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## 14. Abstract

This is a final report for a 10 year project (7/1/1999-8/31/2008). The goal of this epidemiologic and neuropathologic research program was to determine neurotoxic and preventive/ameliorative risk factors for Parkinson's disease (PD). Results related to prediction of incident PD from the Honolulu-Asia Aging Study (HAAS) supported by this project were as follows: coffee drinking may be protective against PD, milk consumption is associated with an increased risk of PD, Low LDL cholesterol is a predictor of PD among men aged 71-75. Increased triceps skinfold thickness, constipation, excessive daytime sleepiness, and olfactory dysfunction, are indicators that may precede the motor syndrome by years. Using neuropathological endpoints: olfactory dysfunction, constipation, and increased reaction predict incidental Lewy bodies. The Braak staging system for Lewy pathology is supported by evaluation of the HAAS autopsy series. One-third of elderly men without PD or dementia with Lewy bodies (DLB) have Lewy pathology in the olfactory bulb. Several preclinical indicators of PD are associated with this stage of synuclein deposition. Lewy pathology in the olfactory bulb is also associated with decreased neuron density in the substantia nigra. These findings indicate that several of the preclinical indicators of Parkinson's disease are expressed very early in the process of synuclein deposition and that neuron density in the substantia nigra also begins very early in this process. Genetic studies in collaboration with the Parkinson's institute noted an association of a polymorphism of the MDR1 transporter gene with PD. Analysis of frozen brain samples for organochlorines indicate that several of the organochlorines are detected more frequently in brains of men who also had Lewy bodies or Alzheimer changes at death. The identification of early markers for PD and susceptibility genes could identify individuals at high risk for the development of PD. Persons so identified would be candidates to participate in drug studies aimed at disease prevention and/or might be preferentially excluded from subsequent exposure to agricultural or military chemicals having possible neurotoxicity.

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## INTRODUCTION

This is a final report covering September 21, 1998 – August 31, 2008 for the project entitled “Neurotoxins and neurodegenerative disorders in Japanese-American men living in Hawaii”. The goal of this epidemiologic and neuropathologic research program is to determine neurotoxic and preventive/ameliorative risk factors for Parkinson’s disease, parkinsonism, and other neurodegenerative conditions. The research is an extension of the Honolulu Heart Program/Honolulu-Asia Aging Study (HHP/HAAS), a longitudinal study of heart disease, stroke, and dementia in a cohort of Japanese-American men born 1900-1919 who were living in Hawaii when the study began in 1965. Additionally, this project builds on a National Institute of Neurological Disease and Stroke funded study during which all cases of Parkinson’s disease (PD) were identified in the HHP/HAAS cohort up to 1994 and smoking and dietary antecedents of Parkinson’s disease were examined.

Components **1** and **2** of this research are identification of risk factors for PD (**1**) and parkinsonism (**2**) using existing data from the longitudinal HHP/HAAS. The work seeks to confirm previous reports<sup>1-6</sup> of an association of pesticide exposure with PD by examining the role of exposure to neurotoxins through occupational exposures such as sugar or pineapple plantation work (pesticides, herbicides) and self reported exposures to pesticides, metals, and other chemicals. Cases of PD in the HAAS cohort were initially identified 1991-1994. Since then new cases have been identified through self report, record review, and direct examinations by a neurologist. Work is currently ongoing (through additional funding) to re-screen the HAAS cohort to identify new cases of PD and parkinsonism through separate funding source.

The neuropathological component, (**3**) currently has access to over 773 brains from deceased HHP/HAAS participants. Lewy bodies in the brainstem pigmented nuclei are being identified and used as an endpoint in risk factor analyses. Among the 506 brains with completed microscopic evaluations, there are 105 brains that have Lewy bodies in either the substantia nigra or the locus ceruleus. Among these 24 had Parkinson’s disease during life.

The **4th** and final component of the research involves genetic determinants of Parkinson’s disease. These were initially investigated with collaborators at Stanford University in a case control study aimed at determining polymorphisms of the CYP2D6, dopamine transporter, CYP1A2, parkin, adenosine receptor, dopamine D2 receptor, paraoxonase 1, and VMAT genes that may be associated with Parkinson’s disease. Results from this study were negative. We have more recently begun collaboration with the Parkinson’s institute to evaluate transporter gene polymorphisms.

The **Supplement** to this research awarded in 2005 was to determine markers of brain injury such as glial fibrillary acidic protein and levels of organochlorine compounds in frozen brain tissue.

**BODY:** (numbers refer to items in the statement of work)

**Components 1 & 2: Evaluation of epidemiological risk factors and preclinical indicators as predictors of Parkinson’s disease and parkinsonism (see appendix 1 for copies of manuscripts and abstracts)**

1. Coffee and caffeine: This work was published in the Journal of the American Medical Association in 2000. Coffee drinking assessed at the baseline examination in 1965 and at the third examination in 1971 among the participants in the Honolulu Heart Program was found to be inversely related to the future development of Parkinson’s Disease. A dose response pattern was found. Based on 30 years of follow-up since baseline examination, age-adjusted 10-year incidence of Parkinson’s disease declined consistently with increased amounts of coffee intake from 9.4/1000 in men who drank no coffee to 1.7/1000 in men who drank  $\geq 28$  oz. per day ( $p < 0.001$ ). Similar relationships were observed with total caffeine intake and caffeine from non-coffee sources. Consumption of increasing amounts of coffee was also associated

with lower risk of Parkinson's disease in men who were never, past, and current smokers at baseline. Other nutrients in coffee, including niacin, were unrelated to Parkinson's disease incidence. The relationship between caffeine and Parkinson's disease was unaltered by intake of milk and sugar and was independent of alcohol consumption.<sup>7</sup>

2. Frequency of Bowel Movements: A manuscript was published in 2001 showing that Age-adjusted incidence of Parkinson's disease (PD) declined consistently from 18.9/10,000 person-years in men with <1 bowel movement/day to 3.8/10,000 person-years in those with >2/day ( $p=0.005$ ). After adjustment for age, pack-years of cigarette smoking, coffee consumption, laxative use, jogging, and the intake of fruits, vegetables, and grains, men with <1 bowel movement/day had a 2.7-fold excess risk of PD versus men with 1/day (95% CI: 1.3, 5.5;  $p=0.007$ ). The risk of PD in men with <1 bowel movement/day increased to a 4.1-fold excess when compared with men with 2/day (95% CI: 1.7, 9.6;  $p=0.001$ ) and to a 4.5-fold excess versus men with >2/day (95% CI: 1.2, 16.9;  $p=0.025$ ).<sup>8</sup>
3. Pesticides and herbicides / years worked on a plantation: A poster was presented at the 5<sup>th</sup> International Conference on Progress in Alzheimer's and Parkinson's disease held in Kyoto, Japan March 31 to April 5, 2001. A manuscript prepared from these data was later published in the Archives of Neurology in 2002. For this manuscript age-adjusted PD incidence was examined by years worked on either a pineapple or sugar plantation, assessed at the baseline examination in 1965 and by pesticide exposure assessed at the 1971 examination. A dose response effect was observed for years worked on a plantation with age adjusted incidence of PD highest in men who worked more than 10 years on a plantation. The relative risk of PD adjusted for age, pack-years of cigarette smoking, and coffee intake was 1.0 (95% CI = .6-1.6), 1.7 (95% CI = .8-3.7), and 1.9 (95% CI = 1.0-3.5) for men who worked on a plantation 1-10 years, 11-20 years, and more than 20 years compared to men who never did plantation work ( $p=0.006$ , test for trend). Years of exposure to pesticides beyond one year appeared to increase the risk of PD although this relationship was not statistically significant. A dose response relationship of PD incidence increasing with increasing years of exposure to pesticides was suggested but not statistically significant ( $p=0.101$  test for trend).<sup>9</sup>
4. Midlife Adiposity: A manuscript was published in 2002 demonstrating that age-adjusted incidence of PD increased threefold from 3.7/10,000 person-years in the bottom quartile of midlife triceps skinfold thickness (TSF) (1 to 5 mm) to 11.1/10,000 person-years in the top quartile (11 to 32 mm,  $p < 0.001$ ). Effects of TSF on PD were independent of cigarette smoking, coffee consumption, physical activity, daily caloric and fat intake, and the other measures of adiposity ( $p < 0.001$ ).<sup>10</sup>
5. Summary of Environmental, Life-style, and physical precursors of PD: In 2003 a manuscript was published summarizing all precursors of PD evaluated in the HHP/HAAS. The findings were that precursors of PD included constipation, adiposity, years worked on a sugar or pineapple plantation, years of exposure to pesticides, and exposure to sugar cane processing. Factors showing an inverse association with PD included coffee intake and cigarette smoking. Among dietary factors, carbohydrates increased the risk of PD while the intake of polyunsaturated fats appeared protective. Total caloric intake, saturated and monounsaturated fats, protein, niacin, riboflavin, beta-carotene, vitamins A, B, and C, dietary cholesterol, cobalamin, alpha-tocopherol, and pantothenic acid showed no clear relation with clinical PD.<sup>11</sup>
6. Milk and Calcium: A manuscript was published in *Neurology* in 2005 demonstrating a 2.3 fold excess of PD (95%CI 1.3 to 4.1) in the individuals who drank >16 oz. of milk per day compared to those who did not drink milk (95% CI 1.3 – 4.1).<sup>12</sup>

7. Excessive Daytime Sleepiness: A manuscript was published in *Neurology* in 2005 showing that excessive daytime sleepiness was associated with a higher risk of future PD (OR=2.8, 95% CI= 1.1 to 6.4).<sup>13</sup>
8. Depressive symptoms: An abstract was published in *Movement Disorders* in 2007 demonstrating that depression was more common in prevalent and incident PD compared to individuals who did not develop PD. However the association was not statistically significant.<sup>14</sup>
9. Pre-Clinical Predictors of PD: In an invited presentation given at the World Parkinson Congress, February 23, 2006 in Washington DC, Dr. Ross presented data showing that Factors associated with increased PD risk were mid-life constipation, adiposity, and impaired olfaction. Deficits in olfaction and reaction time in later life were associated with an increased likelihood of Lewy bodies noted in the autopsy series. This topic has been the subject of additional invited presentations. See reportable outcomes section.
10. Occupational Exposures and Movement Abnormalities: A manuscript was published in *Neuroepidmiology* in 2006 demonstrated that higher exposure to any metal and specifically mercury was associated with abnormal facial expression.<sup>15</sup>
11. Olfactory Dysfunction: A manuscript was published in *Annals of Neurology* in 2008 showing that during the first 4 years of follow-up, age adjusted incidence of PD declined from 54.5/10,000 person years to in the lowest quartile of odor identification to 26.6, 8.2 and 8.4/10,000 person years in the second, third, and highest quartile of odor identification (p<0.001 test for trend).<sup>16</sup>
12. Low LDL Cholesterol: A manuscript was published in 2008 in *Movement Disorders* demonstrating that although incidence of Parkinson's disease increased with decreasing LDL-C in a dose-dependent manner, the association was only significant for men aged 71 to 75 years. In the latter group, risk of Parkinson's disease declined from 38.5/10,000 person-years in men with LDL-C levels <80 mg/dL to less than 9/10,000 person-years for concentrations that were ≥ 140 mg/dL. After adjustment for age, smoking, coffee intake, and other factors, the relative odds of PD for men at the 80<sup>th</sup> versus the 20<sup>th</sup> percentile of LDL-C (135 versus 85 mg/dL) was 0.4 (95% confidence interval: 0.2, 0.9).<sup>17</sup>

**Component 3: Evaluation of neuropathology related to Parkinson's diseases. (see appendix 2 for copies of manuscripts and abstracts)**

1. Computerized Reaction Time and Incidental Lewy Bodies: among 96 brains analyzed, 8 had incidental Lewy bodies. The percent of brains with incidental Lewy bodies increased consistently from 0% (0/24, fastest quartile to 16.7%, 4/24, slowest quartile) among subjects classified into 4 quartiles by reaction time (p=0.037, age adjusted test for trend). These data were presented in a poster at the Seventh International Congress of Parkinson's Disease and Movement Disorders to be held in Miami, Florida November 10-14, 2002.<sup>18</sup>
2. Parkinsonian Signs and Substantia Nigra Neuron Density: A manuscript was published in *Annals of Neurology* 2004. Substantia nigra (SN) neurons were counted on single, transverse caudal midbrain sections from 217 male participants in the Honolulu-Asia Aging Study, aged 74–97 years at death. Quadrants areas within the SN were determined with a planimeter and neuronal density was expressed as neurons/mm<sup>2</sup> for 10 Parkinson's disease (PD) cases, 29

incidental Lewy body cases, and 178 controls with neither condition. Mean densities in all quadrants were significantly lower in the PD group compared with the other groups ( $p = 0.006$ ). This relationship was strongest in the ventrolateral quadrant. In a subgroup of 50 controls who were examined with the Unified Parkinson's Disease Rating Scale an average of 2.1 years prior to death, there was an association of stooped posture ( $p = 0.009$ ), postural instability ( $p = 0.013$ ), body bradykinesia ( $p = 0.048$ ), and gait disturbance ( $p = 0.05$ ) with neuron density in the dorsolateral quadrant; and impaired speech ( $p = 0.014$ ), abnormal facial expression ( $p = 0.022$ ), and difficulty rising from a chair ( $p = 0.032$ ) with neuron density in the dorsomedial quadrant. There was a significant association of increasing number of signs present with decreasing neuron density in both quadrants ( $p = 0.001$  for trend). Low SN neuron density may be the basis for parkinsonian signs in the elderly without PD.<sup>19</sup>

3. Olfactory dysfunction and incidental Lewy bodies: A manuscript was published in *Movement Disorders* in 2006 showing that the age-adjusted percent of brains with incidental Lewy bodies increased from 1.8% in the highest tertile of odor identification to 11.9% in the mid tertile, and to 17.4 in the lowest tertile ( $p = 0.019$ ).<sup>20</sup>
4. Bowel movement frequency and incidental Lewy bodies: A manuscript was published in *Movement Disorders* in 2007 showing that after age adjustment, the percent of brains with incidental Lewy bodies declined with increasing bowel movement frequency ( $p = 0.013$ ). For men with <1, 1, and >1 bowel movement per day the corresponding percents were 24.1, 13.5, and 6.5.<sup>21</sup>
5. Bowel Movement Frequency and Substantia Nigra Neuron Density: A manuscript has been accepted for publication in *Movement Disorders*. In non-smokers, neuron densities (counts/mm<sup>2</sup>) 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.
6. Assessing the validity of the Braak LB staging system in a population based study: An abstract was presented at the 11<sup>th</sup> International Congress of the Movement Disorder Society in 2007 entitled "Lewy Pathology Progression Suggested by Braak Staging System is Supported by Analysis of a Population-based Cohort of Patients" For this analysis 126 brains with complete neuropathological data were used. Dementia with Lewy body cases were Braak LB stages 5 or 6, PD cases were stages 3, 5, or 6, and Incidental LB (ILB) cases were stages 1-6. 94.4% of the cases were consistent with the progression of pathology as outlined by Braak. Seven cases were inconsistent with the Braak LB staging system with no pathology in any representative foci for a stage preceding the last stage with pathology. Six of these inconsistent cases were ILB cases and one had PD. The most common inconsistencies were absence of pathology in foci representative of Braak LB stages 2 and 4.<sup>22</sup>
7. Assessing the number of elderly men without a clinical history of Parkinson's disease or dementia with Lewy bodies who may have Lewy pathology in the olfactory bulb indicating early synuclein pathology. Presented at the 12<sup>th</sup> International Congress of the Movement Disorder Society, June 22-28, 2008 Chicago, The work of Braak suggests that the olfactory bulb is one of the earliest areas to develop synuclein deposits in PD, perhaps explaining the early occurrence of olfactory deficits in PD cases. Sensitive alpha-synuclein immunostaining was



performed to examine the olfactory bulbs for 21 brains from men with PD, 8 men with DLB, 158 with neither disorder. All PD and DLB cases had Lewy pathology in the olfactory bulb. Lewy pathology was identified in 51 (32%) olfactory bulbs from the 158 cases with no clinical history of PD or DLB, so that they were defined as having incidental Lewy pathology (ILP). This indicates that Incidental Lewy pathology in the elderly is more common than previously recognized.<sup>23</sup>

8. The association of olfactory dysfunction with synuclein pathology in the olfactory bulb Presented at the 12<sup>th</sup> International Congress of the Movement Disorder Society, June 22-28, 2008 Chicago. Findings indicated that the percent of bulbs with Lewy pathology in the highest olfactory score tertile (8-12) was 15.4 compared to 43.5 for the middle tertile (score 6-7) and 41.5 for the lowest tertile (score 0-5). The per cent of decedents with Lewy pathology in the olfactory bulb decreased significantly with increasing olfactory score without ( $p=0.04$ ) and with ( $p=0.015$ ) adjustment for age at olfaction testing, time from testing to death, midlife smoking, and coffee intake. The process responsible for alpha-synuclein deposition in the olfactory structures may cause the olfactory deficits that occur in PD as well as in some non-diseased elderly.<sup>24</sup>
9. Lewy pathology in the olfactory bulb is associated with decreased neuron density in the substantia nigra. Presented at the 12<sup>th</sup> International Congress of Parkinson's Disease and Movement Disorders, June 22-28, 2008 Chicago. The mean substantia nigra neuron density was highest among 124 brains with no Lewy pathology ( $18.9/\text{mm}^2$ ) compared to 30 individuals without PD who had Lewy pathology restricted to the olfactory bulb ( $15.6/\text{mm}^2$ ,  $p=.02$ ) and 40 individuals with Lewy pathology in the LC or SN ( $14.8/\text{mm}^2$ ,  $p=.001$ ). Density was lowest in 20 PD cases ( $7.7/\text{mm}^2$ ). In order to examine the association of early Braak stage synuclein deposition with SN neuron density an analysis was performed with the 154 brains that had either no Lewy pathology ( $N=124$ ) or Lewy pathology restricted to the olfactory bulb ( $N=30$ ). In this group percent of brains with olfactory bulb Lewy pathology was 33.3% in the lowest quartile of SN neuron density, 18% in the 2<sup>nd</sup> quartile, and 13.2% in the 3<sup>rd</sup> and top quartiles. The percent of decedents with Lewy pathology in the olfactory bulb decreased significantly as SN neuron density increased with and without adjustment for age at death, midlife pack-years of cigarette smoking, and midlife intake of coffee ( $p=0.025$ ). Findings suggest that Lewy pathology in the olfactory bulb is associated with lower neuron density in the SN in individuals who do not have Parkinson's disease. In some instances, there is already significant neuronal loss in the SN at the earliest stages of alpha-synuclein deposition in the brain.<sup>25</sup>
10. Association of Preclinical Indicators of Parkinson's Disease With Early Stages of Synuclein Deposition; Accepted for presentation at the 6<sup>th</sup> International Congress on Mental Dysfunctions & Other Non-Motor features in Parkinson's Disease, Dresden, Germany October 16-19, 2008. There were 60 decedents who had no synuclein pathology (stage 0), 10 with synuclein pathology restricted to the olfactory bulb (early stage), 26 with involvement of the LC or SN but without clinical PD during life (mid-stage), and 30 with clinical PD during life (late stage). Those in early stage had significantly fewer bowel movements per day (1.6) than those in stage 0 (2.3) ( $p<0.05$ ). Excessive daytime sleepiness and poor olfaction were more common in mid-stage than in earlier stages. Olfaction and grip strength decreased significantly across the stages from 0 to late stage ( $p<0.04$ ). Bowel movement frequency, grip strength, olfaction, and likelihood of excessive daytime sleepiness appear to be affected early in the course of synuclein deposition prior to the classical motor features of PD.

11. The Effects of Lewy Bodies, Alzheimer’s Lesions, and Vascular Lesions on Cognition: The effect of Lewy bodies on cognitive function in a community based population is unclear. The population for this analysis consisted of 365 deceased men from the HAAS who had cognitive screening within three years of death using the cognitive abilities screening instrument (CASI). CASI scores range from 0 to 100 with 100 being the highest. A standardized protocol was used to quantify LB in the limbic and cortical regions to determine LB score; cerebral infarcts; microvascular lesions; neocortical neurofibrillary tangles and neuritic plaques; and a measure of atrophy. A general linear regression model adjusting age at time of CASI and education was used to determine the independent effects on CASI score of the neuropathologic features. An increase of one standard deviation for each neuropathologic feature resulted in the following point decreases in CASI score: atrophy 8.4 points (P<0.001), neurofibrillary tangles 7.6 (P<0.001), Lewy body score 5.2 (P<0.001), microvascular lesions 3.9 (P=0.007), lacunes 2.9 (P<0.04). Plaques and large infarcts fell out of the model due to collinearity with tangles and microvascular lesions respectively. To better understand additive effects of the lesions, four groups were formed. In a subset with no AD or vascular pathology progression of LB score from brainstem to cortical had no apparent effect on cognition while in subsets with AD, vascular or both the presence of limbic and cortical LB was associated with lower CASI score compared to no LB or brainstem predominant. Lewy body score is a significant independent predictor of cognitive function in the HAAS. While small numbers make interpretation difficult, it appears that Lewy bodies have the greatest effect on cognition when AD or vascular lesions are also present. Accepted for presentation at the 6<sup>th</sup> International Congress on Mental Dysfunctions & Other Non-Motor features in Parkinson’s Disease, Dresden, Germany October 16-19, 2008.

