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Bowel Movement Frequency in Late-Life and Substantia Nigra Neuron Density at Death

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Abstract

Constipation is associated with future risk of Parkinson's disease (PD) and with incidental Lewy bodies (LB) in the locus ceruleus or substantia nigra (SN). Our purpose is to examine the independent association between bowel movement frequency in late-life and post mortem SN neuron density. Bowel movement frequency was assessed in the Honolulu-Asia Aging Study from 1991 to 1993 in 414 men aged 71 to 93 years with later postmortem evaluations. Brains were examined for LB in the SN and locus ceruleus and neurons were counted in four quadrants from a transverse section of SN. In non-smokers, neuron densities (counts/mm²) for men with >1, 1, and <1 bowel movement daily were 18.5, 18.8, 10.1 ($p<0.001$) for dorsomedial; 15.3, 16.4, 10.2 ($p<0.03$) for ventromedial; and 18.6, 18.3, 10.9 ($p=0.011$) for ventrolateral quadrants. Relationships were not significant in the dorsolateral quadrant or in any quadrant among smokers. After adjustment for age, time to death, coffee drinking, tricep skinfold thickness, excessive daytime sleepiness, cognitive function, PD, and incidental LB, density ratios in nonsmokers with 1 or more bowel movement(s) daily were significantly higher compared to those with <1 daily. Constipation is associated with low SN neuron density independent of the presence of LB.

Keywords

Parkinson's disease; constipation; Lewy body; substantia nigra; neuron density

Introduction

Constipation is frequent in patients with Parkinson's disease (PD),^{1–18} and may precede the extrapyramidal symptoms of clinical PD by many years.^{1,2,11,12} A previous report from the Honolulu-Asia Aging Study (HAAS) showed that infrequent bowel movements assessed in mid-life were associated with an elevated risk of incident PD during 24 years of follow-up.¹ Constipation was also associated with the presence of incidental Lewy bodies (LB) in decedents who did not have PD during life.¹⁹ Assuming that incidental LB represent a pre-motor stage of PD, this is further evidence that constipation precedes the classic PD motor signs.

Prior to onset of clinical PD, LB develop in pigmented nuclei of the brainstem with loss of dopaminergic neurons in the substantia nigra (SN). Nigral neuron counts are diminished by 50% or more before parkinsonian symptoms and motor signs appear.^{20,21} We have previously reported that parkinsonian signs in elderly without diagnosed PD during life are associated with lower neuron density in the SN. This may explain the presence of subtle parkinsonian signs in otherwise normal elderly.

In this report, we examine the relationship of bowel movement frequency in late life with nigral neuron density at death. To determine whether the relationship is independent of the LB process, further analyses are provided that include adjustments for clinical PD and incidental LB. We also seek to expand this idea further by investigating whether constipation is correlated with parkinsonian signs and low neuron density in deceased individuals without LB in the SN or locus ceruleus.

Methods

Study population

The Honolulu Heart Program was initiated from 1965–1968 when 8,006 men of Japanese ancestry, born 1900 to 1919, and living on the island of Oahu, Hawaii were enrolled in a prospective study of cardiovascular disease.^{22–24} For this report, follow-up for incident PD began at a repeat examination that occurred from 1971 to 1974. The HAAS was launched from 1991–1993 as a continuation of the Honolulu Heart Program with a focus on neurodegenerative diseases and cognitive function.¹ Informed consent was obtained from the study participants and the study was approved by the Kuakini Medical Center Institutional Review Board.

Frequency of Bowel Movements and Confounding Information

At the time when follow-up began (1991–1993), study participants were asked about their usual daily bowel movement frequency and categorized as having <1, 1, and >1 bowel movement/day. Demented participants were excluded at baseline because of possible inaccurate self-reporting. Additional confounding information was selected based on possible or putative associations with constipation or PD. The information included age, time from assessment of bowel movement frequency to death, mid-life pack-years of smoking,²⁵ mid-life coffee intake,²⁶ triceps skinfold thickness,²⁷ excessive daytime sleepiness,²⁸ and cognitive function. Mid-life pack-years of cigarette smoking and coffee intake were measured at initiation of the Honolulu Heart Program (1965–1968) and

considered to be markers of typical lifetime exposures to these factors. Coffee intake was not determined at the time of the questionnaire on bowel movement frequency (1991–1993) and current cigarette smoking was too rare in this elderly sample to be useful in the analysis. Other characteristics were determined at the time of collection of data on bowel movements (1991–1993). At that time excessive daytime sleepiness was assessed by questionnaire, triceps skinfold thickness was measured with calipers, and cognitive function was measured using the Cognitive Abilities Screening Instrument (CASI).²⁹

