Mild Parkinsonian Signs and Plasma Homocysteine Concentration in Community-Dwelling Elderly Individuals

Elan D. Louis, MD, MSc; Nicole Schupf, PhD, DrPH; Ming X. Tang, PhD; Karen Marder, MD, MPH; Jose A. Luchsinger, MD, MPH

Objective: To determine whether plasma homocysteine (Hcy) concentration is associated with mild parkinsonian signs (MPS) in community-dwelling elderly individuals.

Design: Cross-sectional analyses of a population-based cohort study.


Patients: Persons without dementia 65 years and older.

Main Outcome Measure: Participants underwent an abbreviated motor portion of the Unified Parkinson’s Disease Rating Scale. Each participant was assigned an MPS score (range, 0-40). The Hcy concentration was measured from plasma. All analyses were cross-sectional.

Results: There were 369 participants (mean±SD age, 77.8±6.0 years; mean±SD MPS score, 1.51±2.55; mean±SD plasma Hcy concentration, 17.3±6.5 µmol/L). Mean±SD MPS scores in plasma Hcy concentration quintiles were as follows: lowest quintile, 1.15±1.77; second quintile, 1.18±1.88; third quintile, 1.64±2.93; fourth quintile, 1.45±2.17; and highest quintile, 2.12±3.49 (84.3% higher than 1.15) (P=.02). In an unadjusted linear regression model, plasma Hcy concentration was associated with log MPS score (dependent variable) (P=.008). In a linear regression model that adjusted for confounding variables, plasma Hcy concentration was associated with log MPS score (P=.04).

Conclusions: These data indicate that MPS are associated with higher plasma Hcy concentrations. Prospective neuroimaging as well as clinical-pathological studies would further our understanding of several mechanisms that could underlie the observed association.

Arch Neurol. 2007;64(11):1646-1651

MILD PARKINSONIAN signs (MPS), which include impaired gait and balance, rigidity, bradykinesia, and rest tremor, are commonly found during the clinical examination of older people who do not have any diagnosed neurological disease.1-7 These signs, which are highly prevalent, are associated with functional disability8,9 and increased risks for dementia5,7 and mortality.2,10 The processes that underlie MPS are not clear but could include an age-associated decline in nigrostriatal dopaminergic activity, the development of degenerative (eg, Lewy body or Alzheimer type) pathological changes, or the accumulation of vascular pathological changes in the cerebral subcortical white matter or other brain regions. Elevated homocysteine (Hcy) concentration is a cerebrovascular risk factor but it has also been associated with treated and untreated Parkinson disease (PD).11,12 With one possible exception,12 to our knowledge plasma Hcy concentrations have not been assessed in elderly individuals with MPS nor have we previously assessed the association between these concentrations and MPS in our cohorts in northern Manhattan, New York. A recent cross-sectional study in 3 French cities demonstrated an association between elevated plasma Hcy concentration and slower walking speed in elderly individuals.12 In that study,12 however, investigators focused on gait speed rather than MPS. We studied a sample of elderly persons without dementia living in Washington Heights–Inwood, New York, to determine specifically whether plasma Hcy concentration is associated with MPS.