**Component 4: Genetic Determinants of Parkinson’s Disease**

A subcontract was established with Stanford University to use DNA samples from approximately 117 Parkinson’s disease cases and 240 controls without Parkinson’s disease matched for age for genotyping for the following five polymorphisms. In the tables below, PD case refers to DNA from subjects with Parkinson’s disease while Incidental LB refers to DNA from deceased subjects without a history of Parkinson’s disease whose brains have Lewy bodies in the substantia nigra or locus ceruleus.

1. CYP2D6 HhaI polymorphism in exon 6. CYP2D6 is a subfamily of the cytochrome P-450 enzyme system in the liver. This enzyme system catalyzes breakdown of many potential environmental neurotoxins and medications. Mutant alleles of the gene lead to poor or slow metabolism of debrisoquine and similar medications.

**CYP2D6**

Genotype frequency for PD Case plus Incidental LB

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
<b>Case</b>	117	83	70.9%	27	23.1%	5	4.3%	2	1.7%
<b>Control</b>	240	167	69.6%	63	26.3%	8	3.3%	2	0.8%

Genotype frequency for PD Case

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
<b>Case</b>	89	67	75.3%	18	20.2%	3	3.4%	1	1.1%
<b>Control</b>	184	127	69.0%	49	26.6%	7	3.8%	1	0.5%

Genotype frequency for Incidental LB

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
<b>Case</b>	28	16	57.1%	9	32.1%	2	7.1%	1	3.6%
<b>Control</b>	56	40	71.4%	14	25.0%	1	1.8%	1	1.8%

Allele frequency for PD Case plus incidental LB

	N	C	N (%)	T	N (%)	N/D	N (%)
<b>Case</b>	234	193	82.5%	37	15.8%	4	1.7%
<b>Control</b>	480	397	82.7%	79	16.5%	4	0.8%

Allele frequency for PD Case

	N	C	N (%)	T	N (%)	N/D	N (%)
<b>Case</b>	178	152	85.4%	24	13.5%	2	1.1%
<b>Control</b>	368	303	82.3%	63	17.1%	2	0.5%

Allele frequency for Incidental LB

	N	C	N (%)	T	N (%)	N/D	N (%)
<b>Case</b>	56	41	73.2%	13	23.2%	2	3.6%
<b>Control</b>	112	94	83.9%	16	14.3%	2	1.8%

2. CYP1A2 promoter polymorphism. This is a genetic polymorphism in the 5'-flanking region of human CYP1A2 gene that has a major effect on the elimination of caffeine. The purpose of this analysis is to investigate a possible genetic explanation for our finding that caffeine consumption is inversely associated with PD incidence.

**CYP1A2**

Genotype frequency for PD Case plus incide

	N	A/A	N (%)	A/G	N (%)	G/G	N (%)	N/D	N (%)
<b>Case</b>	117	6	5.1%	46	39.3%	63	53.8%	2	1.7%
<b>Control</b>	240	17	7.1%	85	35.4%	138	57.5%	0	0.0%

Genotype frequency for PD Case

	N	A/A	N (%)	A/G	N (%)	G/G	N (%)	N/D	N (%)
<b>Case</b>	89	4	4.5%	33	37.1%	51	57.3%	1	1.1%
<b>Control</b>	184	14	7.6%	64	34.8%	106	57.6%	0	0.0%

Genotype frequency for Incidental LB

	N	A/A	N (%)	A/G	N (%)	G/G	N (%)	N/D	N (%)
<b>Case</b>	28	2	7.1%	13	46.4%	12	42.9%	1	3.6%
<b>Control</b>	56	3	5.4%	21	37.5%	32	57.1%	0	0.0%

Allele frequency for PD Case plus Incidental LB

	N	A	N (%)	G	N (%)	N/D	N (%)
<b>Case</b>	234	58	24.8%	172	73.5%	4	1.7%
<b>Control</b>	480	119	24.8%	361	75.2%	0	0.0%

Allele frequency for PD Case

	N	A	N (%)	G	N (%)	N/D	N (%)
<b>Case</b>	178	41	23.0%	135	75.8%	2	1.1%
<b>Control</b>	368	92	25.0%	276	75.0%	0	0.0%

Allele frequency for Incidental LB

	N	A	N (%)	G	N (%)	N/D	N (%)
<b>Case</b>	56	17	30.4%	37	66.1%	2	3.6%
<b>Control</b>	112	27	24.1%	85	75.9%	0	0.0%

3. Dopamine transporter (DAT) 1215 A/G. This is a polymorphism in exon 9 of the dopamine transporter gene. In a study of Japanese subjects, this polymorphism was found to be significantly less frequent among PD cases compared to controls.

**DAT1215**

**Genotype** frequency for PD Case plus Incidental LB

	N	A/A	N (%)	A/G	N (%)	G/G	N (%)	N/D	N (%)
<b>Case</b>	117	97	82.9%	16	13.7%	2	1.7%	2	1.7%
<b>Control</b>	240	201	83.8%	35	14.6%	3	1.3%	1	0.4%

Genotype frequency for PD Case

	N	A/A	N (%)	A/G	N (%)	G/G	N (%)	N/D	N (%)
<b>Case</b>	89	78	87.6%	9	10.1%	1	1.1%	1	1.1%
<b>Control</b>	184	153	83.2%	28	15.2%	2	1.1%	1	0.5%

Genotype frequency for Incidental LB

	N	A/A	N (%)	A/G	N (%)	G/G	N (%)	N/D	N (%)
<b>Case</b>	28	19	67.9%	7	25.0%	1	3.6%	1	3.6%
<b>Control</b>	56	48	85.7%	7	12.5%	1	1.8%	0	0.0%

Allele frequency for PD Case plus incidental LB

	N	A	N (%)	G	N (%)	N/D	N (%)
<b>Case</b>	234	210	89.7%	20	8.5%	4	1.7%
<b>Control</b>	480	437	91.0%	41	8.5%	2	0.4%

Allele frequency for PD Case

	N	A	N (%)	G	N (%)	N/D	N (%)
<b>Case</b>	178	165	92.7%	11	6.2%	2	1.1%
<b>Control</b>	368	334	90.8%	32	8.7%	2	0.5%

Allele frequency for Incidental LB

	N	A	N (%)	G	N (%)	N/D	N (%)
<b>Case</b>	56	45	80.4%	9	16.1%	2	3.6%
<b>Control</b>	112	103	92.0%	9	8.0%	0	0.0%

4. Parkin Arg366Trp. This is a polymorphism in exon 10 of the parkin gene that has been found to be significantly lower in PD cases compared to controls.

**PARKIN**

Genotype frequency for PD Case plus Incidental LB

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
<b>Case</b>	117	113	96.6%	3	2.6%	0	0.0%	1	0.9%
<b>Control</b>	240	238	99.2%	2	0.8%	0	0.0%	0	0.0%

Genotype frequency for PD Case

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
<b>Case</b>	89	87	97.8%	2	2.2%	0	0.0%	0	0.0%
<b>Control</b>	184	182	98.9%	2	1.1%	0	0.0%	0	0.0%

Genotype frequency for Incidental LB

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
<b>Case</b>	28	26	92.9%	1	3.6%	0	0.0%	1	3.6%
<b>Control</b>	56	56	N/A	0	0.0%	0	0.0%	0	0.0%

Allele frequency for PD Case plus Incidental LB

	N	C	N (%)	T	N (%)	N/D	N (%)
<b>Case</b>	234	229	97.9%	3	1.3%	2	0.9%
<b>Control</b>	480	478	99.6%	2	0.4%	0	0.0%

Allele frequency for PD Case

	N	C	N (%)	T	N (%)	N/D	N (%)
<b>Case</b>	178	176	98.9%	2	1.1%	0	0.0%
<b>Control</b>	368	366	99.5%	2	0.5%	0	0.0%

Allele frequency for incidental LB

	N	C	N (%)	T	N (%)	N/D	N (%)
<b>Case</b>	56	53	94.6%	1	1.8%	2	3.6%
<b>Control</b>	112	112	N/A	0	0.0%	0	0.0%

5. Adenosine A2A Receptor 1083 T/C polymorphism. This is a polymorphism in exon 2 of the human A2A human adenosine receptor gene. Caffeine, an adenosine A2A receptor blocker, has been associated with a decreased risk of developing Parkinson's disease. It is hypothesized that polymorphisms of the A2A receptor gene may alter risk for Parkinson's disease.

**ADENOSINE A2A RECEPTOR**

Genotype frequency for PD Case plus Incidental LB

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
<b>Case</b>	117	25	21.4%	56	47.9%	34	29.1%	2	1.7%
<b>Control</b>	240	55	22.9%	111	46.3%	72	30.0%	2	0.8%

Genotype frequency for PD Case

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
<b>Case</b>	89	20	22.5%	44	49.4%	24	27.0%	1	1.1%
<b>Control</b>	184	38	20.7%	84	45.7%	61	33.2%	1	0.5%

Genotype frequency for Incidental LB

	N	C/C	N (%)	C/T	N (%)	T/T	N (%)	N/D	N (%)
<b>Case</b>	28	5	17.9%	12	42.9%	10	35.7%	1	3.6%
<b>Control</b>	56	17	30.4%	27	48.2%	11	19.6%	1	1.8%

Allele frequency for PD Case plus Incidental LB

	N	C	N (%)	T	N (%)	N/D	N (%)
<b>Case</b>	234	106	45.3%	124	53.0%	4	1.7%
<b>Control</b>	480	221	46.0%	255	53.1%	4	0.8%

Allele frequency for PD Case

	N	C	N (%)	T	N (%)	N/D	N (%)
<b>Case</b>	178	84	47.2%	92	51.7%	2	1.1%
<b>Control</b>	368	160	43.5%	206	56.0%	2	0.5%

Allele frequency for Incidental LB

	N	C	N (%)	T	N (%)	N/D	N (%)
<b>Case</b>	56	22	39.3%	32	57.1%	2	3.6%
<b>Control</b>	112	61	54.5%	49	43.8%	2	1.8%

6. Paraoxonase 1 Met-Leu 54 polymorphism. Two polymorphisms of the paraoxonase 1 gene affect the hydrolysis of toxic oxons and are thought to increase the toxic effects of environmental chemicals as they relate to the etiology of PD. One report has shown an association of the Met-Leu 54 polymorphism with PD.

**PARAOXONASE 1 MET-LEU POLYMORPHISM**

Genotype frequency for PD Case plus Incidental LB

	N	T/T	N (%)	A/T	N (%)	A/A	N (%)	N/D	N (%)
<b>Case</b>	116	96	82.8%	19	16.4%	1	0.8%	0	0%
<b>Control</b>	240	212	88.3%	26	10.8%	2	0.8%	0	0.0%

Genotype frequency for PD Case

	N	T/T	N (%)	A/T	N (%)	A/A	N (%)	N/D	N (%)
<b>Case</b>	88	72	81.8%	16	18.2%	0	0%	0	0%
<b>Control</b>	184	163	88.6%	19	10.3%	2	1.1%	0	0.0%

Genotype frequency for Incidental LB

	N	T/T	N (%)	A/T	N (%)	A/A	N (%)	N/D	N (%)
<b>Case</b>	28	24	85.7%	3	10.7%	1	3.8%	0	0%
<b>Control</b>	56	49	87.5%	7	12.5%	0	0%	0	0%

7. Dopamine receptor D2 *TaqIA* polymorphisms affect risk of developing motor fluctuations in PD. Individuals carrying the A1 allele of the D2 *TaqIA* polymorphism have been reported to have reduced striatal D2 dopamine receptor numbers. A recent report found an excess of the A1A1 genotype in PD patients with motor fluctuations. Here, we investigated an association of the D2 *TaqIA* polymorphism with PD

#### Dopamine Receptor d2 *TaqIA* Polymorphism

Genotype frequency for PD Case plus Incidental LB

	N	A2/A2	N (%)	A2/A1	N (%)	A1/A1	N (%)	N/D	N (%)
<b>Case</b>	116	54	46.6%	40	34.5%	18	15.5%	4	3.4%
<b>Control</b>	240	102	42.5%	100	41.7%	36	15.0%	2	0.8%

Genotype frequency for PD Case

	N	A2/A2	N (%)	A2/A1	N (%)	A1/A1	N (%)	N/D	N (%)
<b>Case</b>	88	39	44.3%	33	37.5%	13	14.8%	3	3.4%
<b>Control</b>	184	79	42.9%	75	40.8%	29	15.8%	1	0.5%

Genotype frequency for Incidental LB

	N	A2/A2	N (%)	A2/A1	N (%)	A1/A1	N (%)	N/D	N (%)
<b>Case</b>	28	15	53.6%	7	25%	5	17.9%	1	3.6%
<b>Control</b>	56	23	41.1%	25	44.6%	7	12.5%	1	1.8%

8. Vesicular monoamine transporter 2 (VMAT2) polymorphism may be associated with abnormality of the VMAT 2 protein that is responsible for packaging and transport of monoamines within the cell. Disruption of this system could cause toxic levels of monoamines resulting in cell death.

#### Vesicular Monoamine Transporter 2 Polymorphism

Genotype frequency for PD Case plus Incidental LB

	N	T/T	N (%)	T/G	N (%)	G/G	N (%)	N/D	N (%)
<b>Case</b>	116	93	80.2%	21	18.1%	1	0.8%	1	0.8%
<b>Control</b>	240	207	86.3%	32	13.3%	1	0.4%	0	0.0%

Genotype frequency for PD Case

	N	T/T	N (%)	T/G	N (%)	G/G	N (%)	N/D	N (%)
<b>Case</b>	88	70	79.5%	18	20.5%	0	0%	0	0%
<b>Control</b>	184	159	86.4%	24	13.0%	1	0.5%	0	0.5%

Genotype frequency for Incidental LB

	N	T/T	N (%)	T/G	N (%)	G/G	N (%)	N/D	N (%)
<b>Case</b>	28	23	82.1%	3	10.7%	1	3.6%	1	3.6%
<b>Control</b>	56	48	85.7%	8	14.3%	0	0%	0	0%

None of the polymorphisms studied have shown a significant relationship with Parkinson's disease or with incidental Lewy bodies in the HHP/HAAS cohort.

### **Additional Genetic Work with New Funding:**

Additional genetics work that is pertinent to this grant (although now separately funded) is a collaboration with The Parkinson's Institute in Sunnyvale, CA. The long-term goal is to determine the relative contributions of genetic and environmental factors in the etiology of typical Parkinson's disease (PD). This plan to obtain specific combined data from ongoing projects to extend investigations of both risk and protective factors for PD in four unique populations: the NAS/NRC World War II Veteran Twins cohort (TWINS), the Agricultural Health Study of Farming and Movement Evaluation (FAME), the Honolulu Asia Aging Study (HAAS), and the PD Epidemiology at Kaiser project (PEAK).

One of the aims is to determine if the risk of PD is increased or decreased in individuals carrying polymorphic variants of genes encoding xenobiotic-specific membrane transporters, especially in combination with exposure to the xenobiotic substrates of these transporters. A set of 112 cases and 224 controls for whom DNA was available at the Honolulu center were selected. The DNA was extracted and aliquoted in Honolulu and then shipped to the Parkinson's institute for analysis without identifiers. Initial analyses have been completed and preliminary results are being prepared for presentation. These reveal an association of a polymorphism of the MDR1 transporter gene with Parkinson's disease ( $p=0.0061$ ).

### **Supplement: Organochlorines in the Brain and Parkinson's Disease**

**Brain Organochlorine Levels and Lewy Pathology:** In Hawaii, organochlorines were commonly used in the agricultural, industrial, and home settings against a variety of pests including mosquitoes. Chlordane (termite control) and mirex (ant control) were commonly used around homes. Methoxy, dieldrin, and heptachlor had more specific agricultural/industrial uses. Considering this use, and the evidence that organochlorines may play a role in PD etiology, we performed a pilot study to measure organochlorine compounds in frozen frontal and occipital brain tissue in 15 HAAS brains from participants with the highest exposure history to pesticides. Organochlorine pesticide and lipid analysis were performed on each of these at the Analytical and Chemical Sciences Lab, Research Triangle Institute, Research Triangle Park, in North Carolina under the direction of Dr. Edo D. Pellizzari.

Results of this pilot study indicated that several samples had levels of one or more organochlorine compounds higher than 10 ppb, levels that would be considered significantly high if found in the blood. DDE, g-chlordane, and methoxychlor were especially prominent. It is safe to say that detectable levels of these substances in any individual brain reflect exposure to those compounds during the



time that the compounds were in use. Furthermore, matching pairs of samples from frontal and occipital lobes had values that were very close suggesting that one sample is representative of total brain levels. With these promising results, organochlorine levels were then obtained on previously frozen occipital lobe brain samples from an additional 445 cohort decedents using the following method.

For the measurement of organochlorines in Dr. Pellizzari's lab, a surrogate compound (PCB congener 198) is added to the sample to monitor extraction and cleanup efficiency. The tissue is dried by grinding with one gram of anhydrous sodium sulfate. The dried sample is extracted three times with 5 mL of hexane. The extracts are combined and extract volume is adjusted to 15 mL. A known aliquot of the extract is then removed for lipid analysis, while the remainder is concentrated to 1 mL. The extract is cleaned while being eluted with three solvent systems on a column of activated Florisil (partially deactivated before use with water). The first eluate (25 mL hexane) contains DDE and other non-polar pesticides. The second eluate (25 mL 10% ether in hexane) contains the more polar pesticides, including dieldrin and endrin. The third eluate (25 mL dichloromethane) contains pesticides such as endosulfan II. The fractions are concentrated individually to 1.0 mL each. A quantitation standard (PCB congener 119) is added, and the extracts analyzed by gas chromatography using an electron capture detector.

The following analyses are preliminary. Assays for several of the organochlorines had many samples that were below the limit of calibration. For compounds where data are sufficient, examples are given in Table 3 that describes existing associations between organochlorine quartiles and the relative odds of PD/LB. Presence of PD/LB was defined as brains with LB in the SN or locus ceruleus regardless of a clinical PD diagnosis. Decedents without PD/LB were without a clinical diagnosis of PD, and all decedents were without AD

**Table 3. Relative odds of PD/LB for decedents in the top 3 quartiles of an organochlorine compound vs. decedents in the bottom quartile.**

Quartile of <b>b-BHC</b>	PD/LB		OR (95%CI)	P-value
	Absent n	Present n		
4th	19	42	1.56(0.73-3.31)	0.25
3rd	24	45	1.84(0.89-3.78)	0.10
2nd	23	55	1.44(0.70-2.95)	0.32
1st	18	62	reference	
Overall	84	204	Test for trend	0.17

Quartile of <b>g-BHC</b>	PD/LB		OR (95%CI)	P-value
	Absent n	Present n		
4th	21	44	1.91(0.84-4.33)	0.12
3rd	25	45	2.22(1.00-4.94)	0.05
2nd	26	67	1.55(0.71-3.38)	0.27
1st	12	48	reference	
Overall	84	204	Test for trend	0.08

Quartile of <b>hepox-b</b>	PD/LB		OR (95%CI)	P-value
	Absent n	Present n		
4th	24	45	1.53(0.72-3.26)	0.27
3rd	23	51	1.30(0.61-2.75)	0.50
2nd	19	58	0.94(0.44-2.03)	0.88
1st	16	46	reference	
Overall	84	204	Test for trend	0.17

Quartile of <b>oxychlordane</b>	PD/LB		OR (95%CI)	P-value
	Absent n	Present n		
4th	24	45	1.55(0.76-3.15)	0.23
3rd	18	49	1.07(0.51-2.24)	0.87
2nd	22	52	1.23(0.60-2.50)	0.57
1st	20	58	reference	
Overall	84	204	Test for trend	0.30

Quartile of <b>a-chlordane</b>	PD/LB		OR (95%CI)	P-value
	Absent n	Present n		
4th	24	52	1.85(0.88-3.89)	0.10
3rd	24	43	2.23(1.05-4.75)	0.04
2nd	21	49	1.71(0.80-3.67)	0.16
1st	15	60	reference	
Overall	84	204	Test for trend	0.09

Quartile of <b>t-nonachlor</b>	PD/LB		OR (95%CI)	P-value
	Absent n	Present n		
4th	25	54	1.31(0.63-2.71)	0.47
3rd	26	49	1.50(0.72-3.11)	0.28
2nd	16	53	0.85(0.39-1.87)	0.69
1st	17	48	reference	
Overall	84	204	Test for trend	0.25

Quartile of <b>DDD</b>	PD/LB		OR (95%CI)	P-value
	Absent n	Present n		
4th	20	44	2.03(0.94-4.38)	0.07
3rd	24	46	2.33(1.10-4.92)	0.02
2nd	25	47	2.38(1.13-4.98)	0.02
1st	15	67	reference	
Overall	84	204	Test for trend	0.08

Quartile of <b>methoxychlor</b>	PD/LB		OR (95%CI)	P-value
	Absent n	Present n		
4th	20	48	1.63(0.73-3.64)	0.23
3rd	21	52	1.58(0.72-3.50)	0.25
2nd	30	53	2.22(1.04-4.73)	0.04
1st	13	51	reference	
Overall	84	204	Test for trend	0.49

These preliminary analyses indicate that b-BHC, g-BHC, a-chlordane, DDD, and methoxychlor all show promise for having relationships with Lewy pathology. We are applying for funding to continue analysis of the organochlorine data using neuron density and dopamine/dopamine metabolite concentrations (obtained under a separately funded grant) as continuous and more heterogeneous end points rather than a discrete response (PD/LB), we are hopeful that associations with organochlorine compounds will strengthen.