PD Case Finding

Prior to 1991, cases of PD were identified through a review of all hospital records of cohort members for new and preexisting diagnoses of PD, review of all Hawaii death certificates, and a review of medical records at the offices of local neurologists for all cohort members with PD identified within the previous 25 years. After 1991, PD cases were identified during examinations from 1991 to 1993, 1994 to 1996, and 1997 to 1999. Participants with a history of PD, PD medications, or parkinsonian symptoms were referred to a study neurologist. Final diagnosis was determined by consensus of two neurologists using published criteria.^{1,30}

Autopsy Methods and determination of neuron density and incidental LB

Autopsy consent was obtained from an authorized legal representative. To avoid bias, HAAS neuropathologists are shielded from clinical information.³¹ Neuron counts in the SN are performed on a 30X, scaled, microprojector tracing of a single, transverse H&E stained, ten micron thick section of the nucleus at the level of the roots of the oculomotor nerve. Dorsal and ventral borders of the nucleus are formed by the midbrain tegmentum and the crus cerebri, respectively. The margin of the cerebral peduncle adjacent to the emerging roots of the 3rd nerve and the lateral mesencephalic sulcus, respectively, mark the medial and lateral extent of the nucleus. Maximum transverse dimension of the traced nucleus is measured with a ruler. The midpoint of this measurement is used to draw a line perpendicular to the transverse dimension dividing the tracing into medial and lateral halves. A series of lines parallel to midline are drawn along the medial to lateral extent of the traced nucleus at points where changes in contour of the nucleus cause variations in dorsal/ventral nuclear dimensions. Midpoints of these lines are connected dividing the tracing into dorsal and ventral halves forming four quadrants: dorsomedial, ventromedial, dorsolateral, and ventrolateral. Neurons are counted in each quadrant. The area of each quadrant is determined from the known magnification of the tracing and the planimetric measurement of the traced quadrant. Neuron density is calculated and expressed as neurons/mm².

Single H&E stained sections from both midbrain and pons were examined for LB in neurons of the SN and locus ceruleus (LC). Individuals who had LB in either the SN or LC without history of PD or dementia with LB were defined as having incidental LB.

Statistical Methods

Results were stratified by cigarette smoking status because cigarette smoking in the HAAS has a strong inverse relationship with clinical PD²⁵ and a positive relationship with neuron density in the SN that is independent of clinical PD and LB.³² Features of the study sample are compared across the bowel movement frequencies using binary logistic and general linear regression models. Average neuron densities (and standard deviations) within each quadrant are also provided as average counts/mm². To examine differences in neuron density across the bowel movement frequencies, neuron density was modeled as an over dispersed integer response following a negative binomial distribution.³³ Here, generalized linear models were used with bowel movement frequencies serving as an independent indicator variable that provides a means for comparing neuron densities in decedents with 1

and >1 bowel movement/day to decedents with <1 bowel movement/day. Statistical models were also adjusted for age, time to death, and other study characteristics. Regression coefficients from the estimated models also yield a ratio of neuron densities (and 95% confidence intervals) between bowel movement strata. Count ratios >1 indicate an excess in neuron density in one bowel movement strata versus another while ratios <1 represent a deficit. As an example, a count ratio of 1.86 would represent an 86% excess in neuron counts/mm² for one bowel movement group versus another. All reported p-values were based on two-sided tests of significance.

Results

The study sample includes 414 men with available information on bowel movement frequency who subsequently died and have completed microscopic autopsy data. Prevalent cases of dementia were excluded at the time of questioning about bowel movement frequency (1991–1993). The average age when bowel movement frequency was assessed was 78.3 ± 4.7 years (range: 71–93). The average time from assessment of bowel movement frequency to death was 8.0 ± 3.5 years (range: 6 months – 14.2 years).

Table 1 shows the relationship of number of bowel movements per day to study characteristics that could act as confounding variables for associations with neuron counts in the SN. Prevalence of PD declined significantly with increasing bowel movement frequency. None of the other characteristics were significantly related to bowel movement frequency.