METHODS

STUDY SAMPLE

Participants in the Washington Heights–Inwood Columbia Aging Project cohort were drawn by random stratified sampling of healthy Medicare beneficiaries 65 years or older residing within a geographically defined area of northern Manhattan. The sampling procedures have...
been described elsewhere. The participants were recruited between 1992 and 1994, and 2126 were enrolled. Their mean ± SD age was 77.2 ± 7.2 years, mean ± SD education was 8.2 ± 4.9 years, 69.4% were women, and 20.7% were non-Hispanic white. Each participant underwent a structured interview of health and function at the time of study entry and, over the ensuing year, was referred for the same standardized neurological examination, which included an abbreviated, 10-item version of the motor portion of the Unified Parkinson’s Disease Rating Scale (UPDRS). We excluded data from 676 individuals who screened negative for dementia, PD, or stroke or who had died or were lost to follow-up and therefore did not receive the standardized neurological examination. Second, data on an additional 44 participants were excluded because of incomplete neurological examinations. Third, we excluded data from 53 participants who were taking a neuroleptic medication because parkinsonian signs can result from the use of these medications. Fourth, we assigned a diagnosis of PD or Parkinson plus syndrome based on research criteria and participants were considered to have PD or Parkinson plus syndrome if (1) they had previously received a diagnosis of PD or Parkinson plus syndrome or (2) they had on the standardized neurological examination 2 or more cardinal signs of parkinsonism. Cardinal signs were bradykinesia, rigidity, postural changes, and rest tremor. A cardinal sign was considered to be present when 1 UPDRS rating was 2, 3, or 4. Fourteen (1.0%) of the remaining 1353 participants had a diagnosis of PD or a Parkinson plus syndrome, which is consistent with a prevalence of 0.7% to 1.2% for PD, which has been reported for persons 65 years and older in northern Manhattan. These 14 participants were excluded because our intention was to study a community population of older people without these diseases. Fifth, all participants also underwent a standardized neuropsychological battery. Using these test results as well as clinical information, a consensus diagnosis of dementia was assigned if participants met established criteria and if functional difficulties could be attributed to cognitive rather than physical disability. A Clinical Dementia Rating (CDR) score was assigned as well. We excluded 227 participants with dementia. After the exclusion of 1014 participants, the remaining sample comprised 1112 potential participants.

A sample of 909 participants from the original cohort of 2126 had been selected randomly at the time of their baseline assessment to conduct a study of Hcy concentration in elderly individuals. Of the 1112 remaining potential participants, 369 (33.2%) had been selected for the plasma Hcy concentration study. Therefore, our final sample comprised these 369 participants. The study was approved by our institution’s internal review board and written consent was obtained from all participants.

EVALUATION

As noted earlier, a standardized neurological examination was conducted by 1 of 3 neurologists, including an abbreviated, 10-item version of the motor portion of the UPDRS. The 10 items included speech, facial expression, tremor at rest (1 item), rigidity (rated separately in the neck, right arm, left arm, right leg, and left leg), posture, and body (axial) bradykinesia. Each of the 10 items was rated from 0 to 4. A rating of 1 indicated a mild abnormality and a rating of 2 or more indicated an abnormality of moderate or greater severity. Reliability of assessment of parkinsonian signs by 3 neurologists has been reported and was acceptable (intraclass correlation coefficient for total motor score = 0.82). An MPS score (range, 0-40) was calculated for each participant.

Demographic data were collected, including age and sex. Ethnic group was based on self-report using the format of the 1990 US census. Individuals were asked whether they were of Hispanic origin. Participants were then assigned to 1 of the following ethnic groups: non-Hispanic black, Hispanic, non-Hispanic white, and other. Number of years of education was obtained by self-report. Presence of a history of diabetes mellitus, hypertension, stroke, heart disease (myocardial infarction or congestive heart failure), or arthritis was assessed by self-report, as was use of nonsteroidal anti-inflammatory drugs (ever vs never). Participants were asked whether they smoked cigarettes (coded as ever [including current or past smokers] vs never) or regularly used ethanol (ever vs never).

PLASMA Hcy AND OTHER PLASMA AND SERUM CONCENTRATIONS

Fasting blood was drawn at baseline in EDTA tubes. Within 2 hours of collection, it was centrifuged, separated into plasma aliquots, and stored at −70°C. The Hcy concentration was measured from stored plasma using high-performance liquid chromatography with fluorescence detection. Plasma concentrations of folate and vitamin B12 were determined by radioassay (Simul outrac; ICN Pharmaceuticals, Costa Mesa, California). Serum creatinine concentration was measured by spectrophotometric assay (Sigma, St Louis, Missouri).

STATISTICAL ANALYSES

These cross-sectional statistical analyses were performed in SPSS (version 13.0; SPSS Inc, Chicago, Illinois). The MPS score had a value of 0 in 204 of 369 participants, which could violate one of the assumptions that justify the use of linear regression modeling. Therefore, for our linear regression models, we added the value 1 to the value in all 369 participants and then log transformed (log,10 ) this modified MPS score.