## KEY RESEARCH ACCOMPLISHMENTS

1. Coffee drinking as well as caffeine consumption from non-coffee sources measured prospectively at two separate HHP examinations are associated with a decreased risk of developing Parkinson's disease up to 30 years later
2. The set of clinical features from the Unified Parkinson's Disease Rating Scale most strongly related to the presence of incidental Lewy-bodies (Lewy bodies in subjects without Parkinson's

disease) include slow “rapid alternating movements of hands”, slow “hand movements”, “action or postural tremor of hands”, and “tremor at rest”.

3. Years working on a plantation (significant) and years exposed to pesticides (non-significant) are associated with PD
4. Impaired olfaction measured approximately three years prior to death is associated with the presence of incidental Lewy bodies in the substantia nigra or locus ceruleus.
5. Service in the military during World War II and working for the military were not associated with increased risk of PD.
6. Exposure to metals was not associated with increased risk of PD in the HAAS cohort.
7. Occupational exposure to welding was not associated with increased risk of PD in the HAAS cohort.
8. Constipation measured as bowel movement frequency is associated with the future development of PD. Manuscript published in Neurology
9. Increased triceps skinfold thickness is associated with the future development of Parkinson’s disease.
10. The presence of brainstem or cortical Lewy bodies does not predispose an individual to have Alzheimer type pathology. The clinical overlap of the dementia syndromes associated with Alzheimer type pathology and Lewy bodies may be related in part to the common brain regions damaged by processes leading to these lesions.
11. Consumption of fruit but not vitamin C is associated with increased PD risk after adjustment for other PD risk factors.
12. Dietary carbohydrate intake is associated with increased risk of PD.
13. Dietary polyunsaturated fats are associated with lower risk of PD
14. There is no relationship between risk of PD and intake of total calories, saturated and monounsaturated fats, protein, niacin, riboflavin, beta carotene, vitamins A,B,C and E, and dietary cholesterol, cobalamin, and pantothenic acid.
15. There was no relationship of fertilizer exposure, years worked on a plantation, cigarette smoking, coffee consumption, or service in the military with number of parkinsonism signs present or with the total sum of the UPDRS score among elderly participants in the HAAS without PD.
16. Milk drinkers have higher risk of developing PD.
17. Olfactory Dysfunction is a predictor of future PD
18. Excessive Daytime Sleepiness is a predictor of PD
19. Depressive symptoms were not at statistically significant predictor of PD
20. Low LDL Cholesterol is a predictor of PD among men aged 71-75 at baseline.
21. Higher exposure to any metal and specifically mercury was associated with abnormal facial expression.
22. Olfactory dysfunction was associated with and incidental Lewy bodies.
23. Infrequent bowel movement frequency was associated with incidental Lewy bodies.
24. A polymorphism of the MDR1 transporter gene is associated with Parkinson’s disease.

25. BHC, g-BHC, a-chlordane, DDD, and methoxychlor found in brain tissue at death may be associated with Parkinson's disease or Lewy bodies.
26. Lewy pathology progression as suggested by the Braak staging system is supported by analysis using the HAAS population-based cohort autopsy series.
27. Preclinical indicators of Parkinson's disease are associated with early stages of synuclein deposition in the brain.
28. One-third of elderly men without a history of Parkinson's disease or dementia with Lewy bodies have Lewy pathology in the olfactory bulb at death.
29. Lewy pathology in the olfactory bulb is associated with decreased neuron density in the substantia nigra.
30. Olfactory dysfunction during life is associated with synuclein pathology in the olfactory bulb at death.

## REPORTABLE OUTCOMES

### Manuscripts

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13. Ross GW, Petrovitch H, Abbott RD, Tanner CM, Popper J, Masaki K, Launer L, White LR. Association of olfactory dysfunction with risk for future Parkinson's disease. *Ann Neurol*. 2008 Feb;63(2):167-73<sup>1</sup>
14. Huang X, Abbott RD, Petrovitch H, Mailman RB, Ross GW. Low LDL cholesterol and increased risk of Parkinson's disease: prospective results from Honolulu-Asia Aging Study. *Mov Disord*. 2008 May 15;23(7):1013-8.
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### Abstracts

1. Ross GW, White LR, Petrovitch H, Davis DG, Hardman J, Nelson J, Markesbery W, Morens DM, Grandinetti A. Association of Midlife Smoking and Coffee Consumption with Presence of Lewy Bodies in the Locus Ceruleus or Substantia Nigra at Autopsy. *Neurology* 1999;52(Suppl 2):A539
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### Invited Presentations

1. Ross GW. Pre-clinical indicators of Parkinson's disease: recent findings from the Honolulu-Asia Aging Study. Invited presentation: World Parkinson Congress, February 23, 2006. Washington DC.

2. Ross GW. Caveats in the Interpretation of Longitudinal Studies. The Second International Brainstorming Conference on Parkinson's Disease, Louisville, Kentucky, May 16-17, 2008.
3. Ross GW. The association of olfactory dysfunction with future risk of Parkinson's disease. The International Symposium on Olfaction and Taste, July 25, 2008. San Francisco, CA
4. Ross GW. Debate: Pre-Motor Parkinson's Disease Can be Diagnosed – Con. National VA Parkinson's Disease Consortium Conference, Pittsburgh, September 3-5, 2008.
5. Ross GW. Keynote Speaker, Prodromal Features of Parkinson's Disease in the Honolulu-Asia Aging Study. 22<sup>nd</sup> Annual Symposium on Etiology, Pathogenesis and Treatment of PD and OMD - Parkinson Study Group. Salt Lake City, September 21, 2008.
6. Ross GW. Honolulu-Asia Aging study (HAAS): Relationship of Organochlorine Levels with Parkinson's Disease Risk. Society of Toxicology, March 15-19, 2009, Baltimore, Maryland

## **CONCLUSIONS**

The work accomplished with our DOD funding has led to numerous opportunities for dissemination of our research findings at scientific meetings.

We have previously reported that coffee drinking may be protective against PD<sup>7</sup> and that milk consumption<sup>12</sup> is associated with an increased risk of PD. The new finding that Low LDL cholesterol is a predictor of PD among men aged 71-75 at baseline may indicate that dietary factors are related to PD risk.

In the past we have shown that increased triceps skinfold thickness, a measure of peripheral adiposity,<sup>10</sup> constipation<sup>8</sup> during mid-life, excessive daytime sleepiness<sup>13</sup> may portend the onset of the motor syndrome of PD by years. We have now added associations of olfactory dysfunction<sup>16</sup> and low blood levels of LDL-cholesterol<sup>17</sup> with incident PD and shown that these indicators may precede the extrapyramidal syndrome by years. This suggests that metabolic differences in those at higher risk for developing PD may be present years before the motor syndrome develops.

The finding that olfactory dysfunction and constipation (along with increased reaction time reported in the past<sup>18</sup>) are predictors of incidental Lewy bodies suggests that these characteristics may be useful in the early detection of Parkinson's disease. New findings indicate that Lewy pathology progression as suggested by the Braak staging system is supported by evaluation of the HAAS population-based cohort autopsy series.<sup>22</sup> According to the Braak system, one of the earliest regions affected by synuclein deposition is the olfactory bulb. One-third of elderly men without a history of Parkinson's disease or dementia with Lewy bodies have been found to have Lewy pathology in the olfactory bulb at death. Several preclinical indicators of Parkinson's disease including impaired olfaction have now been shown to be associated with this stage of synuclein deposition. Lewy pathology in the olfactory bulb has also been associated with decreased neuron density in the substantia nigra. These findings indicate that several of the preclinical indicators of Parkinson's disease are expressed very early in the process of synuclein deposition and that neuron density in the substantia nigra also begins very early in this process.



The identification of such early markers could be used to identify individuals at high risk for the development of PD. Persons so identified would be candidates to participate in drug studies aimed at disease prevention and/or might be preferentially excluded from subsequent exposure to agricultural or military chemicals having possible neurotoxicity.

Frozen samples from brains of deceased HHP/HAAS participants have been analyzed and results indicate that several of the organochlorines are detected more frequently in brains of men who also had Lewy bodies or Alzheimer changes at death. The organochlorine exposure in most of these brains took place as long as 30 years ago. We are beginning to examine the association of these levels with clinical endpoints (Parkinson's disease, parkinsonism, Alzheimer's disease, cognitive impairment) and continuing our evaluation of pathological endpoints (Lewy bodies, neuritic plaques, neurofibrillary tangles, cell counts in the substantia nigra, and striatal dopamine levels). Our ability to measure levels of organochlorine compounds in the brains of deceased participants is important for several reasons. Such data could provide direct evidence linking specific neurotoxin exposures to neurodegenerative conditions, prominently including Parkinson's disease. Although many epidemiological studies have implicated insecticides through self report, few studies have been performed that directly measure specific organochlorines in brain and report an association between these levels and PD.

Through our collaboration with the Parkinson's Institute we have noted an association of a polymorphism of the MDR1 transporter gene with Parkinson's disease. This indicates that polymorphic variants of genes encoding xenobiotic-specific membrane transporters may be associated with PD.

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### **Invited Presentations**

Ross GW. Caveats in the Interpretation of Longitudinal Studies. The Second International Brainstorming Conference on Parkinson's Disease, Louisville, Kentucky, May 16-17, 2008.

Ross GW. Debate: Pre-Motor Parkinson's Disease Can be Diagnosed – Con. National VA Parkinson's Disease Consortium Conference, Pittsburgh, September 3-5, 2008.

Ross GW. Honolulu-Asia Aging study (HAAS): Relationship of Organochlorine Levels with Parkinson's Disease Risk. Society of Toxicology, March 15-19, 2009, Baltimore, Maryland.

Ross GW. Keynote Speaker, Prodromal Features of Parkinson's Disease in the Honolulu-Asia Aging Study. 22<sup>nd</sup> Annual Symposium on Etiology, Pathogenesis and Treatment of PD and OMD - Parkinson Study Group. Salt Lake City, September 21, 2008.

Ross GW. Pre-clinical indicators of Parkinson's disease: recent findings from the Honolulu-Asia Aging Study. Invited presentation: World Parkinson Congress, February 23, 2006. Washington DC.

Ross GW. The association of olfactory dysfunction with future risk of Parkinson's disease. The International Symposium on Olfaction and Taste, July 25, 2008. San Francisco, CA.

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# Appendix 1

# NEUROLOGY

## **Frequency of bowel movements and the future risk of Parkinson's disease**

R. D. Abbott, H. Petrovitch, L. R. White, K. H. Masaki, C. M. Tanner, J. D. Curb, A. Grandinetti, P. L. Blanchette, J. S. Popper and G. W. Ross  
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# Frequency of bowel movements and the future risk of Parkinson's disease

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**Article abstract**—*Background:* Constipation is frequent in PD, although its onset in relation to clinical PD has not been well described. Demonstration that constipation can precede clinical PD could provide important clues to understanding disease progression and etiology. The purpose of this report is to examine the association between the frequency of bowel movements and the future risk of PD. *Methods:* Information on the frequency of bowel movements was collected from 1971 to 1974 in 6790 men aged 51 to 75 years without PD in the Honolulu Heart Program. Follow-up for incident PD occurred over a 24-year period. *Results:* Ninety-six men developed PD an average of 12 years into follow-up. Age-adjusted incidence declined consistently from 18.9/10,000 person-years in men with <1 bowel movement/day to 3.8/10,000 person-years in those with >2/day ( $p = 0.005$ ). After adjustment for age, pack-years of cigarette smoking, coffee consumption, laxative use, jogging, and the intake of fruits, vegetables, and grains, men with <1 bowel movement/day had a 2.7-fold excess risk of PD versus men with 1/day (95% CI: 1.3, 5.5;  $p = 0.007$ ). The risk of PD in men with <1 bowel movement/day increased to a 4.1-fold excess when compared with men with 2/day (95% CI: 1.7, 9.6;  $p = 0.001$ ) and to a 4.5-fold excess versus men with >2/day (95% CI: 1.2, 16.9;  $p = 0.025$ ). *Conclusions:* Findings indicate that infrequent bowel movements are associated with an elevated risk of future PD. Further study is needed to determine whether constipation is part of early PD processes or is a marker of susceptibility or environmental factors that may cause PD.

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Constipation is frequent in patients with PD.<sup>1-17</sup> Case reviews further suggest that constipation can precede the extrapyramidal symptoms of clinical PD by many years.<sup>1,2</sup> Such reviews, however, are subject to uncertain recall of constipation histories and to confounding due to episodes of constipation that can occur naturally with advancing age.

Clear demonstration that constipation can precede clinical PD is important because it suggests that recognition of pathogenic mechanisms in the PD process could occur before the emergence of motor symptomatology. Identification of constipation as a risk factor for PD could also help identify early or suspected disease and provide for opportunities to develop or investigate intervention strategies. Unfortunately, there are no prospective follow-up studies that confirm that constipation can precede the clinical manifestations of PD. The purpose of this report is to examine the association between the frequency of bowel movements and the future risk of PD based on 24 years of follow-up of a cohort of asymptomatic men enrolled in the Honolulu Heart Program.

**Materials and methods.** *Study sample.* From 1965 to 1968, the Honolulu Heart Program began following 8006 men of Japanese ancestry living on the island of Oahu, HI, for the development of cardiovascular disease.<sup>18-20</sup> At the time of study enrollment, subjects were aged 45 to 68 years. Initial screening consisted of a baseline physical examination and documentation of cardiac and neurologic conditions to identify prevalent cases of cardiovascular disease. Procedures were in accordance with institutional guidelines and approved by an institutional review committee. Informed consent was obtained from the study participants.

The Honolulu Heart Program is now in its 36th year of follow-up. During this period, surviving members of the original cohort participated in repeat examinations and were tracked for morbidity and mortality outcomes through a comprehensive system of surveillance that included a review of hospital discharges, death certificates, and autopsy records. As of 1990, less than 1% of the original cohort had moved off the island of Oahu resulting in an out-migration rate of about one per thousand per year. Validity studies have indicated that nearly 100% of hospital discharge events have been identified.

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For this report, follow-up for incident PD began at a repeat examination that occurred from 1971 to 1974 when information on the frequency of bowel movements was first collected. Subjects examined included 6860 men, approximately 90% of the surviving members of the original cohort. After exclusion of 64 men with missing bowel movement data and six men with prevalent PD, 6790 men remained for follow-up.

*Frequency of bowel movements and confounding information.* At the time when follow-up began (1971 to 1974), study participants were asked about their usual daily bowel movement frequency and categorized as having <1, 1, 2, and >2 bowel movements/day. Information on the use of laxatives was also collected. Other confounding information collected at the beginning of follow-up and known to be related to PD included age, pack-years of cigarette smoking, and intake of coffee.<sup>21,22</sup> Participants were also asked about jogging and intake of fruits, vegetables, and grains. Men were defined to be joggers if they reported that they jogged or ran intermittently or regularly without further characterization in terms of distance and intensity. While other measures of physical activity were not available when follow-up began (1971 to 1974), a physical activity index (an overall measure of 24-hour metabolic output) that was measured at the time of study enrollment (1965 to 1968) was also assessed.<sup>23</sup> Measurement of food and coffee intake was based on a food frequency questionnaire in which subjects were asked about consumption of these items during the previous week.<sup>21</sup>

*PD case finding and diagnosis.* For this report, 24 years of follow-up data are available on incident PD after collection of information on bowel movement frequency (1971 to 1974). 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, an ongoing 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.

Beginning in 1991, the diagnosis of PD was based on a complete screening of the participating cohort at examinations that occurred from 1991 to 1993 and again from 1994 to 1996. All subjects were questioned about diagnoses of PD, symptoms of parkinsonism (any two of rest tremor, bradykinesia, rigidity, or postural instability), and PD medications by a structured interview. Those with a history or sign of parkinsonism were referred to a study neurologist who administered standardized questions about symptoms and onset of parkinsonism, previous diagnoses, and medication use, followed by a comprehensive and standardized neurologic examination. A diagnosis of PD was based on consensus among the study neurologists according to published criteria.<sup>24</sup> These required that the subject have the following: 1) parkinsonism; 2) a progressive disorder; 3) any two of a marked response to levodopa, asymmetry of signs, asymmetry at onset, or initial onset tremor; and 4) absence of any etiology known to cause similar features. Cases of parkinsonism related to progressive supranuclear palsy, multisystem atrophy, cerebrovascular disease, drug-induced parkinsonism, post-encephalitic parkinsonism, or post-traumatic parkinsonism were not included among the cases of PD. Further description of PD case finding and diagnosis is described elsewhere.<sup>21,25</sup>

*Statistical methods.* Crude and age-adjusted incidence rates of PD in person-years were estimated according to bowel movement frequency based on the 24 years of follow-up available in the 6790 men who were examined from 1971 to 1974.<sup>26</sup> Age-adjusted risk factors across levels of bowel movement frequency were also derived.<sup>26</sup> To test for an independent effect of bowel movement frequency on PD after adjusting for age and the other covariates, proportional hazards regression models were used.<sup>27</sup> In this instance age, coffee intake, pack-years of cigarette smoking, and combined intake of fruits, vegetables, and grains were modeled as continuous variables, while jogging and laxative use were modeled as dichotomous variables (yes versus no). While frequency of bowel movements was modeled as a continuous risk factor, relative risks of PD (and associated confidence intervals) were also estimated comparing the risk of PD for men with <1 bowel movement/day to men with 1, 2, and >2/day. All reported *p* values were based on two-sided tests of significance.

**Results.** The average age of the 6790 men was 60 years (range: 51 to 75) at the time when questions were asked about usual bowel movement frequency (1971 to 1974). Over the 24-year course of follow-up, 96 men developed PD. The average age at the time of diagnosis was 73 years (range: 55 to 90), and the average time to diagnosis was 12 years (range: 2 months to 24 years).

Table 1 shows the percent of men with <1, 1, 2, and >2 bowel movements/day and the use of laxatives according to age when follow-up began. Approximately two-thirds of the men reported having 1 bowel movement/day while a quarter reported having 2/day. Overall, 4.3% of the men had <1 bowel movement/day and 6.3% had >2/day. The percent of men with infrequent bowel movements (<1/day) rose from 3.6% in men aged 51 to 55 years to 9.1% of men aged 71 to 75 (*p* < 0.001) whereas the percent of men with >2/day declined from 6.8 to 3.6% (*p* = 0.015). Although associations appear modest, the percent of men with 1 bowel movement/day also increased with age whereas the percent of men with 2/day declined. Along with the increase in infrequent bowel movements with advancing age (<1/day), there was also an increase in the reported use of laxatives. Laxative use increased from 16.4% in the youngest group of men to 29.6% in those who were the oldest (*p* < 0.001).