Table 2 demonstrates that neuron densities are significantly lower in 3 of the four quadrants of the SN in nonsmokers who had the fewest bowel movements per day versus those whose bowel movements were more frequent. This was true when comparing those with <1 bowel movement per day to those with 1 per day and also when comparing those with <1 bowel movement per day to those who had >1 bowel movement per day. In smokers, neuron densities were unrelated to bowel movement frequency.

Table 3 shows adjusted neuron density ratios in nonsmokers with 1 and >1 bowel movements per day compared to those with <1 (reference strata). Results are shown for an age-adjusted model and a model adjusted for the presence of clinical PD, incidental LB, and other potential confounders. As in table 2, in all but the dorsolateral quadrant of the SN, neuron densities are significantly higher in those who had >1 bowel movement per day versus those whose bowel movements were the least frequent (<1/day). In the ventrolateral quadrant, compared to decedents with <1 bowel movement per day, those with 1 bowel movement per day had 71% excess in neuron density, while neuron density was nearly doubled (91%) in those with >1 bowel movement per day.

Discussion

Although constipation is common in PD and can precede its motor symptoms by 12 years or more years¹, the pathologic association between impaired gastrointestinal motility and PD is poorly understood. The motility impairment might be caused by the same processes that produce the motor symptoms of PD, but in different regions of the nervous system. This would suggest a pathologic process preceding the sequence of CNS injuries proposed by Braak.³⁴ Evidence for this includes findings of dopaminergic neuron depletion in the colon and LB in the myenteric plexus in decedents who had PD.^{6,17}

It is interesting that an association between bowel movements and neuron density was absent in decedents who smoked cigarettes during mid-life. Cigarette smoking, is known to be associated with a low frequency of PD. In our data, cigarette smoking was also associated with higher neuron densities in all quadrants of the SN and across all bowel movement

frequencies as compared to non-smokers (see table 2). It may be that an association between bowel movement frequency and neuron density is more subtle in cigarette smokers where neuron density seems uniformly high.³⁴ The mechanism by which cigarette smoking changes the relationship between bowel movement frequency and neuron density in the SN is clearly in need of further study.

Data from the HAAS autopsy study demonstrate that low counts in the SN can occur with or without LB being present.³⁵ Among non-smokers, our data suggest that nigral neuron counts are lower in individuals with a history of infrequent bowel movements. The relationship is strongest in the ventrolateral quadrant where there is a preponderance of SN injury in PD³⁶ but was also present in the dorsomedial and ventromedial quadrants. This relationship appears to be independent of the presence of LB in the SN or LC or clinical diagnosis of PD.

In addition to statistically adjusting for LB, we repeated our analyses after removal of all cases of PD and incidental LB. Although our sample has been reduced and our statistical methods become less precise, decedents with >1 bowel movement/day continued to have a significant excess of neuron counts in the dorsomedial and ventrolateral quadrants relative to those with <1 bowel movement/day ($p=0.018$ and $p=0.019$). This is also true after adjustment for all other factors.

There were 29 men with no LB in the SN or LC who also had exams that included the Unified Parkinson's Disease Rating Scale (UPDRS) at the time of assessment of bowel movement frequency. Unfortunately, this sample size is too limited to provide a careful assessment with additional adjustment for the UPDRS. Among the 29 men, however, 2 had <1 bowel movement per day, 19 had 1 bowel movement per day, and 8 had >1 bowel movement per day. The 2 men with <1 bowel movement per day fell in the highest tertile of UPDRS scores. They also had average neuron densities in all quadrants that were lower than any other combination of bowel movement frequency and UPDRS score.

Although the cause of constipation in PD remains unknown, data presented here suggest that constipation in non-smoking men is associated with low neuron counts in the SN and can occur without LB in the SN. Current initiatives in the HAAS are now investigating whether individuals with constipation and low nigral neuron density have synuclein deposition in the nervous system of the gut or the dorsal motor vagal nucleus.