In a linear regression model, we examined the association between MPS score categories (0, 1, 2, 3, 4, ≥ 5) and plasma Hcy concentration. In an additional analysis, plasma Hcy concentration was divided into quintiles (≤ 12.115 µmol/L, > 12.115 to ≤ 14.080 µmol/L, > 14.080 to ≤ 16.555 µmol/L, > 16.555 to ≤ 20.111 µmol/L, and > 20.111 µmol/L [to convert to milligrams per liter, divide by 7.1371]); and in a linear regression model, we examined the association between plasma Hcy concentration quintile and log MPS score (dependent variable).

An unadjusted linear regression analysis was performed with log MPS score as the dependent variable and plasma Hcy concentration as the independent variable. We then considered possible covariates that were confounders or mediators; in these analyses, because MPS score was not normally distributed, Spearman correlation coefficients (r) were used to assess associations between the MPS score and continuous covariates (eg, age) and Mann-Whitney U tests or Kruskal-Wallis tests were used to assess associations between the MPS score and categorical covariates (eg, sex). Covariates that we considered were age in years; sex; ethnic group (non-Hispanic black; Hispanic, non-Hispanic white, and other); years of education; regular ethanol use (ever vs never); cigarette use (ever vs never); history of stroke, arthritis, diabetes, hypertension, or heart disease; nonsteroidal anti-inflammatory drug use (ever vs never); CDR score (0 or 0.5); plasma folate and vitamin B12 concentrations; and serum creatinine concentration. These analyses identified 4 variables that were associated with MPS score: age, diabetes mellitus, heart disease, and CDR score (Table). Each of these 4 variables was then placed in a linear regression model along with plasma Hcy concentration (log MPS score = dependent variable), and in these 4 models (each with 2 independent variables: plasma Hcy concentration and 1 of the 4 confounding variables), the association between plasma Hcy concentration...
and log MPS score remained robust, indicating that these confounding variables were not likely to be mediators. We then performed linear regression analyses (log MPS score = dependent variable) that adjusted simultaneously for age, diabetes mellitus, heart disease, and CDR score. To further test whether vascular disorders (diabetes mellitus, heart disease, hypertension, and stroke) were mediators, in 4 linear regression analyses, we excluded participants with these vascular disorders and examined the association between plasma Hcy concentration and log MPS score. In some analyses, we also stratified by age into 4 strata.

RESULTS

There were 369 participants. Their mean±SD age was 77.8±6.0 years, mean±SD education was 7.9±4.7 years, and 68 (18.4%) were non-Hispanic white, which was similar to the original cohort of 2126 from which they were derived. Two-hundred seventy-five (74.5%) were women, which is 5.1% higher than the original cohort (69.2%); because of the large size of the comparison groups, this modest difference reached marginal significance (P=.046). None had PD or dementia and none was receiving levodopa therapy. The mean±SD MPS score was 1.51±2.55 (range, 0-18). The MPS score was 0 in 204 participants (55.3%), 1 in 39 (10.6%), 2 in 45 (12.2%), 3 in 27 (7.3%), 4 in 18 (4.9%), and 5 or higher in the remaining 36 participants (9.8%). The mean±SD plasma Hcy concentration was 17.3±6.5 µmol/L (range, 8.3-54.1 µmol/L). The median latency between the 10-item version of the motor portion of the UPDRS and phlebotomy was 41 days (interquartile range, 18-71 days), indicating that the 2 events were separated by only a modest interval.

Log MPS score was associated with age and was higher in participants with diabetes mellitus, heart disease, or a CDR score of 0.5 (Table). Plasma Hcy concentration was higher in men and ethanol users; it was also associated with plasma concentrations of folate and vitamin B₁₂ and serum creatinine concentration (Table).