Table 2 describes the way in which age and the age-adjusted covariates varied according to bowel movement frequency. Age, coffee intake, and use of laxatives declined with increasing number of bowel movements/day (*p* < 0.001). Daily consumption of coffee in men with <1 bowel movement/day was (on average) 3.4 oz more than in men with >2/day (14.0 versus 10.6 oz/day). The percent of men who used laxatives was more than doubled in men with infrequent bowel movements (<1/day) as compared with men who had >2/day (44.7 versus 18.0%). Pack-years of smoking appeared to increase with frequency of bowel movements (*p* = 0.033), although there was no relation with the percent of men who were current and past cigarette smokers. Although jogging was not significantly related to bowel movement frequency, the percent of men who jogged was nearly doubled in men with >2 bowel movements/day (11.1%) versus men with <1/day (5.9%). Intake of fruits, vegetables, and grains increased significantly but modestly with increasing bowel movement frequency.

**Table 1** Percent of men with <1, 1, 2, and >2 bowel movements/day and percent of men who used laxatives according to age at the beginning of study follow-up (1971 to 1974)

Age	Sample size	Bowel movements/d				Laxative use (1402)
		<1 (289)*	1 (4371)	2 (1704)	>2 (426)	
51–55	1642	3.6	61.7	27.9	6.8	16.4
56–60	2421	3.8	63.4	26.2	6.6	19.3
61–65	1353	3.4	65.7	23.9	7.0	21.2
66–70	1011	5.7	69.0	20.6	4.7	26.8
71–75	363	9.1	65.0	22.3	3.6	29.6
Test for trend	—	$p < 0.001†$	$p < 0.001†$	$p < 0.001‡$	$p = 0.015‡$	$p < 0.001†$
Overall	6790	4.3	64.4	25.1	6.3	20.6

\* Sample size.

† Significant increase with age.

‡ Significant decrease with age.

The incidence of PD according to frequency of bowel movements is shown in table 3. Both unadjusted and age-adjusted incidence increased consistently with decreasing bowel movement frequency. The age-adjusted incidence of PD increased from 3.9/10,000 person-years in men with >2 bowel movements/day to 18.9/10,000 person-years in men with <1/day ( $p = 0.005$ ). Although modest, the average age at PD diagnosis declined consistently with decreasing bowel movement frequency. Men with infrequent bowel movements (<1/day) had a diagnosis of PD that was an average of 18 months sooner than those with >2 bowel movements/day. This latter association was not significant.

Table 4 further describes the estimated relative risk of PD in men with <1 bowel movement/day versus men whose bowel movements were more frequent. After adjustment for age and the other covariates, men with <1 bowel movement/day had a 2.7-fold excess risk of PD versus men with 1/day (95% CI: 1.3, 5.5;  $p = 0.007$ ). The risk of PD in

men with <1 bowel movement/day increased to a 4.1-fold excess when compared with men with 2/day (95% CI: 1.7, 9.6;  $p = 0.001$ ) and to a 4.5-fold excess versus men with >2/day (95% CI: 1.2, 16.9;  $p = 0.025$ ).

Although data may be too limited to carefully assess time-varying effects, the association between the frequency of bowel movements and the risk of PD appeared similar for diagnoses made early versus later into follow-up. As compared with men with  $\geq 1$  bowel movement/day, men whose bowel movements were less frequent had a 2.9-fold excess risk of PD in the first 12 years of follow-up (95% CI: 1.1, 7.6;  $p = 0.030$ ) and a similar 3-fold excess for diagnoses that were made in the 12-year period that followed (95% CI: 1.0, 8.6;  $p = 0.042$ ).

**Discussion.** Recall bias is a major weakness of case-control studies in describing an association between constipation and future risk of clinical PD.<sup>1,2</sup>

**Table 2** Average age and age-adjusted covariates according to bowel movement frequency at the beginning of study follow-up (1971 to 1974)

Covariate	Bowel movements/d			
	<1 (289)*	1 (4371)	2 (1704)	>2 (426)
Age¶	61.5 ± 6.3†	60.3 ± 5.5	59.6 ± 5.4	59.5 ± 5.0
Coffee intake, oz/d¶	14.0 ± 11.6	12.9 ± 11.1	12.0 ± 11.8	10.6 ± 9.3
Pack-years of smoking‡	34.4 ± 35.5	34.2 ± 32.5	34.5 ± 32.8	39.1 ± 35.5
Current smoking status				
Past, %	32.4	37.3	36.9	32.0
Current, %	36.3	34.3	32.9	39.5
Laxative use, %¶	44.7	20.7	17.0	18.0
Jogging, %	5.9	9.1	8.8	11.1
Intake of fruits, vegetables, and grains, g/d§	438 ± 246	432 ± 242	443 ± 250	468 ± 303

\* Sample size.

† Mean ± standard deviation.

‡ Significant increase with increasing bowel movement frequency ( $p = 0.033$ ).

§ Significant increase with increasing bowel movement frequency ( $p = 0.011$ ).

¶ Significant decrease with increasing bowel movement frequency ( $p < 0.001$ ).

**Table 3** Incidence of PD according to frequency of bowel movements

Bowel movements/d	Sample size	Incident PD cases	Incidence, rate/10,000 person-years	
			Unadjusted	Age-adjusted
<1	289	10	19.6	18.9
1	4371	66	8.0	7.9
2	1704	17	5.2	5.4
>2	426	3	3.8	3.9
Test for trend	—	—	$p = 0.002$	$p = 0.005$
Overall	6790	96	7.5	—

Patients with PD may be prone to errors in reporting of past symptoms because of uncertain recall of constipation histories that may have predated a diagnosis of PD by many years.

The major strength of this report is that data are from individuals who were asked about usual bowel movement frequency an average of 12 years prior to the development of PD. Bias that might have been introduced through the use of medications for PD is also absent. Although constipation has always been known to coexist in patients with PD, this is the first large-scale prospective study to show a significant association between infrequent bowel movements and an elevated risk of PD in later life. In addition, the risk of PD increased consistently as frequency of bowel movements decreased. Although not significant, data further suggest that infrequent bowel movements (<1/day) are also associated with an ear-

lier age at onset of PD. Among the men with PD, a diagnosis was made before the age of 60 years in two of the 10 men (20%) with <1 bowel movement/day, six of the 66 men (9.1%) with 1/day, and in none of the 20 men with  $\geq 2$ /day. Infrequent bowel movements also appeared to be associated with an elevated risk of PD for diagnoses made early and late into the 24-year follow-up considered in this report. Whether these findings can be duplicated in other prospective studies and extended to include women and other ethnic groups warrants further study.

Although there exists the possibility of diagnostic misclassification among the PD cases, with some having multiple-system atrophy rather than PD, the number of such instances is likely to be small.<sup>28</sup> In the current report, the chance of a diagnosis of an atypical parkinsonism syndrome is further reduced by consensus agreement on the presence of any two of a marked response to levodopa, asymmetry of signs, asymmetry at onset, or initial onset tremor, signs generally thought to be more specific for PD. Incidence of PD in the Honolulu Heart Program is also in general agreement with rates observed in Europe and the United States.<sup>25,29</sup> In addition, among the men with PD, 10 cases had an autopsy. Seven cases were confirmed by the presence of Lewy bodies in the substantia nigra, while pathologic examination of the remaining three has not been completed.

Although bowel movement and constipation questionnaires vary among study samples, frequency of bowel movements and use of laxatives in the men in the Honolulu Heart Program are also similar to frequencies described elsewhere.<sup>30-35</sup> In the National Health Interview Survey on Digestive Diseases, 64% reported having 7 to 13 bowel movements/week and 11.7% reported having 14 to 20/week.<sup>30</sup> Although use of laxatives in this cohort was less than in the Honolulu Heart Program, it was not markedly less (increasing from 7.4% in men aged 50 to 59 years to 19.3% in men aged 70 to 79). In the National Health and Nutrition Examination Survey, 64 to 74% recorded daily defecation.<sup>31</sup> In an industrial community, 5.1% reported having <5 bowel movements/week, 68% reported having 5 to 7/week, and 26% reported having 2/day.<sup>32</sup> The latter corresponds well with the 25.1% of men in the current cohort who reported having 2 bowel movements/day, although laxative use in this industrial community was high (27.9% in subjects aged 50 to 59 years to 50% in those who were older). In one report in which bowel movement frequency was recorded in the same manner as in the current sample, 58.9% reported having 1 bowel movement/day, approximately 30% had 2/day, with the remaining sample being evenly divided between those with <1 and >2/day.<sup>33</sup> Use of laxatives was also reported by 18.5% of the sample, similar to the Honolulu cohort.

As might be expected, men in the Honolulu Heart Program also reported using a variety of different types of laxatives. Preference for a specific laxative, however,

**Table 4** Estimated relative risk of PD in men with <1 bowel movement/day versus men whose bowel movements were more frequent

Adjustment	Risk of PD in men with <1 bowel movement/d as compared with men with 1, 2, and >2/d		
	1/d	2/d	>2/d
Age-adjusted	2.3‡ (1.2, 4.5)†	3.4§ (1.6, 7.5)	4.8   (1.3, 17.3)
Risk factor adjusted*	2.7   (1.3, 5.5)	4.1** (1.7, 9.6)	4.5†† (1.2, 16.9)

\* Adjusted for age, pack-years of cigarette smoking, coffee consumption, laxative use, jogging, and intake of fruits, vegetables, and grains.

† 95% confidence interval.

‡ Excess of PD versus men with 1 bowel movement/d ( $p = 0.013$ ).

§ Excess of PD versus men with 2 bowel movements/d ( $p = 0.002$ ).

|| Excess of PD versus men with >2 bowel movements/d ( $p = 0.018$ ).

|| Excess of PD versus men with 1 bowel movement/d ( $p = 0.007$ ).

\*\* Excess of PD versus men with 2 bowel movements/d ( $p = 0.001$ ).

†† Excess of PD versus men with >2 bowel movements/d ( $p = 0.025$ ).

did not seem to vary greatly by frequency of bowel movements or among cases and non-cases of PD. Among those who used laxatives, approximately 25% were taking milk of magnesia, citrate of magnesia, or magnesium sulfate. Over-the-counter stimulants were used by 12.1% of laxative users whereas 9.2% used prunes, 7.1% used enemas or suppositories, 5.5% used bulk-forming laxatives, and 2.5% used lubricants. Rarely used laxatives included an assortment of fruits, vegetables, cereals, tea, and coffee. Use of laxatives was not associated with the risk of PD.

Although constipation is the most common gastrointestinal disorder among patients with PD, careful comparisons with matched controls are few and equivocal. Variation among reported rates is also high. In four case-control studies, prevalence of constipation ranged from 28 to 61% in patients with PD as compared with 6 to 33% in controls.<sup>3-6</sup> Others report that constipation or prolonged transit time can afflict up to 80% of patients with PD.<sup>7</sup> Among studies suggesting that constipation can precede PD, one source reported that in a series of 178 patients with PD, 46% had constipation prior to the first symptoms of PD, whereas in spouse controls (largely women), 28% had complaints of constipation.<sup>1</sup> In another study, constipation was reported to have occurred prior to a diagnosis of PD in 10 of 12 patients by an average of 16 years.<sup>2</sup> In the Honolulu cohort, among the men with infrequent bowel movements (<1/day) who later developed PD, onset occurred an average of 10 years into follow-up (range: 5 months to 19 years).

Despite a history of documentation of an association between constipation and PD since first described by James Parkinson in 1817,<sup>36</sup> pathologic mechanisms relating constipation and PD remain poorly understood. Increased colonic transit time may be a manifestation of the same processes that cause the motor symptoms of PD. Evidence supporting this includes the findings of depletion of dopamine-producing neurons in the colon and the presence of Lewy-bodies in the myenteric plexus.<sup>8,9</sup> Importantly, delayed colonic transport in PD has been found to be independent of age, physical activity, and medication.<sup>10</sup> Additionally, CNS derangements may lead to abnormalities in skeletal muscle of the pelvic floor and anal sphincter that control defecation. Evidence for this includes radiologic studies in patients with PD demonstrating paradoxical anal sphincter muscle contraction during defecation and anorectal manometry showing hypercontractility of the external sphincter.<sup>4,8,11,12</sup> As a result, it appears that both autonomic and CNS abnormalities contribute to constipation in PD, and it is possible that these changes may be prodromal symptoms of the impending extrapyramidal syndrome.

Effects of diet and physical activity on gastrointestinal symptomatology and PD may also exist, although none has been clearly identified.<sup>8,13,14</sup> In one report based on 19 patients with PD, dietary intake of insoluble fiber was associated with improvements

in constipation, extrapyramidal function, and bioavailability of levodopa.<sup>15</sup> In the current study, adjustments for jogging and intake of fruits, vegetables, and grains had no effect on the association between bowel movement frequency and the risk of PD. Although not measured when the frequency of bowel movements was first assessed, adjustment for the overall physical activity index<sup>23</sup> that was measured at the time of study enrollment (1965 to 1967) also failed to alter the observed relation between bowel movement frequency and PD in the Honolulu sample.

As noted by others and confirmed here, defecatory dysfunction can precede the clinical diagnosis of PD, suggesting that constipation could be one of the earliest features of PD processes that predate motor symptomatology by an average of 10 years or longer.<sup>2</sup> Defecatory dysfunction is also thought to be associated with severity and duration of PD,<sup>8</sup> although such a relation has not been confirmed.<sup>16</sup> It has further been suggested that frequent and severe constipation that is resistant to therapy could be a symptom of the early signs of PD, although a careful distinction must be made from constipation that occurs naturally with advancing age.<sup>1,6,7</sup>

Failure for constipation to be relieved by laxatives could be a marker of autonomic dysfunction that precedes PD pathology, or it could be a sign that PD processes have already begun. Although data in the current report are too limited to examine constipation that is not relieved by laxatives, the risk of PD appeared highest (26.6/10,000 person-years) in the cohort of men who reported using laxatives and continued to have <1 bowel movement/day. Insufficient data also prevent a clear assessment of interaction effects between frequency of bowel movements and use of laxatives.

It may also be that bowel movement frequency in the elderly has a weaker association with future PD compared with those who are younger, simply because infrequent bowel movements in the elderly become common relative to the incidence of PD. Unfortunately, repeat measurement of bowel movement frequency in the Honolulu cohort did not occur until late into the 24-year follow-up (1991 to 1993). Although bowel movement frequency declined over this period (simply because of the effects of age), bowel movement frequencies reported at the baseline and the later examination were positively related ( $p < 0.001$ ). Using data from the later examination (1991 to 1993), future PD continued to be elevated in men with <1 bowel movement/day. In 3,397 men (aged 71 to 93 years) without PD in whom repeated bowel movement data were available, incident PD was observed in nine men. Among those who reported having <1 bowel movement/day, 1% developed PD (2/223) whereas 0.2% (7/3174) developed PD in those whose bowel movements were more frequent. Although far from conclusive based on the small number of cases, additional follow-up of this sample is expected to improve the opportunity to

more carefully assess the association between bowel movements and the future risk of PD in this elderly sample of men. In addition, the effects of infrequent bowel movements (<1/day) that may have appeared at the baseline examination (1971 to 1974) and persisted until the later examination (1991 to 1993) can also be examined.

In terms of clinical implications in the elderly, demonstration that the association between bowel movement frequency and the risk of PD weakens with advancing age means that information on bowel movement frequency must be measured as early in life as possible. The use of more comprehensive instruments for the collection of constipation histories may also be warranted. Although clinical implications must be defined, combining information on constipation with other factors, such as a positive family history and other motor deficits, could have some uses for identifying high-risk individuals for future PD. It may be worthwhile to document constipation histories from the suspected appearance of PD to its clear clinical presence. Combining constipation that is resistant to therapy with other factors could also provide a means for broadening enrollment into neuroprotective trials by including high-risk groups of asymptomatic individuals with early motor symptomatology.

In light of the observed findings from the Honolulu Heart Program and elsewhere,<sup>1-17</sup> it remains to be determined whether constipation is related to the underlying pathophysiologic processes in PD development, is a sign of early PD, or is a disease marker linked to other susceptibility or environmental factors. Identifying constipation as a risk factor for PD could lead to more effective strategies for identifying early or suspected disease and could provide for opportunities for prevention and intervention.

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# Time trends in the incidence of parkinsonism in Olmsted County, Minnesota

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**Article abstract**—*Objective:* To investigate time trends in the incidence of parkinsonism and PD over a 15-year period (1976 to 1990). *Methods:* The authors used the medical records–linkage system of the Rochester Epidemiology Project to identify incidence cases of parkinsonism in Olmsted County, MN, over three 5-year periods, 1976 to 1980, 1981 to 1985, and 1986 to 1990. PD and other types of parkinsonism were classified using defined criteria. Population denominators were derived from census data and were corrected by removing prevalent cases of parkinsonism. *Results:* Over the 15 years of the study, 364 cases of parkinsonism were identified; 154 (42%) of them had PD. The incidence of parkinsonism remained stable over the three 5-year periods for the age classes 0 to 39, 40 to 59, and 60 to 69 years. For the age class 70 to 99 years, there was some increase over time mainly owing to an increased incidence of drug-induced parkinsonism. The incidence of PD remained stable over the three 5-year periods for all age classes. Results were similar when considering men and women separately. No birth-cohort effect was present for parkinsonism. Comparison with three previous studies in the same population did not reveal any major long-term secular trends in the incidence of parkinsonism. *Conclusions:* The findings for PD over 15 years and comparison of the findings with historical data for parkinsonism over half a century suggest that no major environmental risk factors for PD (e.g., environmental toxins, drugs, diet constituents, or infectious agents) were introduced or removed from this population during these periods.

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The independent role of susceptibility genes and environmental risk factors and their interactions in the etiology of PD remain controversial.<sup>1–3</sup> Time trends in the incidence of PD may contribute to generating new etiologic hypotheses or may serve as a reference against which to test etiologic hypotheses based on laboratory findings (e.g., the recent suggestion that the pesticide rotenone could be an environmental cause of PD).<sup>4</sup> In addition, time trends in the incidence of PD and parkinsonism have public health uses for projecting the future burden of these disabling conditions and for planning medical services. Unfortunately, data on time trends are limited because it is difficult to measure the incidence of PD over time in a defined population. The limited current data are derived from counts of existing diagnoses obtained through medical record review and physician surveys or from a records-linkage system.<sup>5–9</sup>

We investigated time trends in the incidence of parkinsonism and PD over three 5-year periods (quinquennia) in Olmsted County, MN. In addition, we explored long-term trends in the incidence of parkinsonism by comparing our findings with those from three previous studies in the same population.<sup>7–9</sup> This study was made possible by the records-linkage system serving this community and was part of a broader project partly described elsewhere.<sup>10,11</sup>

**Methods.** *Case ascertainment.* We ascertained cases of parkinsonism through the records-linkage system of the Rochester Epidemiology Project, which provides the infrastructure for indexing and linking essentially all medical information of the population of Olmsted County, MN.<sup>12,13</sup> Each provider in the community employs a dossier system (or unit record) whereby all medical information for each individual is accumulated in a single record. Medical diag-

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**Frequency of bowel movements and the future risk of Parkinson's disease**  
R. D. Abbott, H. Petrovitch, L. R. White, K. H. Masaki, C. M. Tanner, J. D. Curb, A.  
Grandinetti, P. L. Blanchette, J. S. Popper and G. W. Ross  
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2002 ✓

ORIGINAL CONTRIBUTION

# Plantation Work and Risk of Parkinson Disease in a Population-Based Longitudinal Study

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**Context:** Parkinson disease (PD) has an unknown cause; however, convincing evidence is emerging that indicates pesticides can selectively injure the dopaminergic system in laboratory animals. Retrospective studies in humans demonstrate a link between exposure to agricultural lifestyle factors and PD.

**Objective:** To determine whether working on a plantation in Hawaii and exposure to pesticides are associated with an increased risk of PD decades later.

**Design and Setting:** Prospective cohort study based on the island of Oahu, Hawaii, with 30 years of follow-up. Years of work on a plantation were assessed by questionnaire at study enrollment in 1965. Self-reported information on pesticide exposure was collected at a separate examination 6 years later.

**Participants:** Participants were 7986 Japanese American men born between 1900 and 1919 who were enrolled in the longitudinal Honolulu Heart Program.

**Main Outcome Measures:** Incident PD was determined by medical record review or by an examination conducted by a study neurologist at a later date.

**Results:** During follow-up, 116 men developed PD. Age-adjusted incidence increased significantly among men who worked more than 10 years on a plantation. The relative risk of PD was 1.0 (95% confidence interval, 0.6-1.6), 1.7 (95% confidence interval, 0.8-3.7), and 1.9 (95% confidence interval, 1.0-3.5) for men who worked on a plantation 1 to 10 years, 11 to 20 years, and more than 20 years compared with men who never did plantation work ( $P = .006$ , test for trend). Age-adjusted incidence of PD was higher in men exposed to pesticides than in men not exposed to pesticides although this was not statistically significant ( $P = .10$ , test for trend).