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Table 1

Study characteristics according to frequency of daily bowel movements

Study characteristic ^{//}	Bowel movements/day		
	<1 (33) [*]	1 (255)	>1 (126)
Baseline age in years (1991–1993)	78.8 ± 4.3 [†]	78.1 ± 4.9	78.7 ± 4.3
Time to death (years)	6.7 ± 3.4	8.2 ± 3.4	7.9 ± 3.6
Mid-life pack-years of smoking	32.9 ± 32.1	26.8 ± 28.2	25.8 ± 27.7
Mid-life coffee intake (oz/day)	15.4 ± 14.3	13.9 ± 14.4	13.2 ± 11.6
Tricep skinfold thickness [§] (mm)	10.7 ± 4.5	10.2 ± 4.0	9.9 ± 3.2
Cognitive Abilities Screening Instrument	82.9 ± 9.7	84.7 ± 10.3	85.2 ± 10.5
Excessive daytime sleepiness [§] (%)	14.3 (4/28) [‡]	11.3 (27/239)	9.0 (11/122)
Parkinson's disease (%)	24.2 (8/33)	5.1 (13/255)	4.0 (5/126)

^{*} Sample size

[†] Mean ± standard deviation

[‡] Cases/sample size

[§] One decedent is missing data on tricep skinfold thickness and 25 have missing data on excessive daytime sleepiness.

^{//} Prevalence of Parkinson's disease declined significantly with increasing bowel movement frequency (p=0.002). None of the other characteristics were significantly related to bowel movement frequency.

Table 2

Mean neuron density (neurons/mm²) according to frequency of daily bowel movements and mid-life smoking status within quadrants of the substantia nigra.

Quadrant	Bowel movements/day			p-value for comparing	
	<1	1	>1	<1 vs 1	<1 vs >1
<u>Non-Smokers</u>					
Sample size	12	90	48		
Dorsomedial	10.1 ± 5.4*	18.8 ± 7.6	18.5 ± 10.2†	<0.001	<0.001
Dorsolateral	10.2 ± 8.0	12.3 ± 6.3	12.4 ± 5.7	0.254	0.244
Ventromedial	10.2 ± 7.4	16.4 ± 7.8	15.3 ± 8.9	0.007	0.026
Ventrolateral	10.9 ± 12.1	18.3 ± 9.5	18.6 ± 10.4‡	0.011	0.011
<u>Smokers</u>					
Sample size	21	165	78		
Dorsomedial	18.0 ± 8.1	19.4 ± 8.9	19.4 ± 8.8	0.503	0.519
Dorsolateral	11.5 ± 7.2	14.0 ± 8.7	13.6 ± 6.6	0.133	0.214
Ventromedial	21.3 ± 13.0	18.4 ± 9.5	18.5 ± 9.1	0.218	0.251
Ventrolateral	18.1 ± 7.9	18.7 ± 8.6	19.9 ± 9.5	0.761	0.441

* Mean ± standard deviation

Table 3

Count ratios of neuron densities (neurons/mm²) in mid-life nonsmokers with 1 and >1 bowel movement/day versus those with <1 bowel movement/day.

Bowel movements/day	Adjusted count ratios	
	Age-adjusted	Adjusted [†]
<u>Dorsomedial quadrant</u>		
<1	reference	reference
1	1.86 [‡] (1.38, 2.50) [*]	1.30 (0.93, 1.82)
>1	1.85 [‡] (1.35, 2.53)	1.47 [§] (1.03, 2.09)
<u>Ventrolateral quadrant</u>		
<1	reference	reference
1	1.66 ^{//} (1.12, 2.46)	1.71 ^{//} (1.13, 2.60)
>1	1.70 ^{//} (1.12, 2.57)	1.91 ^{**} (1.23, 2.98)
<u>Ventromedial quadrant</u>		
<1	reference	reference
1	1.61 ^{††} (1.14, 2.28)	1.44 (0.99, 2.10)
>1	1.51 ^{‡‡} (1.05, 2.17)	1.52 ^{§§} (1.02, 2.26)
<u>Dorsolateral quadrant</u>		
<1	reference	reference
1	1.21 (0.87, 1.67)	1.00 (0.70, 1.43)
>1	1.22 (0.87, 1.72)	0.94 (0.65, 1.38)

* 95% confidence interval

[†] Adjusted for age, time to death, mid-life coffee drinking, tricep skinfold thickness, excessive daytime sleepiness, cognitive function, Parkinson's disease, and incidental Lewy bodies.

[‡] Significant excess versus men with <1 bowel movement/day (p<0.001)

[§] Significant excess versus men with <1 bowel movement/day (p=0.034)

^{//} Significant excess versus men with <1 bowel movement/day (p=0.012)

^{**} Significant excess versus men with <1 bowel movement/day (p=0.004)

^{††} Significant excess versus men with <1 bowel movement/day (p=0.007)

^{‡‡} Significant excess versus men with <1 bowel movement/day (p=0.026)

^{§§} Significant excess versus men with <1 bowel movement/day (p=0.039)