Higher MPS score was associated with higher plasma Hcy concentration (Figure 1). For example, for an MPS score of 0, the mean±SD plasma Hcy concentration was 16.2±6.7 µmol/L; MPS score of 1, 17.3±7.7 µmol/L; MPS score of 2, 18.5±8.4 µmol/L; MPS score of 3, 17.0±8.3 µmol/L; MPS score of 4, 15.1±3.3 µmol/L; and MPS score of 5 or higher, 20.2±1.10.0 µmol/L (linear regression analysis, β=0.56; P=.02). Plasma Hcy concentration was stratified into quintiles (Figure 2); the mean±SD MPS score in each quintile was as follows: lowest plasma Hcy concentration quintile, 1.15±1.77; second quintile,
Hcy concentration was associated with log MPS score (de-
crease 74–77 years; 0.59±1.73 vs 1.69±3.35 (175.3% dif-
ference) (age 74–77 years); 0.80±1.24 vs 2.28±3.68 (184.7% dif-
ference) (age 78–82 years); and 2.33±2.10 vs 3.00±4.00 (28.6% dif-
ference) (>82 years). Lowest quintiles are depicted by dashed bars and
highest quintiles, by solid bars.

Figure 3. Within each of the 4 age strata (<74, 74–77, 78–82, and >82
years), mean±SD mild parkinsonian signs (MPS) scores in the lowest vs
highest plasma homocysteine concentration quintile were: 1.25±1.80 vs
1.47±2.70 (17.3% difference) (<74 years), 0.59±1.73 vs 1.69±3.35 (175.3% difference) (age 74–77 years), 0.80±1.24 vs 2.28±3.68 (184.7% dif-
ference) (age 78–82 years), and 2.33±2.10 vs 3.00±4.00 (28.6% dif-
ference) (>82 years). Lowest quintiles are depicted by dashed bars and
highest quintiles, by solid bars.

COMMENT

We studied a group of community-dwelling elderly per-
sons without dementia in northern Manhattan. None had
PD or a Parkinson plus syndrome and none were using
levodopa. Our data show that elevated plasma Hcy con-
centration was associated with MPS.

The mean difference in MPS score between the high-
est vs lowest plasma Hcy concentration quintile was 0.97
points (2.12 vs 1.15), which was an 84.3% increase. This
difference is greater than the difference we have re-
ported in the MPS scores of smokers vs nonsmokers (a
77.8% difference).1 We have previously shown that an
increase in MPS score from 1 to 2 is associated with a
reduction in several functional measures (15% reduc-
tion in Active Life Expectancy Score and 10% increase
in the timed chair stand test score), indicating that this
small absolute difference in MPS score can have func-
tional consequences.8

The mechanism that underlies the observed associa-
tion is not clear but there are several possibilities. First,
elevated plasma Hcy concentration could result in MPS.
This could either be a direct cause or through an inter-
mediate step (eg, vascular disease [strokes] or some other
unknown factor). Arguing against strokes are our data,
which suggest that vascular factors are not mediating
this association. Second, MPS could result in elevated plasma
Hcy concentrations. However, this scenario does not seem
Elevated plasma Hcy concentration has also been recognized as an independent risk factor for cerebrovascular disease. \( \frac{12}{12} \) In one study, elevated plasma Hcy concentration was associated with a global measure of physical performance. That measure included a variety of tests of balance, gait, lower body strength and coordination, and manual dexterity, although not MPS per se. \( \frac{32}{32} \) While slow walking speed should not necessarily be equated with parkinsonism, patients with parkinsonism often manifest slow walking speed. In another study, \( \frac{12}{12} \) Hcy concentration was associated with a global measure of physical performance. That measure included a variety of tests of balance, gait, lower body strength and coordination, and manual dexterity, although not MPS per se.