**Conclusion:** These longitudinal observations regarding plantation work in Hawaii support case-control studies suggesting that exposure to pesticides increases the risk of PD.

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**T**HE CAUSE of Parkinson disease (PD) is unknown. There is no treatment that prevents the disease or slows progression, and there are no confirmed modifiable risk factors. However, the description in 1983 of parkinsonism secondary to exposure to the protoxin MPTP (*N*-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine) intensified the search for environmental risk factors.<sup>1</sup> The chemical structure of MPP<sup>+</sup> (1-methyl-4-pyridinium), the toxic metabolite of MPTP, is similar to the herbicide paraquat.<sup>2</sup> Additionally, the toxic mechanism of action of MPP<sup>+</sup>, inhibition of mitochondrial respiration at complex I, is similar to that of the insecticide rotenone.<sup>3</sup> Supporting a possible role for these compounds in the cause of PD are recent reports of decreased motor activity commensurate with dopaminergic system damage in rats

given rotenone and mice given paraquat and the dithiocarbamate fungicide maneb in combination.<sup>2,3</sup> In humans, there are reports of increased levels of the organochlorine compound dieldrin in brains of patients with PD compared with healthy controls and controls with Alzheimer disease.<sup>4,5</sup> These discoveries have focused suspicion on exposure to agricultural chemicals as a risk factor for PD.

Numerous case-control studies in humans have found well water drinking, farming, rural living, and exposure to pesticides and herbicides to be associated with an increased risk of PD.<sup>6-15</sup> Although these findings have been consistent, retrospective assessment of exposure can be subject to recall bias. In this article, prospectively collected data about sugarcane and pineapple plantation work among participants in the Honolulu Heart Program are used to examine the

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# NEUROLOGY

## **Midlife adiposity and the future risk of Parkinson's disease**

R. D. Abbott, G. W. Ross, L. R. White, J. S. Nelson, K. H. Masaki, C. M. Tanner, J. D. Curb, P. L. Blanchette, J. S. Popper and H. Petrovitch  
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## Midlife adiposity and the future risk of Parkinson's disease

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**Abstract—Background:** Evidence suggests that nigrostriatal system disorders are associated with PD and adiposity. Whether patterns of adiposity coexist or predate clinical PD is unknown. This report examines the relation between midlife adiposity and the risk of PD. **Methods:** Measurement of adiposity occurred from 1965 to 1968 in 7,990 men in the Honolulu Heart Program (aged 45 to 68 years and without PD). Adiposity measures included body mass index (BMI), subscapular skinfold thickness (SSF), and triceps skinfold thickness (TSF). Follow-up for incident PD occurred over a 30-year period. **Results:** During the course of follow-up, PD was observed in 137 men. Among the measures of adiposity, age-adjusted incidence of PD increased threefold from 3.7/10,000 person-years in the bottom quartile of TSF (1 to 5 mm) to 11.1/10,000 person-years in the top quartile (11 to 32 mm,  $p < 0.001$ ). Effects of TSF on PD were independent of cigarette smoking, coffee consumption, physical activity, daily caloric and fat intake, and the other measures of adiposity ( $p < 0.001$ ). Whereas rates of PD were lowest in the bottom quartile of BMI and SSF vs higher quartiles, associations with PD were weaker than they were for TSF. The effect of TSF on clinical onset before age 65 years was similar to the effect that was observed in later life. **Conclusions:** Increased triceps skinfold thickness measured in midlife is associated with an elevated risk of future PD. Whether patterns of adiposity reflect a unique metabolic pathology in individuals at a high risk of PD warrants further study.

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The cardinal signs of PD are due in large part to the loss of dopamine-producing neurons in the pars compacta region of the substantia nigra.<sup>1</sup> Nerve cell loss in other regions of the brain, including influences on the autonomic nervous system, is also known to

occur.<sup>1–7</sup> Evidence for an effect of complex nervous system interactions involving autonomic dysfunction on appetite regulation and energy metabolism,<sup>8</sup> as well as recent observations that obesity in humans is related to the depletion of striatal dopamine receptor

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availability,<sup>9</sup> suggests that nigrostriatal system disorders are associated with PD and adiposity. Whether these pathologic processes coexist or whether characteristic patterns of adiposity can predate clinical PD and its early motor symptoms is unknown. Such processes could have associations with specific forms of obesity and contribute to the complexity and heterogeneity in body fat among individuals and to its wide variation in response to exercise, diet, and other interventions. The purpose of this report is to examine the association between measures of adiposity observed in middle-adulthood and the future risk of PD based on 30 years of follow-up of a cohort of asymptomatic men enrolled in the Honolulu Heart Program.

**Materials and methods.** *Study sample.* From 1965 to 1968, the Honolulu Heart Program began following 8,006 men of Japanese ancestry living on the island of Oahu, Hawaii, for the development of cardiovascular disease.<sup>10-12</sup> At the time of study enrollment, subjects were aged 45 to 68 years. Initial screening consisted of a baseline physical examination and documentation of cardiac and neurologic conditions to identify prevalent cases of cardiovascular disease. Procedures were in accordance with institutional guidelines and approved by an institutional review committee. Informed consent was obtained from the study participants.

Since the beginning of the Honolulu Heart Program, surviving members participated in repeat examinations and were tracked for morbidity and mortality outcomes through a comprehensive system of surveillance that included a review of all hospital discharges, death certificates, and autopsy records. For this report, follow-up for incident PD began at the time of study inception (1965 through 1968). After excluding 14 men with missing measures of adiposity and two men with prevalent PD, 7,990 remained available for follow-up.

*Measurement of adiposity and confounding information.* At the time when follow-up began, body mass index (BMI) was used as a standard measure of overall adiposity (weight in kg/height in m<sup>2</sup>). Measures of central and peripheral adiposity included subscapular skinfold thickness (SSF) and triceps skinfold thickness (TSF). For both SSF and TSF, skinfold thicknesses were recorded to the nearest millimeter on the left side in the standing position using Lange calipers (Cambridge Instruments). Measurement of SSF was taken 4 cm below the angle of the scapula. For TSF, arms hung vertically with muscles relaxed while measurements were taken over the triceps muscle midway between the axilla and the elbow.

Other confounding information observed at the time of study enrollment and known to be related to PD included age, pack-years of cigarette smoking, and intake of coffee.<sup>13,14</sup> Data on the intake of caloric and dietary fat were also collected. Information on coffee consumption and daily caloric and fat intake was obtained by a dietitian based on 24-hour dietary recall methods and validated against 7-day diet records in a subset of the original cohort.<sup>15</sup> Assessment of physical activity was also made through the measurement of a physical activity index to quantify overall metabolic output during a typical 24-hour period.<sup>16-19</sup> Low levels of the physical activity index have been shown

to be associated with an increased risk of coronary heart disease and stroke.<sup>16-19</sup>

*PD case finding and diagnosis.* For this report, 30 years of follow-up data were available on incident PD after collection of information on adiposity (1965 through 1968). Before 1991, cases of PD were identified through a review of all hospital records of study participants for new and preexisting diagnoses of PD, an ongoing review of all Hawaii death certificates, and a review of medical records at the offices of local neurologists for all cohort members suspected to have PD.

After 1991, study participants were screened for PD at examinations that occurred from 1991 to 1993. During this time, all subjects were questioned about a diagnosis of PD and the use of PD medications by a structured interview. Study participants received further screening by a technician trained to recognize the clinical signs of parkinsonism (including gait disturbance, tremor, and bradykinesia). Those with a history or sign of parkinsonism were referred to a study neurologist who administered standardized questions about symptoms and the onset of parkinsonism, previous diagnoses, and medication use, followed by a comprehensive and standardized neurologic examination. A diagnosis of PD was made by the study neurologists according to published criteria without access to the risk factor data examined in this report.<sup>20</sup> These required that the subject have the following: 1) parkinsonism (e.g., bradykinesia or resting tremor combined with rigidity or postural reflex impairment); 2) a progressive disorder; 3) any two of a marked response to levodopa, asymmetry of signs, asymmetry at onset, or initial onset tremor; and 4) absence of any etiology known to cause similar features. Cases of parkinsonism related to progressive supranuclear palsy, multisystem atrophy, cerebrovascular disease, drug-induced parkinsonism, postencephalitic parkinsonism, or post-traumatic parkinsonism were not included among the cases of PD. During repeat examinations that were given from 1994 to 1996 and from 1996 to 1998, subjects were again asked about a diagnosis of PD and the use of PD medications. Medical records were further reviewed by the study neurologists who applied the same published criteria used earlier in making a diagnosis of PD.<sup>20</sup> Further description of the diagnosis of PD is described elsewhere.<sup>13,21</sup>

*Statistical methods.* Crude and age-adjusted incidence rates of PD in person-years were estimated according to ranges of BMI, SSF, and TSF based on the 30 years of follow-up in the 7,990 men who were examined from 1965 through 1968.<sup>22</sup> Age-adjusted risk factors across approximate quartiles of each adiposity measure were also derived.<sup>22</sup> To test for an independent effect of BMI, SSF, and TSF on the risk of PD, proportional hazards regression models were examined.<sup>23</sup> Adjustments were made for age, pack-years of cigarette smoking, coffee consumption, daily caloric and fat intake, and the other measures of adiposity. While BMI, SSF, and TSF were modeled as continuous risk factors, relative risks of PD (and 95% CI) were also estimated comparing the risk of PD between men in each of the top three quartiles of an adiposity measure to those in the bottom quartile. All reported *p* values were based on two-sided tests of significance.

**Results.** The average age at study enrollment (1965 through 1968) of the 7,990 men was 54 years (range, 45 to 68). During the 30 years of follow-up, 137 developed PD.

**Table 1** Incidence of PD by quartile of BMI, SSF, and TSF measured at the time of study inception (1965–1968)

Quartile (range)	Sample size	Incident PD cases	Incidence of PD, rate/10,000 person-years	
			Unadjusted	Age-adjusted
<b>BMI, kg/m<sup>2</sup></b>				
1st (14.3–21.7)	1,996	20	4.3	4.1
2nd (21.8–23.8)	1,991	41	8.5†	8.3†
3rd (23.9–25.8)	2,011	45	9.1‡	9.2‡
4th (25.9–39.9)	1,992	31	6.5	6.8
Test for trend, <i>p</i> value*			0.243	0.116
<b>SSF, mm</b>				
1st (2–10)	1,660	14	3.6	3.4
2nd (11–16)	2,656	58	9.0‡	8.9‡
3rd (17–21)	1,789	30	6.8	6.9†
4th (22–51)	1,885	35	7.7†	8.2‡
Test for trend, <i>p</i> value*			0.195	0.098
<b>TSF, mm</b>				
1st (1–5)	1,901	17	3.8	3.7
2nd (6–7)	2,205	35	6.5	6.6
3rd (8–10)	2,298	44	7.9‡	7.8‡
4th (11–32)	1,586	41	10.8§	11.1§
Test for trend, <i>p</i> value*			<0.001	<0.001

\* Based on modeling the adiposity measure as a continuous variable.

† Excess risk of PD vs men in the 1st quartile ( $p < 0.05$ ).

‡ Excess risk of PD vs men in the 1st quartile ( $p < 0.01$ ).

§ Excess risk of PD vs men in the 1st quartile ( $p < 0.001$ ).

BMI = body mass index; SSF = subscapular skinfold thickness; TSF = triceps skinfold thickness.

The average age at the time of diagnosis was 73 years (range, 54 to 89) and the average time to a diagnosis was 19 years (range, 2 to 30).

Incidence of PD by quartile of BMI, SSF, and TSF is shown in table 1. For each adiposity measure, age-adjusted incidence of PD was lowest in the first quartile as compared to quartiles that were higher. Differences in the risk of PD across the top three quartiles of BMI and SSF were not apparent. In contrast, the age-adjusted incidence of PD rose consistently from 3.7/10,000 person-years in men in the bottom quartile of TSF (1 to 5 mm) to a threefold excess (11.1/10,000 person-years) in those in the top quartile (11 to 32 mm,  $p < 0.001$ ).

Associations between potential factors that could confound the relation between an adiposity measure and the risk of PD are described in table 2. Mean ages and age-adjusted covariates that were measured at the time of study enrollment are provided across the quartiles of BMI. Comparisons across quartiles of SSF and TSF were similar.

Among the factors, age, pack-years of cigarette smoking, the percent of men who were current smokers, and the

physical activity index declined with increasing BMI ( $p < 0.05$ ). The percent of men who were past smokers increased with BMI ( $p < 0.001$ ). There was no clear relation between the daily intake of coffee, calories, and fat across the ranges of BMI. As expected, there was a positive association between BMI and the other adiposity measures ( $p < 0.001$ ).

After adjusting for the possible confounding effect of these other factors, only the association between TSF and PD remained significant. Table 3 provides the results of this latter finding. After adjustment for age, pack-years of smoking, coffee consumption, physical activity, and daily caloric and fat intake (column A), the relative risk of PD increased from 1.5 to 2.5 for men in the second to top quartile of TSF as compared to those in the first quartile. As a continuous risk factor, the rise in PD incidence with increasing TSF was significant ( $p < 0.001$ ).

To help determine if the effect of TSF on the risk of PD could be independently attributed to the peripheral location of body fat, additional adjustments were made for BMI and SSF (markers of overall and central adiposity). As seen in column B of table 3, findings suggest that the effect of TSF on PD is independent of the other adiposity measures. The increase in the observed relative risks (column B) as compared with when the effects of BMI and SSF were ignored (column A) is largely due to a small excess of PD in men with an elevated TSF who also had low levels of BMI and SSF. Tests for interaction effects between the adiposity measures on the risk of PD, however, were not significant.

The figure (top panel) further describes the association between TSF and the age-adjusted incidence of early and late PD onset (<65 and ≥65 years of age). Overall, early onset of PD occurred in 19 men (2.3/10,000 person-years), whereas late onset occurred in 118 men (10.7/10,000 person-years). Although the number of early onset cases is small, risk comparisons among the quartiles of TSF were not appreciably different from those made for the later-onset cases. The association between TSF and PD was also not significantly related to the time elapsed from the measurement of TSF to the diagnosis of PD (see the figure, bottom panel). In the first 15 years of follow-up, 42 men were diagnosed with PD (3.7/10,000 person-years), whereas 95 cases were diagnosed in the second 15 years of follow-up (11.9/10,000 person-years). As with early- and late-onset PD, effects of TSF on the risk of PD were similar for diagnoses that occurred during each 15 years of follow-up. Similar findings were also observed within other periods of follow-up.

**Discussion.** Although loss in body fat is common in patients with clinical PD,<sup>24,25</sup> reported findings have been limited to cross-sectional and case-control studies with uncertain recall and timing of anthropometric histories. Rarely are different adiposity measures (BMI, SSF, and TSF) available for the assessment of their effects on future disease. We are not aware of another study that has been able to prospectively examine the association between midlife adiposity and the future risk of PD. A major strength of the current report also includes the measurement of adiposity following a standardized protocol well before the development of PD. Because

**Table 2** Average age and age-adjusted covariates according to quartile of BMI measured at the time of study inception (1965–1968)

Covariates	Quartile of BMI			
	1st	2nd	3rd	4th
Age, y†	55.4 ± 5.8	54.6 ± 5.5	54.0 ± 5.4	53.7 ± 5.5
Coffee intake/d, dL	4.0 ± 3.6	4.0 ± 3.8	4.0 ± 3.7	3.9 ± 3.8
Pack-years of smoking*	33.9 ± 28.4	31.7 ± 29.7	30.0 ± 29.6	31.0 ± 31.2
Current smoking status, %				
Past‡	19.3	25.7	29.3	27.0
Current†	59.4	46.6	40.6	40.4
Physical activity index†	33.4 ± 4.5	32.9 ± 4.5	32.5 ± 4.4	32.4 ± 4.6
Kilocalorie intake/d	2212 ± 653	2245 ± 660	2231 ± 680	2178 ± 715
Fat intake/d, g	75.2 ± 32.7	77.7 ± 32.9	78.0 ± 34.0	77.0 ± 34.8
SSF, mm‡	10.0 ± 3.6	15.0 ± 4.6	18.2 ± 5.1	22.7 ± 6.2
TSF, mm‡	5.6 ± 2.2	7.6 ± 2.7	8.6 ± 2.9	10.1 ± 3.8

Values are mean ± SD unless otherwise indicated.

\* Covariate declines with increasing BMI ( $p < 0.01$ ).

† Covariate declines with increasing BMI ( $p < 0.001$ ).

‡ Covariate increases with increasing BMI ( $p < 0.001$ ).

BMI = body mass index; SSF = subscapular skinfold thickness; TSF = triceps skinfold thickness.

subjects did not have PD when follow-up began, effects of medication for PD on patterns of adiposity are also absent.

These findings suggest that adiposity in middle-adulthood is related to an increased risk of PD in later life. Although PD risk was consistently less in men in the bottom vs higher quartiles of each adiposity measure, associations were strongest for TSF. Here, risk of PD increased consistently with increasing TSF levels after accounting for other risk factors effects, including the simultaneous control for BMI

and SSF. In addition, associations were similar for early and late onset of PD and for diagnoses made 15 years beyond the time of adiposity measurement.

The relation between TSF and PD also seemed to persist for repeated measurements that were made from 1991 through 1993, although statistical testing may be limited because of reductions in available follow-up. During the later examination, measurements of BMI, SSF, and TSF were available in 3,512 surviving members of the original cohort aged 71 to 93 years without PD. Among this group, TSF tended to increase from baseline (1965 through 1968) values by an average of  $2.2 \pm 4.0$  mm, whereas SSF and BMI declined modestly ( $-0.3 \pm 6.7$  mm and  $-0.5 \pm 2.6$  kg/m<sup>2</sup>). Although changes in body composition are expected to occur with age, each baseline measure was positively and strongly predictive of the later (1991 through 1993) measure ( $p < 0.001$ ).

In the remaining years of follow-up, 27 men developed PD (20.3/10,000 person-years). Age-adjusted incidence of PD for men in the top quartile of TSF (12.5 to 34 mm) was 34.3/10,000 person-years vs 16.4/10,000 person-years in those who were leaner (2 to 12 mm). The incidence of PD continued to rise significantly with increasing TSF after adjustment for age, BMI, and SSF ( $p = 0.013$ ). Relations between the other adiposity measures and PD were positive but not significant. In addition, after controlling for TSF at the time of study enrollment (1965 through 1968), risk of PD rose with increasing TSF as the cohort aged, also independent of the other adiposity measures ( $p = 0.048$ ).

Explanations for the observed findings in the Honolulu Heart Program are unclear, particularly for the long-term preclinical effects of adiposity on cases

**Table 3** Estimated risk factor–adjusted relative risk of PD in men in the top three quartiles of TSF as compared to men in the 1st quartile

Quartile comparison	Risk factor–adjusted relative risk	
	A*	B†
2nd vs 1st	1.5 (0.9–2.8)	1.6 (0.9–3.0)
3rd vs 1st	1.8§ (1.0–3.2)	2.0§ (1.1–3.6)
4th vs 1st	2.5¶ (1.4–4.4)	2.8¶ (1.4–5.6)
Test for trend, $p$ value‡	<0.001	<0.001

Values are relative risk (95% CI).

\* Adjusted for age, pack-years of smoking, coffee intake, physical activity index, and daily caloric and fat intake.

† Adjusted for age, pack-years of smoking, coffee intake, physical activity index, daily caloric and fat intake, and the other measures of adiposity.

‡ Based on modeling TSF as a continuous variable.

§ Excess risk of PD vs men in the 1st quartile ( $p < 0.05$ ).

¶ Excess risk of PD vs men in the 1st quartile ( $p < 0.01$ ).

TSF = triceps skinfold thickness.



