.84
UPDRS, Part 1 (Mental subscore)	0.63	0.53	0.80	0.35	0.54	0.65	0.77	0.52	0.47	0.21	0.56	0.31
UPDRS, Part 2 (ADL subscore)	3.88	3.77	0.92	4.65	3.74	0.42	5.15	3.69	0.12	3.78	3.79	0.99
Ambulatory capacity	1.13	1.05	0.85	0.87	1.06	0.70	1.29	1.04	0.54	1.17	1.05	0.77
MMSE	1.09	−0.13	0.08	−2.07	0.01	0.01	0.39	−0.10	0.43	−1.96	0.06	0.001*
Symbol Digit Modalities Test	1.94	0.13	0.30	0.97	0.18	0.66	0.72	0.18	0.73	0.44	0.17	0.86
Selective Reminding Test total recall	2.65	1.47	0.59	4.26	1.41	0.20	4.53	1.31	0.09	1.89	1.49	0.83
New Dot Test	−0.45	−0.07	0.32	−0.20	−0.08	0.77	0.02	−0.10	0.73	−0.55	−0.06	0.15
Hamilton Depression Rating Scale	−0.43	1.06	0.21	−0.53	1.04	0.24	0.32	1.04	0.51	0.73	1.01	0.80
Schwab & England rater	−7.64	−7.72	0.97	−9.46	−7.63	0.49	−8.91	−7.63	0.56	−8.56	−7.69	0.70
BMI	0.17	0.31	0.95	−0.27	0.32	0.81	0.03	0.32	0.89	0.02	0.32	0.89

Models are adjusted for baseline value of the outcome, sex, baseline age, and treatment group. BMI was calculated as weight in kilograms divided by height in meters squared.
*$P < 0.004$.

optional MVI after the baseline visit and/or subjects changing their diets. The improvement in B12 status in nearly 50% of subjects provided an opportunity to explore whether such improvements for those below thresholds were associated with less severe progression according to the UPDRS scores (Table 4 and Supporting Table 2). Although the differences were not significant, the trends for smaller annualized UPDRS changes in those whose B12 status improved provide support for a disease-modifying effect of B12. An improvement in vitamin D status has also been reported in the DATATOP study.[20]

Given that motor and cognitive features attributed to B12 deficiency might stabilize or improve after supplementation and nearly half of the subjects had a >20% increase in B12 level, it is possible that our main findings (Table 2) underestimate the effect of low B12 status in early PD. The clinical benefits attributed to improvement in B12 nutritional status are most likely to occur in individuals with the lowest B12 levels. We suspect that B12 status improvement during the study may explain why outcomes according to thresholds (Table 3) did not reach statistical significance whereas it was observed in the tertile analysis (Table 2). Some support for this interpretation is provided by the exploratory analysis, which segregated subjects according to B12 levels at both study entry and termination (Table 4).

We observed a greater deterioration in ambulatory capacity in the low B12 tertile (Table 2) attributed predominantly to worsening of gait and postural instability. Those in the low B12 tertile had annualized change of 1.53, compared to 0.77 in the upper tertile (difference of 0.76). To provide some context for this magnitude of difference in ambulatory capacity scores, an analysis of the NET-PD LS1 cohort, assessing

TABLE 4. Mean annualized change outcomes by baseline and final B12 deficiency status for subjects with baseline and final serum samples

Mean annualized change	B12 <184 pmol/L Baseline and Follow-up N = 12	B12 <184 pmol/L Baseline, not Follow-up N = 40	B12 <184 pmol/L Follow-up, not Baseline N = 7	B12 <184 pmol/L Neither Baseline Nor Follow-up N = 396
UPDRS Total	14.38	10.11	6.02	7.85
UPDRS, Part 3 (Motor subscore)	9.85	6.92	3.45	5.31
UPDRS, Part 1 (Mental subscore)	−0.04	0.26	0.33	0.23
UPDRS, Part 2 (ADL subscore)	4.57	2.93	2.23	2.28
Ambulatory capacity	0.86	0.76	0.71	0.53
MMSE	−0.05	0.44	0.11	−0.13
Symbol Digit Modalities Test	2.49	−0.14	−0.74	0.44
Selective Reminding Test total recall	1.52	−0.10	−1.31	−0.00
New Dot test	0.15	−0.20	0.12	−0.16
Hamilton depression	0.61	−0.37	0.75	0.56
Schwab & England rater	−7.25	−5.32	−5.63	−4.52

whether baseline features predicted falls, found that mean ambulatory capacity in subjects who fell was 2.17 and was 1.4 for those that did not fall (difference 0.77).[21] Thus, we consider the magnitude of difference to be clinically relevant, particularly given that components of gait dysfunction that develop in PD may not respond to dopaminergic treatments[22] or DBS.[23] Moreover, increased postural instability correlates with greater morbidity[24] and mortality[25] in PD.

Our findings of relationships of low B12 levels with worse study outcomes could be attributed to (1) an independent (comorbid) effect on the central and peripheral nervous systems, (2) a direct effect on PD pathogenesis, and (3) or alternatively, low B12 may be a marker of an unknown associated factor, perhaps correlating with some other aspect of PD or nutritional status. Analysis of annualized outcomes by sextiles (not shown) showed greater increases in the ambulatory capacity in both the first and second sextiles. Because the second sextile range 197 to 234 pmol/L (267-317 pg/mL) is well above the traditional B12 cutoff of <157 pmol/L (212 pg/mL) [16] as well the borderline low threshold used in this study of 184 pmol/L (250 pg/mL), greater motor progression also occurred in participants with "normal" baseline B12 levels. It is appropriate to acknowledge that the established vitamin B12 status determinants cutoffs were determined empirically (not prospectively).