Our findings raise the question as to whether MPS may be associated with one another and, if so, with what other variables. In one study, \( \frac{12}{12} \) Plasma Hcy concentration can be lowered with vitamin B\(_6\), vitamin B\(_12\), and folate supplementation, but results of a trial of these vitamin supplements to lower Hcy concentrations in the secondary prevention of stroke were negative. \( \frac{33}{33} \) As discussed earlier, one of the potential mechanisms linking plasma Hcy concentration to MPS is cerebrovascular disease. Mean plasma Hcy concentration was 1.5 µmol/L higher in participants with a history of stroke vs those without such a history, but the difference was not significant \( (P = .20) \). We believe that the lack of statistical significance was due to the relatively small sample size and the use of clinical history rather than brain imaging data to ascertain cerebrovascular disease. This likely resulted in an underestimation of the true prevalence of cerebrovascular disease, thereby biasing our results toward the finding of a nonsignificant difference in plasma Hcy concentration between participants with vs without stroke. Ideally, our study should be replicated in a sample with brain imaging data to assess subclinical cerebrovascular disease.

This study had a number of limitations. First, our analyses were cross-sectional. This does not lessen the validity of the association we observed but places limitations on our ability to study its underlying mechanisms. More specifically, longitudinal analyses would have allowed us to assess whether higher plasma Hcy concentration is a risk factor for subsequent development of MPS, or conversely, whether MPS are a risk factor for subsequent development of elevated plasma Hcy concentration. Biologically, the former rather than latter explanation is more plausible but this cannot be directly addressed by the current analyses. Brain imaging studies (eg, magnetic resonance imaging) or postmortem studies would also allow one to further examine the possible biological bases (vascular vs Lewy body) for the association we observed. However, we do not have these types of data. Second, only about one-third of our sample of 1112 potential participants had plasma Hcy concentration measurements, raising the issue of selection bias. Arguing most strongly against this is that the participants who underwent phlebotomy for Hcy concentration had been selected randomly. Furthermore, the age and educational characteristics of our final sample of 369 participants were similar to those of the initial sample of 2126. A small percentage difference in the proportion of women reached statistical significance in these large sample size comparisons. Sex, however, was not associated with our outcome variable, MPS. Women did have lower plasma Hcy concentrations (and less variance in these concentrations) than men. Hence, in a sample that was slightly enriched in women, as ours was, this lower variance in one of the primary variables (plasma Hcy concentration) may have made it more difficult for us to detect the association between Hcy concentrations and MPS. Third, our blood samples were centrifuged, aliquoted, and frozen within 2 hours rather than 30 minutes of collection. This 2-hour processing time could have resulted in elevated Hcy concentrations. However, we have no reason to suspect that processing time was differential across MPS strata. Finally, prior work has indicated that MPS comprise several discrete factors (eg, tremor, rigidity, bradykinesia). \( \frac{9}{9} \) While it would have been interesting to have examined the association between MPS and each of these factors, our study was not sufficiently powered for these analyses. Despite these limitations, this study is one of
few that have examined the associations between plasma Hcy concentrations and motor signs in elderly individuals. Its strengths include the population-based design, the careful assessment of MPS in several hundred elderly persons, the exclusion of participants with dementia and parkinsonism, and the adjustment for a variety of potential confounding variables.

In summary, we provide evidence of an association between MPS and elevated plasma Hcy concentrations. Prospective neuroimaging as well as clinical-pathological studies are now needed to further our understanding of mechanisms that may underlie this association.

Accepted for Publication: March 30, 2007.

Correspondence: Elan D. Louis, MD, MSc, Neurological Institute, 710 West 168th St, Unit 198, New York, NY, 10032 (EDL2@columbia.edu).

Author Contributions: Dr Louis had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Louis, Schupf, Marder, and Luchsinger. Analysis and interpretation of data: Louis, Schupf, Tang, Marder, and Luchsinger. Drafting of the manuscript: Louis and Luchsinger. Critical revision of the manuscript for important intellectual content: Louis, Schupf, Tang, Marder, and Luchsinger. Statistical analysis: Louis, Schupf, Tang, Marder, and Luchsinger. Study supervision: Schupf.

Financial Disclosure: None reported.

Funding/Support: This work was supported by National Institutes of Health grants P01 AG07232, R01 NS42859, and RR00645 (General Clinical Research Center).

Additional Contributions: Ralph Greene, MD, and Joshua Miller, PhD, conducted the homocysteine measurements.

REFERENCES