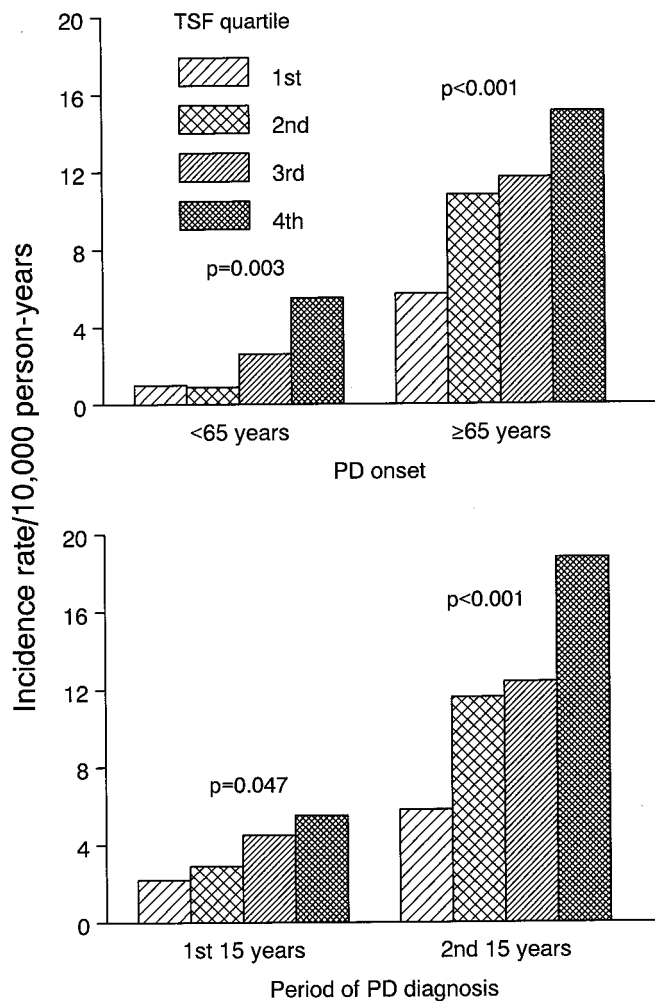


Figure. Age-adjusted incidence of early and late PD onset (<65 and ≥65 years of age) and the incidence of PD in the first and second 15 years of follow-up according to quartile of triceps skinfold thickness (TSF) measured at the time of study inception (1965 through 1968). *p* Values represent a test for trend based on modeling TSF as a continuous variable.

of PD that were diagnosed well after the time of study enrollment. Excesses in adiposity could have been the consequence of physical inactivity induced by bradykinesia and undiagnosed early PD, reflecting an insidious pathogenic process with a long latency period of more than 15 years in many instances. Such a long latency period is in contrast to the estimated 3- to 6-year preclinical period based on neuroimaging and neuropathology studies in PD.<sup>26,27</sup> Although further explanation is needed, the long interval between recorded measures of adiposity and the diagnosis of PD in our study may provide some insights into the pathogenesis of PD and related histologic changes that begin as early as 25 years of age.<sup>28</sup> Whether the mechanisms associated with the long-term preclinical effects observed in the current report are different from those that result in weight loss in patients with clinical PD is unknown.<sup>24,25</sup> In the current report, physical activity also had no effect

on the risk of PD, nor did it modify the observed relation between PD and the measures of adiposity.

Adipose tissue is also known as an important site of estrogen metabolism.<sup>29,30</sup> In light of the suggestion that endogenous estrogen in women may have a neuroprotective effect against PD,<sup>31</sup> it might be expected that the risk of PD could be reduced in those who are obese. The neuroprotective effect of estrogen, however, has not been clearly established. Others have found no such effect and further hypothesize that estrogen may be antidopaminergic.<sup>32</sup> Even in the presence of a protective effect of estrogen, estrogen levels resulting from excesses in adiposity in men may fail to reach critical levels to allow for the appearance of an inverse relation between body fat and PD. Long-term exposure to estrogen concentrations that are normally seen in premenopausal women may also be required. Estrogen metabolism may further vary according to the location of adipose tissue,<sup>30</sup> particularly between peripheral (TSF) and central (SSF) body fat. Whether findings from the Honolulu Heart Program can also apply to women warrants further study.

In addition, extensions to other population segments is also unknown, although the rate of PD described in the current report is in general agreement with rates that have been observed in Europe and the United States.<sup>21,33</sup> Nevertheless, men in the Honolulu Heart Program are unique. For example, early childhood experiences were often difficult. Study participants were either immigrants or the progeny of immigrants from Japan who migrated to Hawaii as contract laborers to serve in the sugar and pineapple industries. As a possible consequence of these experiences at an important time of physical development, subjects tended to be smaller than men of similar age in the United States.<sup>34</sup>

There also exists the possibility that mortality from other causes could have resulted in a poor estimate of the true effect of adiposity on PD, although such an effect is likely to be small. The best description of the true association between TSF and PD might actually appear in the left side of the top panel of the figure (for PD cases diagnosed <65 years). In this instance, competing risks occur too infrequently in this long-lived sample to account for the pattern of association that was observed between TSF and PD. Even within the first 15 years of follow-up, the effect of early mortality from other causes is likely to be modest. Early mortality in the top or bottom quartiles of TSF also does not explain the findings observed in this report. After adjustment for age and the other risk factors, including BMI and SSF, excluding men in the top and bottom quartiles of TSF failed to alter the observed association between TSF and PD ( $p = 0.0291$  and  $p = 0.007$  when men in the top and bottom quartiles were excluded). Duplicate analyses for BMI and SSF also failed to alter the observed associations that these body composition measures have with PD.

Although corroborating data are limited, a recent

animal study suggests that the association between adiposity and PD could be due to an increased susceptibility to environmental factors that lead to PD. In transgenic mice with genetically determined obesity, increased vulnerability to the neurotoxicants methamphetamine and kainic acid was associated with a greater decrease in levels of striatal dopamine and tyrosine hydroxylase and to elevated levels of glial fibrillary acidic protein, a sensitive indicator of neuronal damage.<sup>35</sup> Body fat may also act as a reservoir for lipid-soluble neurotoxins that selectively damage dopamine-producing neurons in the substantia nigra. Regional differences in fatty tissue turnover and neurotoxin release from these regions may also explain the stronger association of peripheral body adiposity (TSF) to PD as compared to overall (BMI) and central (SSF) adiposity.

Obesity could also be directly linked with derangements in dopaminergic systems that increase the risk of PD. Recently, obesity in humans has been associated with a decrease in dopamine receptor availability in a study using [*c*-11] raclopride to measure D<sub>2</sub> dopamine receptors with PET.<sup>9</sup> Increases in appetite and weight have also been associated with drugs that block dopamine D<sub>2</sub> receptors,<sup>36,37</sup> whereas treatment with levodopa is often associated with weight loss and appetite suppression.<sup>38</sup> It is possible that decreased D<sub>2</sub> receptors in obese individuals could lead to compensatory increases in dopamine turnover, consequent increases in oxidative metabolites, and eventually, to an increase in oxidative stress and neuronal death.

High caloric and fat intake, including the intake of dietary cholesterol, has also been observed in patients with PD,<sup>39,40</sup> although an association between these dietary items (including iron and animal fat) and the future risk of PD was not observed in the Honolulu sample. There also exists the possibility that such effects were not apparent because of the underreporting of dietary intake in subjects who were overweight.<sup>41,42</sup> Such underreporting in the current study, however, does not seem to explain the relation that was observed between adiposity and PD because excesses in PD were also observed in the leaner men in the second quartile of each body composition measure as compared to the first quartile (see table 1).

Although clinical implications are difficult to address based on findings from the current report, identifying patterns of adiposity that predate clinical PD could suggest that subtle PD processes have the potential for being recognized before the emergence of motor symptomatology. Combining information on adiposity with other factors, such as a positive family history or early signs of developing movement abnormalities, could have some uses for identifying high-risk individuals for future PD. In light of the evidence that pathologic processes in PD may include effects on adiposity,<sup>8,9,36-40</sup> further studies of susceptibility and environmental factors that may

increase the risk of PD in individuals with characteristic patterns of adiposity appear to be warranted.

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**Midlife adiposity and the future risk of Parkinson's disease**

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## Environmental, life-style, and physical precursors of clinical Parkinson's disease: recent findings from the Honolulu-Asia Aging Study

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■ **Abstract** *Background* Increased westernization with Japanese migration to the U. S. in the early 20<sup>th</sup> century is thought to have altered the risk of cardiovascular disease. Whether similar effects include changes in the risk of Parkinson's disease (PD) is not clear. This report describes the relations between environmental, life-style, and physical attributes and the incidence of PD that have been observed in the Honolulu-Asia Aging Study. *Methods* Beginning in 1965, environmental, life-style, and physical attributes were recorded at selected examinations in a cohort of 8,006 Japanese-American men. Subjects were followed for clinical

PD. *Findings* During 30 years of follow-up, PD was observed in 137 men. Overall incidence (7.1/10,000 person-years) was generally higher than in Asia and similar to rates observed in Europe and the U. S. Precursors of PD included constipation, adiposity, years worked on a sugar or pineapple plantation, years of exposure to pesticides, and exposure to sugar cane processing. Factors showing an inverse association with PD included coffee intake and cigarette smoking. Among dietary factors, carbohydrates increased the risk of PD while the intake of polyunsaturated fats appeared protective. Total caloric intake, saturated and monounsaturated

fats, protein, niacin, riboflavin, beta-carotene, vitamins A, B, and C, dietary cholesterol, cobalamin,  $\alpha$ -tocopherol, and pantothenic acid showed no clear relation with clinical PD. *Interpretation* Findings suggest that several environmental, life-style, and physical attributes appear to be precursors of PD. Whether patterns of precursors can be used to identify individuals at high risk of future PD or can broaden the scope of early interventions or recruitment into neuroprotective trials warrants further study.

■ **Key words** Parkinson's disease · risk factor · epidemiology

## Introduction

Increased westernization with Japanese migration to the U. S. in the early 20<sup>th</sup> century is thought to have altered the incidence of cardiovascular disease through changes in diet, behavior, and the environment [12, 20, 36, 38, 44, 46]. Whether similar effects include alterations in the risk of Parkinson's disease (PD) is not known, although worldwide differences in the incidence of PD suggest that geographic variation in unknown risk factor exposures may have a role in its etiology [29, 47]. This report describes the relations between environmental, life-style, and physical attributes and the incidence of PD that have been observed in the Honolulu-Asia Aging Study.

## Background and resources

### ■ Study sample

From 1965 to 1968, the Honolulu Heart Program began following 8,006 men of Japanese ancestry living on the island of Oahu, Hawaii for the development of cardiovascular disease [15, 19, 45]. At the time of study enrollment, subjects were aged 45 to 68 years. Initial screening included a baseline physical examination and documentation of cardiac and neurologic conditions to identify prevalent cases of cardiovascular disease. Additional follow-up included repeat examinations and the tracking of morbidity and mortality outcomes through a comprehensive system of surveillance based on a review of all hospital discharges, death certificates, and autopsy records. Within the Honolulu Heart Program, the Honolulu-Asia Aging Study was established in 1991 for dedicated research on neurodegenerative diseases and cognitive function in the elderly. Procedures were in accordance with institutional guidelines and approved by an institutional review committee. Informed consent was obtained from the study participants.

### ■ PD case finding and diagnosis

In this report, 30 years of follow-up data were available on incident PD since the time of study inception [1965–1968]. Prior to 1991, cases of PD were identified through a review of all hospital records for new and pre-existing diagnoses of PD. Ongoing reviews also included a thorough evaluation of Hawaii death certificates and the medical records of local neurologists for cohort members suspected to have PD.

After 1991, study participants were screened for PD at examinations that occurred from 1991 to 1993. All subjects were questioned about a diagnosis of PD and the use of PD medications by a structured interview.

Study participants received further screening by a technician trained to recognize the clinical signs of parkinsonism (including gait disturbance, tremor, and bradykinesia). Those with a history or sign of parkinsonism were referred to a study neurologist who administered standardized questions about symptoms and the onset of parkinsonism, previous diagnoses, and medication use, followed by a comprehensive and standardized neurologic examination. A diagnosis of PD was made by the study neurologists according to published criteria without access to the risk factor data examined in this report [43]. These required that the subject have the following: 1) parkinsonism (e. g., at least two of the four cardinal features: bradykinesia, rest tremor, rigidity, or postural reflex impairment); 2) a progressive disorder; 3) any two of a marked response to levodopa, asymmetry of signs, asymmetry at onset, or initial onset tremor; and 4) absence of any etiology known to cause similar features. Cases of parkinsonism related to progressive supranuclear palsy, multi-system atrophy, cerebrovascular disease, drug induced parkinsonism, post-encephalitic parkinsonism, or post-traumatic parkinsonism were not included among the cases of PD. During repeat exams that were given from 1994 to 1996 and from 1996 to 1998, subjects were again asked about a diagnosis of PD and the use of PD medications. Medical records were further reviewed by the study neurologists who applied the same published criteria used earlier in making a diagnosis of PD [43]. Further description of the diagnosis of PD is described elsewhere [29, 33].

### ■ Statistical methods

After the measurement of an environmental, life-style, or physical attribute, age-adjusted incidence rates of PD in person-years were estimated according to various attribute levels [17, 23]. All subjects were free of PD when follow-up began at the examination when an attribute was first observed. Independent effects of an attribute on the risk of PD were examined through the use of proportional hazards regression models [7]. Relative risks of PD were also estimated comparing the risk of PD between attribute levels. For analyses based on a small number of PD cases, p-values were estimated from permutation tests for exact logistic regression [27]. All reported p-values were based on two-sided tests of significance.

## Findings

Among the men enrolled in the Honolulu-Asia Aging Study, the average age at the time of study inception (1965–1968) was 54 years (range: 45–68). In 30-years of follow-up, 137 developed PD (7.1/10,000 person-years).

The average age at the time of diagnosis was 73 years (range: 54–89), and the average time to a diagnosis was 19 years (range: 2–30). Although we describe new observations from the Honolulu-Asia Aging Study, sample sizes and event counts may vary due to when follow-up began or through updated evidence for a definitive diagnosis of PD. In addition to the presentation of new data, we also expand on findings in earlier manuscripts from the Honolulu-Asia Aging Study based on sample sizes and event counts that were used in those original reports.

### ■ Cigarette smoking and coffee intake

Data from a variety of sources suggest that smoking is protective against PD [13, 33], although the biological basis that underlies the relation between smoking and PD is poorly understood. Identification of a protective effect of smoking is important since it could shed light on the unknown pathogenic mechanisms of PD along with similar relations that have been observed in Alzheimer's disease [14, 41].

Prospective follow-up in the Honolulu-Asia Aging Study confirms that cigarette smoking is inversely related to the risk of clinical PD [13, 33]. In the most recent report from Hawaii [33], 51% of all PD cases (52/102) occurred in 28% of the men who reported that they never smoked cigarettes. Among the 52 cases, only 19 would have been expected to occur had the risk of PD been similar to those who were former or current smokers. The association between smoking and PD is also unexplained by early mortality in men who smoked cigarettes and is independent of other factors that have been linked to PD, including the intake of coffee.

In addition to cigarette smoking, coffee has also been shown to have a protective effect on the risk of PD [33]. An effect further appears to be reproducible for different follow-up periods and with different methods of quantifying coffee intake (24-hour recall methods versus food frequency questionnaires). Based on 30 years of follow-up, nondrinkers of coffee experienced a 5-fold excess in the risk of PD as compared to men who consumed 28 oz/day or more (10.4 versus 1.9/10,000 person-years, respectively). The risk of PD also declined consistently with each increase in amount of coffee consumed ( $p < 0.001$ ). Among all PD cases, 31% (32/102) occurred in the 16% of men who reported that they were nondrinkers of coffee. Among the 32 cases, only 13 would have been expected to occur had the risk of PD been similar to those who consumed any amount of coffee.

For both cigarette smoking and coffee intake, effects are independent and strong. In the Honolulu-Asia Aging Study [33], the highest rate of PD occurred in men who neither smoked cigarettes nor drank coffee (15.1/10,000 person-years) as compared to an absence of PD in current smokers and those who consumed the most amount

of coffee on a daily basis ( $\geq 28$  oz/day). Although cigarette smoking reduced the risk of PD, there was a near constant dose-response relation between coffee intake and PD incidence for men who never smoked cigarettes, for those who were past smokers, and for those who were current smokers.

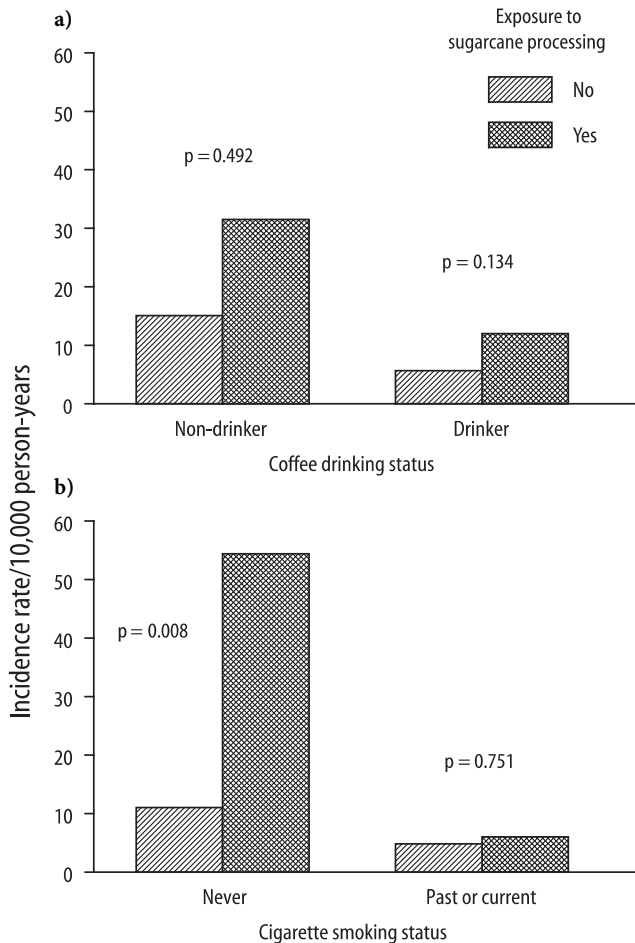
### ■ Plantation work

In 1983, a description of parkinsonism in heroin addicts exposed to the neurotoxin MPTP intensified the search for environmental risk factors for PD [24]. MPTP is a contaminant contained in a synthesized recreational narcotic that has similarities in structure to the herbicide paraquat [37]. MPTP also has a toxic mode of action comparable to the insecticide rotenone [5]. Since these reports first appeared, special efforts have focused on identifying a role of agricultural chemicals in the etiology of PD. Subsequently, numerous case-control studies have found that well water, farming, rural living, and exposure to pesticides are associated with an increased risk of PD [32].

Political and social pressures that led to the migration of Japanese to the U. S. in the early 20<sup>th</sup> century help make the Honolulu-Asia Aging Study a valuable resource for the study of the relation between a constellation of factors associated with agriculture and the risk of PD. Cohort members were either immigrants or the progeny of immigrants from the same regions of Japan who migrated to Hawaii as contract laborers to serve in the sugar and pineapple industries. It provides a useful opportunity to examine the effects of a dominant and relatively homogeneous industry (plantation work) on the risk of PD.

Based on 30-years of follow-up, recent data have demonstrated that differences in the risk of PD are modest for men who spent 10 years or less as a plantation worker, while beyond 10 years, risk of PD nearly doubles [32]. For men who worked 10 years or less on a plantation, incidence of PD ranged from 5 to 6/10,000 person-years as compared to 10.3/10,000 person-years in those who worked more than 20 years ( $p = 0.011$ ). Although findings were based on accurate plantation work histories that were collected at the beginning of study inception [1965–1968], specific data on sugarcane and pineapple plantation exposures were not available.

Nevertheless, at repeat examinations that occurred 6 years into follow-up [1971–1974], participants received an additional exam where inquiries were made about nonspecific exposures to sugarcane processing that lasted for at least a year. Based on 24-years of follow-up after this exam, data suggest that sugarcane processing is associated with the risk of PD. As seen in Fig. 1, however, the association appears most apparent in men who did not smoke cigarettes. For nonsmokers, the incidence



**Fig. 1** Age-adjusted incidence of PD according to exposure to sugarcane processing for at least 1 year as reported at physical examinations received from 1971 to 1974 within coffee drinking (a) and cigarette smoking (b) strata

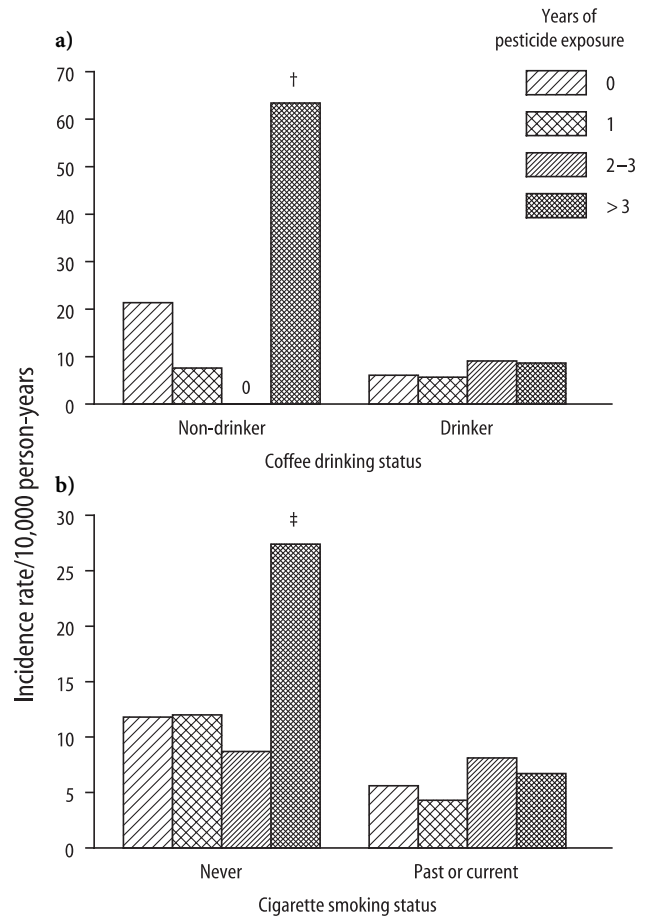
of PD was increased by nearly 5-fold in those exposed to sugarcane processing as compared to those who were not (Fig. 1b,  $p = 0.008$ ). Regardless of coffee drinking status, the corresponding risk was 2-fold (Fig. 1a), although not statistically significant. For non-cigarette smokers, the effect of sugarcane processing on the risk of PD also appeared to be independent of years worked on a plantation. Additional interpretations suggest that cigarette smoking is associated with a reduced susceptibility to PD that might otherwise be attributed to sugarcane processing.