Our analysis provides a novel window into an association of low B12 status with the development of greater morbidity over time. To our knowledge, no similar large study of B12 status and gait impairment or ambulatory capacity has previously been reported in PD or aging cohorts. Our finding of greater morbidity in a tertile range that overlaps with the low-normal range of B12 also suggests that borderline low levels pose a risk for imminent deterioration. An analogous finding has been reported in a cross-sectional study of mild cognitive impairment; those with B12 levels in the low end of the normal range had reduced memory performance compared to subjects with B12 levels in the normal range.[26]

B12 must be obtained from dietary sources, and its absorption depends on a series of steps.[7] Interestingly, a large, prospective, population-based study found no association of dietary intake of B12 with the development of PD.[4] However, PD patients may be more susceptible to low B12 status, not because of differences in dietary intake, but because of a difference in gastrointestinal absorption, attributed to a higher rate of intestinal bacterial overgrowth, which occurs in 25% to 54% of PD patients.[8,27,28]

There are a number of mechanisms by which low vitamin B12 status might affect the nervous system. It is a cofactor for two enzymes: methionine synthase and methylmalonyl-coenzyme A mutase.[9] Impairment of either or both enzymes could explain abnormalities of myelination of the peripheral and central nervous system that occurs in B12 deficiency.[8] Numerous studies have shown associations of B12 deficiency with white matter lesions in the brain and the posterior columns of the spinal cord.[29,30] Moreover, a number of MRI studies describe a greater burden of white matter hyperintensity (WMH) in patients with the postural instability gait disturbance PD phenotype compared to the tremor dominant form. Whereas the cause of WMH is often considered microvascular in origin,[31] a recent study using diffusion tensor imaging describes evidence for demyelination as well.[32] Together with our findings, these imaging findings raise the possibility that low B12 status causes deterioration of motor capacity through demyelination.

We measured tHcy as a surrogate marker of B12 deficiency. Although we found the expected inverse correlation with B12 status, elevated tHcy correlated with lower MMSE scores at baseline and predicted greater declines in MMSE, independent of B12 levels. tHcy levels are determined by a number of factors, some of which are modifiable, such as nutritional deficiencies of B12, folate, and B6. Elevated tHcy may have a number of adverse effects, including: functional modifications of proteins; oxidative stress; deposition of pathological aggregates; mitochondrial dysfunction; as well as toxic effects on dopaminergic neurons.[33] This independent association of tHcy with cognitive outcomes is consistent with other large studies in aging.[34,35] Moreover, it is consistent with findings reported in a small, cross-sectional study of treated PD patients.[36]

Our finding of distinct outcomes (worse baseline and outcome MMSE scores with elevated tHcy vs. greater increase in ambulatory capacity score in the low B12 tertile) is consistent with the notion that elevated tHcy is a risk factor for cognitive progression in PD, and suggests that elevated tHcy exerts its effect by way of a biological mechanism distinct from B12. Given that both serum[36,37] and CSF[38] tHcy levels rise after starting L-dopa treatment, elevated tHcy may become increasingly relevant to cognitive decline as PD progresses. The DATATOP study was performed before folic acid fortification was mandated in the United States in 1998. Because folic acid supplementation reduces tHcy, the rate of moderately elevated tHcy may be lower in contemporary early PD cohorts.

This study has a number of strengths and some weaknesses. Its strengths include the prospective collection of clinical data and that B12 status measurements were obtained in a blinded manner. Its weaknesses include its post-hoc design and the long storage time between collection and analysis. However, because the DATATOP study had strict procedures in place for blood collection and storage and because studies of stored blood specimens have shown stability for B12,[39] MMA,[39] and tHcy,[39,40] over

decades, these blood measurements are likely reliable. Small sample size in this study for those with borderline low B12 levels (<184 pmol/L) at both time points may have contributed to the lack of statistical significance when compared to those with ≥184 pmol/L at both time points, and therefore further verification of the trends that we observed should be confirmed in future studies.

In PD, little is known about what influences the rate of progression. Our results extend observations of associations of these analytes with greater morbidity in moderately advanced PD[11,36,41,42] to early untreated PD. Moreover, this is the first study in PD to show that low B12 levels predict greater motor morbidity and elevated tHcy predicts greater cognitive loss. Because low B12 status is easily reversed with oral supplementation and elevated tHcy can be reduced with B12, B6, and folate supplementation,[43] these findings raise the possibility that prevention or early correction of low B12 and elevated tHcy may slow the development of disability in PD.

These results should be confirmed, perhaps in a completed contemporary prospective study with stored biospecimens. Given that low B12 status is associated with neurological and other medical morbidities and is readily treated, great care would be needed to design an ethically acceptable randomized, prospective study to evaluate the effect of B12 supplementation on PD progression, given that serum measurements collected as part of a prospective study in unsupplemented patients would likely reveal some subjects with B12 deficiency. ■

Acknowledgements: Stephen Catalya performed some of the tHcy assays. Samples from the DATATOP study conducted by the Parkinson Study Group and provided by the DNA and Cell Laboratory at Indiana University with support from the Michael J. Fox Foundation were used in this study. We thank contributors, including all members of the Parkinson's Study Group who collected samples used in this study, as well as patients and their families whose help and participation made this work possible.

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Supporting Data

Additional Supporting Information may be found in the online version of this article.