### ■ Exposure to pesticides

Findings of an association between plantation work and the risk of PD in the Honolulu sample [32] has further suggested that increasing years of exposure to pesticides also elevates the risk of PD, although results were not statistically significant ( $p = 0.101$ ). As with sugarcane

processing, however, additional analyses indicate that cigarette smoking reduces the susceptibility to PD associated with pesticide exposure. Reduced susceptibility also seems to occur for coffee drinkers.

The risk of PD in men who drank coffee (Fig. 2a right panel) or smoked cigarettes (Fig. 2b right panel) appeared unrelated to years of exposure to pesticides. In the absence of these factors, however, susceptibility to pesticides seems to increase. Among nondrinkers of coffee, risk of PD was 3-times higher in men who were exposed to pesticides for more than 3 years (63.4/10,000 person-years) as compared to men with no exposure to pesticides (21.4/10,000 person-years,  $p = 0.044$ ). Risk of PD in nonsmokers also seemed to increase susceptibility to pesticides for exposures beyond 3 years versus men who were not exposed (27.4 versus 11.8/10,000 person-years,  $p = 0.053$ ). While interactions were not statistically significant, such findings suggest that the risk of PD may have multi-factorial origins and variations in susceptibilities.



Significant excess versus unexposed men: † $p = 0.044$ , ‡ $p = 0.053$

**Fig. 2** Age-adjusted incidence of PD according to self-reported years of pesticide exposure reported at physical examinations received from 1971 to 1974 within coffee drinking (a) and cigarette smoking (b) strata



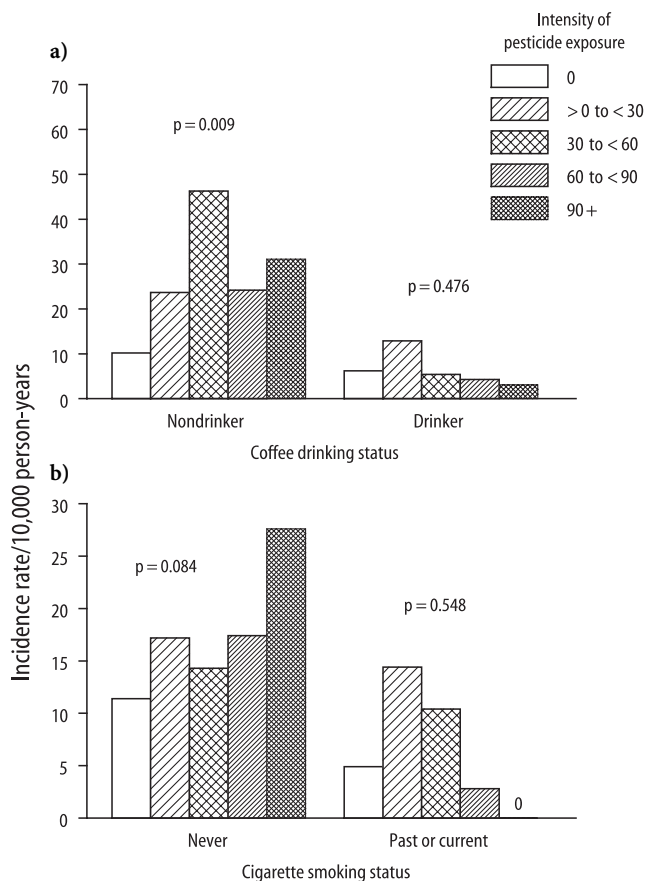
Unfortunately, the data in Fig. 2 are based on self-reported exposures to pesticides at either work or at home. While reported responses can be quite variable, documentation of home exposure is difficult since it depends on individual recall and knowledge about product contents and cumulative exposure experiences. Regular exposure to pesticides at work may also have been more common than perceived, and many who reported not being exposed could have had high levels of exposure.

In response to these issues, exposure to pesticides was independently estimated using occupation and industry codes created by the U. S. Bureau of the Census [40] that were collected among the study participants at the time of study inception [1965–1968]. Through resources available at the U. S. National Institute for Occupational Safety and Health, a measure of exposure was assigned to each occupational and industrial code combination with the following definitions: 0 = none, 1 = low, 2 = moderate, and 3 = high. In addition to data on usual occupation and industry, years spent in these occupations and industries were also collected. Based on these additional data, an overall measure of intensity to pesticide exposure was created by multiplying the exposure associated with an occupational and industrial code combination (0, 1, 2, or 3) by the number of years spent in that job related combination. The average value of the overall intensity measure was 4.6 (range: 0 to 153).

Based on the occupation and industry work histories, an association between pesticide exposure and the risk of PD appears to be confirmed (Fig. 3). As with the self-reported measure, susceptibility to PD seemed reduced in men who smoked cigarettes or drank coffee. For nondrinkers of coffee, however, there is a near linear increase in the incidence of PD with increasing intensity of pesticide exposure ( $p = 0.009$ ). A similar trend also seems to occur in nonsmokers, although it is not statistically significant ( $p = 0.084$ ).

### ■ Constipation

Since the time of James Parkinson, constipation has been known to be common in patients with PD [31]. Recent data suggest that up to 80% of PD patients are afflicted with constipation [18], and some believe that defecatory dysfunction could be associated with PD severity and duration [9]. Although subject to uncertain recall of constipation histories, two case reviews further suggest that constipation may predate PD. In one series, 178 PD patients were asked to recall their bowel habits prior to the diagnosis of PD. Among this group, 46% reported having constipation, while in spouse controls (largely women), 28% had complaints of constipation [22]. In another report, constipation was reported to



**Fig. 3** Age-adjusted incidence of PD according to intensity of pesticide exposures associated with industrial and occupational codes recorded at physical examinations received at the time of study enrollment (1965–1968) within coffee drinking (a) and cigarette smoking (b) strata

have occurred prior to a diagnosis of PD in 10 of 12 patients by an average of 16 years [4].

Recently, the Honolulu-Asia Aging Study has more clearly demonstrated that constipation predates PD based on 24 years of follow-up after data were first collected on bowel movement frequency at examinations that occurred from 1971 to 1974 [1]. A major strength of this finding is that it is based on the collection of bowel movement patterns following a standardized research protocol well before the development of PD. Here, age-adjusted incidence declined consistently from 18.9/10,000 person-years in men with < 1 bowel movement/day to 3.8/10,000 person-years in those with > 2/day ( $p = 0.005$ ). Use of cigarettes and coffee intake failed to alter the association between bowel movement frequency and the risk of PD.

Data further suggest that the greatest risk of PD is likely to occur when constipation is resistant to treatment. In the Honolulu sample, the age-adjusted risk of PD was highest (51.6/10,000 person-years) in the cohort of men who reported using laxatives at least 2 times per

week and continued to have < 1 bowel movement/day (see Fig. 4). Among heavy users of laxatives, rates of PD declined as bowel movement frequency increased ( $p = 0.009$ ), suggesting that the type of constipation associated with PD (unresponsive to therapy) is unique. This seems reasonable since most constipation is unrelated to PD.

### ■ Body fat distribution

While loss in body fat is common in patients with clinical PD [6, 8], reported findings based on cross-sectional and case-control studies (with uncertain recall and timing of anthropometric histories) are far from clear. In a recent mouse study with genetically induced obesity, there was an increased vulnerability to the neurotoxins methamphetamine and kainic acid through reductions in levels of striatal dopamine and tyrosine hydroxylase and to elevated levels of glial fibrillary acidic protein, a sensitive indicator of neuronal damage [35]. Evidence for an effect of complex nervous system interactions involving autonomic dysfunction on appetite regulation and energy metabolism [21], and recent ob-

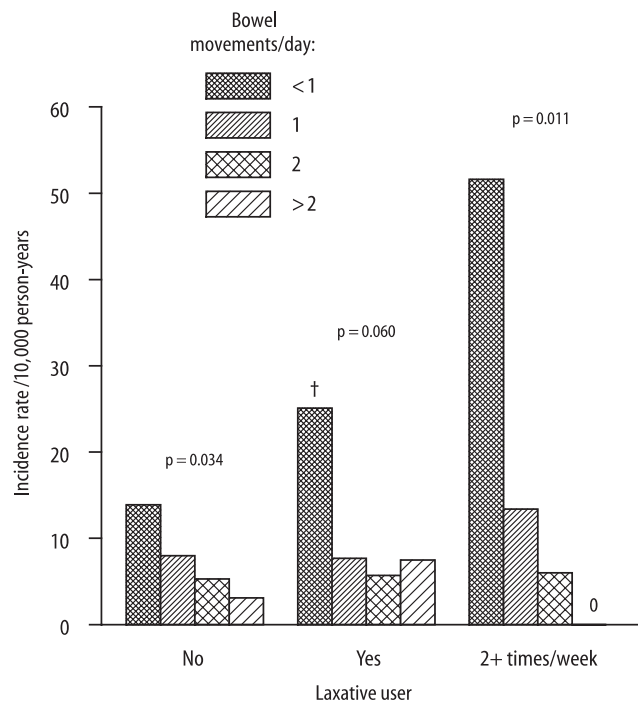
servations that obesity in humans is related to the depletion of striatal dopamine receptor availability suggests that nigrostriatal system disorders have associations with both PD and adiposity [42].

To help address this issue more clearly, the Honolulu-Asia Aging Study was able to access archived data on body composition that was collected at more than one physical examination following standardized procedures of measurement [2]. Based on measurements of body mass index (BMI), subscapular skinfold thickness (SSF), and tricep skinfold thickness (TSF) at the time of study enrollment [1965–1968], the leanest group of men were found to experience the lowest incidence of PD over 30-years of follow-up. Among the measures of adiposity, age-adjusted incidence of PD increased consistently by three-fold from 3.7/10,000 person-years in the bottom quartile of TSF (1–5 mm) to 11.1/10,000 person-years in the top quartile (11–32 mm,  $p < 0.001$ ). Associations of TSF with PD were also independent of cigarette smoking, coffee consumption, physical activity, daily caloric and fat intake, and the other measures of adiposity ( $p < 0.001$ ). While rates of PD were lowest in the bottom quartile of BMI and SSF versus higher quartiles, associations with PD were weaker than they were for TSF. The association of TSF with clinical onset before age 65 years was similar to that observed in later life. Neither cigarette smoking nor coffee intake reduced the susceptibility to PD that was associated with an elevated TSF.

In addition to levels of adiposity observed in middle adulthood [2], those that were measured in later life also appeared to be related to the risk of clinical PD (see Fig. 5).

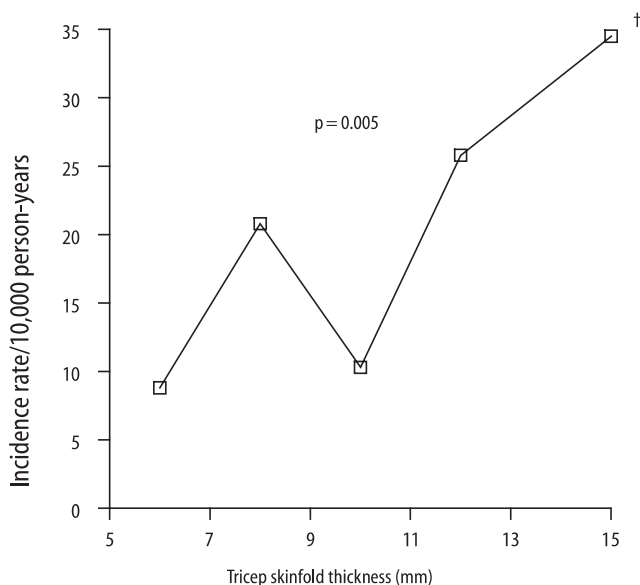
During a repeat physical examination that was given from 1991 to 1993, measurements of BMI, SSF, and TSF were available in 3,512 surviving members of the original cohort aged 71 to 93 years. In the remaining 5 to 7 years of follow-up, 27 men developed PD (20.3/10,000 person-years). Age-adjusted incidence of PD for men in the bottom quintile of TSF (2–6.5 mm) was 8.8/10,000 person-years versus 34.5/10,000 person-years in those in the top quintile (13–30 mm).

Although it might be expected that the small number of PD cases would limit statistical power, the incidence of PD continued to rise significantly with increasing TSF ( $p = 0.005$ ). Effects also remained significant after adjustment for age, BMI, and SSF ( $p = 0.013$ ). As with adiposity measures observed in mid-life, associations between BMI and SSF were not statistically significant. These findings further suggest that the association between adiposity and PD observed in middle adulthood also extends to the elderly.



†Significant excess of PD vs. men with more frequent bowel movements ( $p = 0.009$ ).

**Fig. 4** Age-adjusted incidence of PD according to bowel movement frequency and the use of laxatives reported at physical examinations received from 1971 to 1974. The p-values represent a test for trend based on modeling bowel movement frequency as a continuous variable



† Plotted according to median tricep skinfold thickness within a quintile.

**Fig. 5** Age-adjusted incidence of PD by median levels of tricep skinfold thickness within quintile ranges in men aged 71 to 93 years at physical examinations received from 1991 to 1993. The p-value represents a test for trend based on modeling tricep skinfold thickness as a continuous variable

## ■ Dietary intake

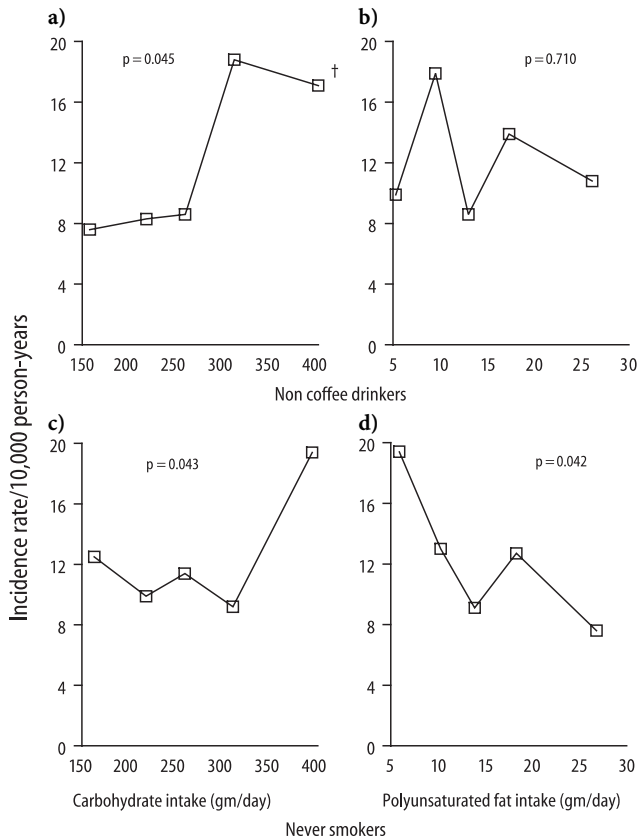
Studies of the relation between diet and PD often report conflicting results. Most are based on case-control designs with the usual limitations involving uncertain recall of past dietary behaviors. In one case-control study, comparisons were made between dietary histories using food-frequency questionnaires [16]. Patients with PD were found to have consumed higher levels of carbohydrates and lower amounts of beta-carotene and niacin prior to disease onset than controls. There were no apparent associations observed between protein and fat intake and PD. In contrast, others report that higher caloric and fat intake consumed during the year prior to study enrollment are associated with PD, while there was no association with carbohydrates [25]. Based on measures of dietary habits followed during most of adult life, an increase in the intake of animal fat and vitamin D was described in patients with PD versus matched controls [3].

Similar dietary data were collected in the Honolulu-Asia Aging Study at the time of study enrollment [1965–1968] with 30 years of follow-up for the first appearance of clinical PD. Here, nutrient intake was determined by a dietitian based on 24-hour recall methods and validated against a full week of dietary records in a subset of the original cohort. Comparisons between the two assessment methods showed no significant differences between the instruments for measuring dietary

intake, and day-to-day variation was less than typical in western cultures [26]. While errors in recall are less of an issue here, it is likely that other errors in measurement (also shared with case-control studies) are not entirely removed. For example, 24-hour recall may not reflect typical dietary patterns, and some groups, particularly obese individuals, often under-report true dietary intake [34, 39]. Nevertheless, these types of studies are often considered to be the best available. In the presence of the errors in data collection in dietary surveys, it is likely that the observed effects of food intake on disease provide an underestimate of true associations. Unfortunately, because of the high level of correlation that exists among dietary intake variables, identifying specific relations is extremely difficult in any cohort or case-control study.

Based on the calculation of micronutrient intake from the 24-hour recall data in the Honolulu-Asia Aging Study, there is some consistency with associations that have been reported elsewhere, while most appear to be absent. Among the latter, total caloric intake, protein, niacin, riboflavin, beta-carotene, vitamins A, B, and C, dietary cholesterol, cobalamin,  $\alpha$ -tocopherol, and pantothenic acid had no clear relation with clinical PD. Although the intake of vitamin E in the Honolulu-Asia Aging Study was modestly related to a reduced odds of PD, legumes (a food rich in vitamin E) were associated with a marked protective effect [30]. Associations appeared for other dietary variables, but most consistently in subjects who were nonsmokers and nondrinkers of coffee. Further work in this area is ongoing in the Honolulu-Asia Aging Study.

Among the associations identified thus far, intake of carbohydrates and polyunsaturated fats appear to have the most consistent relation with the risk of PD. Associations observed in the Honolulu-Asia Aging Study are described in Fig. 6 for those who were nondrinkers of coffee (Fig. 6a, b) and in those who reported never smoking cigarettes (Fig. 6c, d). Here, the age-adjusted incidence of PD is plotted by median intake values within quintile ranges of the daily intake of carbohydrates (Fig. 6a, c) and polyunsaturated fat (Fig. 6b, d) based on dietary intake that was observed at the time of study enrollment (1965–1968). For carbohydrates (Fig. 6a, c), PD incidence rose significantly with increasing intake for both non coffee drinkers and never smokers ( $p < 0.05$ ). Differences in the risk of PD, however, were modest up to the 4<sup>th</sup> and 5<sup>th</sup> quintiles of carbohydrate intake. In contrast, the intake of polyunsaturated fats appeared protective against PD, particularly in men who never smoked cigarettes ( $p = 0.042$ ). For those who were never smokers of cigarettes, the effects of carbohydrates and polyunsaturated fats were also independent of each other. Saturated and monounsaturated fats were unrelated to the risk of PD in this sample of men.



† Plotted according to median intake within a quintile.

**Fig. 6** Age-adjusted incidence of PD by median intake values within quintile ranges of daily intake of carbohydrates and polyunsaturated fats at the time of study enrollment [1965–1968] for nondrinkers of coffee and in nonsmoking men. The p-values represent a test for trend based on modeling each intake value as a continuous variable

## Discussion

While geographic variation in the incidence of PD is consistent with an environmental role in the development of PD, more convincing evidence is based on differences in the risk of PD that have been observed to occur with migration. For example, migration from Asia and western Africa to the U.S. has resulted in an increase in the incidence of PD within these ethnic communities as compared to reported rates from countries of origin [29, 47]. The incidence of PD in the Japanese-American men enrolled in the Honolulu-Asia Aging Study is also higher than in Japan and are typical of rates that have been observed in Europe and the U.S. Although difficulties in how PD is defined can contribute to these differences, findings from the current report suggest that a role of environmental, life-style, and physical attributes on the risk of PD is real. Specifically, observations suggest that precursors associated with PD can include coffee intake, cigarette smoking, plantation work, exposure to pesti-

cides, constipation, body fat distribution, and possibly diet.

Although associations between these precursors and PD are important, it is also noteworthy to draw attention to the long delay from the time of precursor measurement to the time of diagnosis of clinical PD. In many instances, delays in diagnosis exceeded 15 years after risk factor measurement [2]. Such long latency periods are in contrast to the estimated 3 to 6 year preclinical periods based on findings from neuroimaging and neuropathology studies [10, 28]. While further explanation is needed, the long interval between precursor measurement and the diagnosis of PD may provide some insights into the pathogenesis of PD and to Lewy body formation that can begin as early as 25 years of age [11]. The possibility that PD neuropathology has origins in early life suggests that PD progression is slower and more subtle than previously thought. Based on an increased risk of PD due to long-term exposures to pesticides and plantation work in the Honolulu-Asia Aging Study, this may also mean that the development of PD is not inevitable if exposures can be limited or removed. It further suggests that prevention of PD could begin in early adulthood.

Explanations for the observed relations between a precursor and PD are unclear. It must first be noted that the term precursor does not imply that factors associated with PD are casual or are the result of processes leading to PD. At the very best, findings merely suggest that these factors can predate a diagnosis of clinical PD. In some instances, cumulative exposure to a precursor during early life may contribute to increased PD risk indirectly by increasing susceptibility to other factors that cause PD in later life.

In other cases, the effects of these precursors on PD progression could be more direct. Dietary intake of antioxidants could reduce oxidative stress and free-radical damage to neurons in the substantia nigra. Others have suggested that toxic levels of iron and manganese promote oxidative stress [3]. Effects of coffee and cigarette smoking could be important by modulating neurotransmitter and neuroreceptor systems in the brainstem and corpus striatum or by directly interfering with the uptake of neurotoxins [13, 33]. Findings of an association between pesticides and PD by two methods of quantification are consistent with the growing evidence for a neurotoxic role of pesticides on selective nigral injury, Lewy body formation, and responses to levodopa [32].

Based on the observation that coffee intake and cigarette smoking seem to reduce the susceptibility to PD due to other precursors, findings further support the possibility that a high risk of PD requires exposure to a combination of factors. Genetic susceptibility may also have an important role. The complex interaction among a constellation of these factors and their role in PD development may offer a partial explanation for why iden-

tification of risk factors for PD has been illusive. Although discouraging, this also suggests that reduced exposure to any single precursor could sufficiently delay or eliminate neuropathologic processes that lead to PD through the requirement that precursors need to coexist for PD progression to continue.

Whether combinations of precursors, particularly cigarette smoking, coffee intake, and possibly constipation histories can be used as enrollment criteria for the study of PD deserves consideration. Such design strategies could increase the potential for maximizing thera-

peutic effects in a clinical trial. It might also seem reasonable that, in prospective cohort studies of precursors of PD, focus should be on those groups where risk is highest, where accrual of events is quicker, and lengths of follow-up can be reduced. Identifying collections of precursors for PD (in combination with a family history and emerging movement abnormalities) could also lead to more effective strategies for identifying early or suspected disease, as well as provide for different approaches to prevention and intervention.

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# Consumption of milk and calcium in midlife and the future risk of Parkinson disease

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**Abstract—Objective:** To examine the relation between milk and calcium intake in midlife and the risk of Parkinson disease (PD). **Methods:** Findings are based on dietary intake observed from 1965 to 1968 in 7,504 men ages 45 to 68 in the Honolulu Heart Program. Men were followed for 30 years for incident PD. **Results:** In the course of follow-up, 128 developed PD (7.1/10,000 person-years). Age-adjusted incidence of PD increased with milk intake from 6.9/10,000 person-years in men who consumed no milk to 14.9/10,000 person-years in men who consumed >16 oz/day ( $p = 0.017$ ). After further adjustment for dietary and other factors, there was a 2.3-fold excess of PD (95% CI 1.3 to 4.1) in the highest intake group (>16 oz/day) vs those who consumed no milk. The effect of milk consumption on PD was also independent of the intake of calcium. Calcium from dairy and nondairy sources had no apparent relation with the risk of PD. **Conclusions:** Findings suggest that milk intake is associated with an increased risk of Parkinson disease. Whether observed effects are mediated through nutrients other than calcium or through neurotoxic contaminants warrants further study.

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Evidence suggests that diet and nutrient intake may have an important association with the risk of Parkinson disease (PD), although findings are largely from retrospective case-control studies where selection bias and uncertainty in dietary recall are common.<sup>1</sup> Recently, investigators from the Health Professionals Follow-Up Study (HPFS) and the Nurses' Health Study (NHS) provided prospective evidence for an association between the high intake of dairy products and an increased risk of PD.<sup>1</sup> It remains uncertain, however, whether associations include commonly consumed dairy products and related nutrients such as milk and dietary calcium.

We examined the relation between milk and calcium intake in midlife and the future risk of PD. Findings are based on 30 years of follow-up of the sample of men who were enrolled in the Honolulu Heart Program from 1965 to 1968. All men were free of PD when follow-up began.

**Methods.** *Background and study sample.* From 1965 to 1968, the Honolulu Heart Program began following 8,006 men of Japanese ancestry living on the island of Oahu, HI, for the development of cardiovascular disease.<sup>2,3</sup> At the time of study enrollment,

subjects were ages 45 to 68. Initial screening consisted of a baseline physical examination and documentation of cardiac and neurologic conditions to identify prevalent cases of cardiovascular disease. Since the time of study entry, subjects have undergone a comprehensive system of follow-up for morbidity and mortality outcomes through repeat examinations, surveillance for all hospital discharges, and a thorough review of medical records, death certificates, and autopsy reports by a panel of physician experts. Procedures have been in accordance with institutional guidelines and approved by an institutional review committee. Written informed consent has been obtained from the study participants.

For this report, follow-up began at the time of the baseline examination (1965 to 1968). These were the only examinations in the Honolulu Heart Program where data on the intake of milk and dietary calcium were collected. There were two men with prevalent PD at the baseline examination who were excluded from follow-up. Only men whose dietary intake was reported as being "fairly typical" of their usual dietary habits are considered in this report. Here, "fairly typical" is loosely defined as anything other than a major difference in under- or overeating (or drinking). Small variations were not recorded. Based on this latter criterion, an additional 500 men were excluded. The final sample that was available for follow-up included 7,504 men.

*Dietary measurements and confounding information.* Information on the intake of milk and dietary calcium was obtained by a dietitian based on 24-hour recall methods.<sup>4</sup> Dietitians used standardized methods to obtain individual recall of food intake through the use of food models and serving utensils to illustrate portion sizes.<sup>4,5</sup> Collected data were validated against a 7-day diet record in 329 of the 8,006 men in the original cohort.<sup>6</sup> There were

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**Table 1** Incidence of PD over 30-year period of follow-up within ranges of midlife milk intake

Milk intake, oz/d	Sample size	Incident PD cases	Incidence of PD, rate/10,000 person-years	
			Unadjusted	Age adjusted
0	2,674	43	6.8	6.9
>0–8	3,228	47	6.1	5.9
>8–16	1,089	20	7.4	7.4
>16	513	18	14.5*	14.9*
Test for trend†			$p = 0.022$	$p = 0.017$

\* Excess risk of PD vs men in the lowest intake range ( $p < 0.01$ ).

† Test for trend is based on modeling milk as a continuous variable.

PD = Parkinson disease.

no significant differences between the methods of assessing dietary intake for 15 nutrient categories, and day-to-day variation was less than typical among Western cultures.<sup>6</sup> Nutrient intake levels were estimated by grouping foods into standard portions in 54 categories. Levels of nutrient intake for each category were then obtained from the US Department of Agriculture Handbook no. 8 and from a food table specifically designed for the Honolulu Heart Program.<sup>7</sup> Dietary calcium was further stratified as being derived from dairy and nondairy sources. Dairy sources included whole and skim milk, cheese, butter, and ice cream. Calcium intake from nondairy sources was largely from meats, fish, grains, soy products, and fruits and vegetables. Other dietary data considered in this report include the intake of coffee, total kilocalories, and fat. Data on the supplemental intake of calcium through nondairy sources were not available.

To help isolate the independent effect of milk and dietary calcium intake on the risk of PD, several risk factors measured at the time of dietary assessment were also considered as possible confounding variables. They included age, pack-years of cigarette smoking, and years worked on a plantation. Adjustments were also made for triceps skinfold thickness because of the observation that midlife adiposity has been shown to be related to the future risk of PD.<sup>8</sup> As body composition is often associated with levels of physical activity, additional adjustment controlled for the “physical activity index,” a common measure used to quantify overall metabolic output in a typical 24-hour period and known to be inversely related to the risk of coronary heart disease and stroke.<sup>9,10</sup> Further description of the data collection methods for the other risk factors has been published elsewhere.<sup>2,3</sup>

**PD case finding and diagnosis.** For this report, 30 years of follow-up data were available on incident PD after collection of the dietary information (1965 to 1968). Prior to 1991, cases of PD were identified through a review of all hospital records of study participants for new and pre-existing diagnoses of PD, an ongoing review of all Hawaii death certificates, and a review of medical records at the offices of local neurologists for all cohort members suspected to have PD.

From 1991 to 1993, the Honolulu–Asia Aging Study was established for the study of neurodegenerative diseases in the surviving members of the Honolulu Heart Program.<sup>11</sup> During this time, all participants were screened for PD through structured interviews concerning the diagnosis of PD and the use of PD medications. Study participants received further screening by a technician trained to recognize the clinical signs of parkinsonism (including gait disturbance, tremor, and bradykinesia). Those with a history or sign of parkinsonism were referred to a study neurologist who administered standardized questions about symptoms and the onset of parkinsonism, previous diagnoses, and medication use, followed by a comprehensive and standardized neurologic examination. A diagnosis of PD was made by the study neurologists according to published criteria without access to the risk factor data examined in this report.<sup>12</sup> These required that the subject have the following: 1) parkinsonism (e.g., bradykinesia or resting tremor combined with rigidity or postural reflex impairment); 2) a progressive disorder; 3) any two of a marked response to levodopa, asymmetry of signs, asymmetry at onset, or initial onset tremor; and 4) absence of any etiology known to cause similar features.

Cases of parkinsonism related to progressive supranuclear palsy, multisystem atrophy, cerebrovascular disease, drug-induced parkinsonism, postencephalitic parkinsonism, or posttraumatic parkinsonism were not included among the cases of PD. During repeat exams that were given from 1994 to 1996 and from 1997 to 1998, subjects were again asked about a diagnosis of PD and the use of PD medications. Medical records were further reviewed by the study neurologists who applied the same published criteria used earlier in making a diagnosis of PD.<sup>12</sup> Further description of the diagnosis of PD is provided elsewhere.<sup>13,14</sup>

**Statistical methods.** Crude and age-adjusted incidence rates of PD in person-years were estimated according to ranges of milk and calcium intake based on the 30 years of follow-up in the 7,504 men who were examined from 1965 to 1968.<sup>15</sup> Age-adjusted risk factors were also derived across the ranges of dietary intake.<sup>15</sup> To test for an effect of milk and calcium intake on the risk of PD, proportional hazards regression models were examined.<sup>16</sup> Adjustments were also made for age, coffee intake, pack-years of smoking, the physical activity index, triceps skinfold thickness, intake of total kilocalories and fat, and years worked on a plantation. In addition to a test for trend in the changing risk of PD with changes in milk and calcium intake, relative risks (and 95% CIs) were estimated comparing the risk of PD in the higher milk intake ranges vs the lowest. All reported  $p$  values were based on two-sided tests of significance.

**Results.** The average age at study enrollment (1965 to 1968) of the 7,504 men was  $54.5 \pm 5.6$  years (range 45 to 68 years). During the 30 years of follow-up, 128 developed PD (7.1/10,000 person-years). The average age at the time of diagnosis was  $73.4 \pm 7.5$  years (range 54 to 89 years), and the average time to a diagnosis was  $18.4 \pm 7.2$  years (range 2 to 30 years).

Incidence of PD is further described in table 1 according to ranges of milk consumed at the time when follow-up began. For men who consumed no milk, the age-adjusted incidence was 6.9/10,000 person-years. For those who consumed >16 oz/day, incidence more than doubled (14.9/10,000 person-years;  $p < 0.01$ ).

Table 2 describes the incidence of PD within quartiles of dietary calcium intake from dairy and nondairy sources. Whereas risk of PD rose with increasing amounts of calcium consumed from dairy sources, effects of calcium were weak and largely explained by concomitant milk intake. After additional adjustment for milk consumption, calcium had no effect on the risk of PD. Calcium consumed from nondairy sources also had no association with PD incidence ( $p = 0.704$ ), further suggesting that calcium is unrelated to the risk of PD.

Among the possible confounders considered in this report, age, pack-years of smoking, and triceps skinfold



**Table 2** Incidence of PD over 30-year period of follow-up within quartiles of midlife intake of calcium from dairy and nondairy sources

Quartile of calcium intake, mg/d	Sample size	Incident PD cases	Incidence of PD, rate/10,000 person-years	
			Unadjusted	Age adjusted
<b>Dairy sources</b>				
0–1	1,821	32	7.4	7.6
2–126	1,840	27	6.1	5.9
127–315	1,962	28	6.0	5.9
316–2,455	1,881	41	8.9	9.0
Test for trend*			$p = 0.063$	$p = 0.046$
<b>Nondairy sources</b>				
0–200	1,883	32	7.3	7.1
201–262	1,860	37	8.2	8.2
263–340	1,887	34	7.4	7.4
341–1,251	1,874	25	5.5	5.6
Test for trend			$p = 0.537$	$p = 0.704$

\* Test for trend is based on modeling calcium as a continuous variable.

PD = Parkinson disease.

thickness declined with increasing amounts of milk consumed ( $p < 0.001$ ). In contrast, the intake of total calcium, coffee, and total kilocalories and fat increased ( $p < 0.001$ ). Although differences were modest, physical activity increased with increasing amounts of milk consumed ( $p < 0.05$ ). Milk intake was unrelated to years worked on a plantation.

To help determine whether the excess risk of PD in those who consumed milk could be attributed to confounding from other factors, the effect of milk intake on PD was further adjusted for age, coffee intake, pack-years of smoking, physical activity, triceps skinfold thickness, total kilocalories and fat intake, and years worked on a plantation. Findings are shown in table 3 with and without the additional adjustment for the intake of calcium.

**Table 3** Adjusted relative risk of PD for men who consumed milk vs those who consumed no milk

Milk intake, oz/d	Adjusted relative risk* (95% CI)	
	Without adjustment for calcium intake	With adjustment for calcium intake
0	Ref.	Ref.
>0–8	0.9 (0.6,1.4)	1.0 (0.6,1.5)
>8–16	1.2 (0.7,2.0)	1.3 (0.7,2.4)
>16	2.3 <sup>†</sup> (1.3,4.1)	2.6 <sup>‡</sup> (1.1,6.4)
Test for trend§	$p = 0.007$	$p = 0.085$

\* Adjusted for age, coffee intake, pack-years of smoking, physical activity, triceps skinfold thickness, total kilocalories and fat intake, and years worked on a plantation.

<sup>†</sup> Excess risk of PD vs men who consumed no milk ( $p < 0.01$ ).

<sup>‡</sup> Excess risk of PD vs men who consumed no milk ( $p < 0.05$ ).

§ Test for trend is based on modeling milk as a continuous variable.

PD = Parkinson disease.

When unadjusted for total calcium intake, there was a rise in the risk of PD with increased amounts of milk consumed ( $p = 0.007$ ). Although the dose–response relation between milk intake and the risk of PD declined in significance after accounting for calcium intake ( $p = 0.085$ ), a more than twofold excess in the risk of PD persisted in those who consumed the most milk (>16 oz/day) vs those who consumed no milk ( $p < 0.05$ ). The effect of milk intake on PD was also unaltered in the presence of other risk factors including plantation work and elevated triceps skinfold thickness.

Whether milk intake has a different effect on PD that occurs before age 65 vs PD that occurs later is shown in the figure (top). Although sample sizes and statistical power are reduced, men who consumed the most milk (>16 oz/day) continued to have an excess risk of PD vs men who consumed no milk regardless of the age at diagnosis ( $p < 0.05$ ).

Whether milk intake has both long- and short-term effects on PD is also described in the figure (bottom). Although a test for a change in the relation between milk consumption and the risk of PD with time is not significant, it appears that the effect of milk on the risk of PD is strongest in the first 15 years of follow-up ( $p < 0.01$ ) vs PD that was diagnosed in the second 15 years of follow-up. Although there appears to be a nonlinear relation between milk intake and the risk of PD in the first 15 years of follow-up, curvature in this relation could not be carefully assessed owing to the small number of cases that were observed to occur in this time period.

**Discussion.** Findings suggest that milk intake is associated with the future risk of PD that is independent of total kilocalories, dietary calcium, and other confounding variables. An apparent association between dietary calcium and PD is also explained by concomitant milk intake. In addition, calcium intake from nondairy sources was not related to PD, further

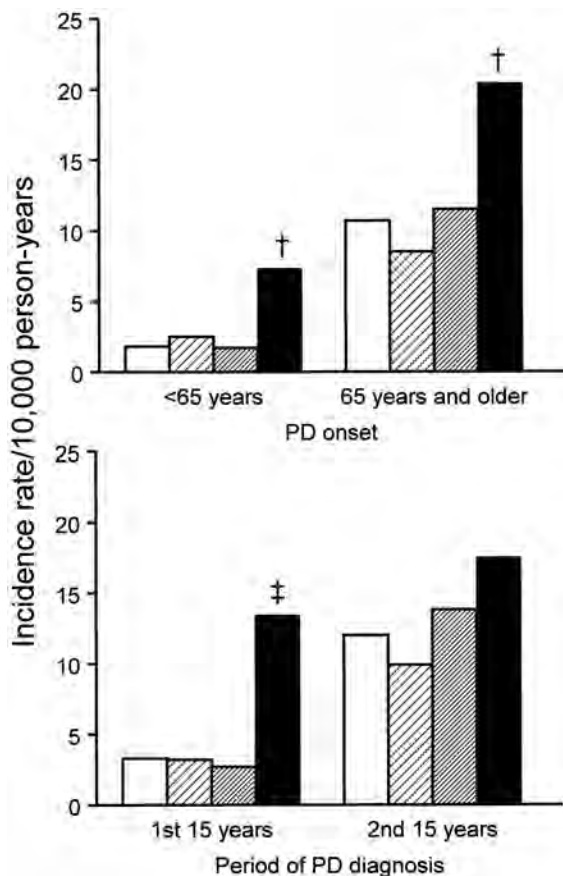


Figure. Age-adjusted incidence of early and late Parkinson disease (PD) onset (<65 and  $\geq 65$  years, respectively) and the incidence of PD in the first and second 15 years of follow-up according to milk intake during midlife. Milk intake increases from 0 (open columns), >0 to 8 (wide-hatched columns), >8 to 16 (thin-hatched columns), to >16 (filled columns) oz/day from left to right within each of the four-bar groupings. †Excess risk of PD vs men who consumed no milk ( $p < 0.05$ ). ‡Excess risk of PD vs men who consumed no milk ( $p < 0.01$ ).

suggesting that a role for calcium in altering PD risk is absent. A recent study from the HPFS and NHS provides similar findings with regard to nondairy calcium.<sup>1</sup> Although the HPFS and NHS investigators found a significant relation between calcium from dairy products and the risk of PD, it is not clear if associations could be attributed to milk intake as it was in the current report. Regardless, general findings of an effect of dairy products on the risk of PD in separate cohorts of men suggest that the observed relation between dairy products and PD could be real. The lack of a similar finding for women in the HPFS and NHS,<sup>1</sup> however, is difficult to resolve, although it could be important.

Although a more specific link between milk and PD was less apparent in the HPFS and NHS than in the Honolulu Heart Program,<sup>1</sup> differences in findings could be attributed (in part) to differences in study methods. For example, the current report assessed data from 24-hour dietary recall methods, whereas

the HPFS and NHS collected data from a food frequency questionnaire.<sup>1</sup> In the latter study, dairy intake is measured as the number of servings consumed during a period of time, whereas in the current report, findings are presented according to amounts of milk or calcium consumed per day. Data from the Honolulu Heart Program, however, suggest that the amount of milk consumed may be more important than its frequency of intake. Although the age-adjusted incidence of PD in the current report increased consistently with increasing frequency of intake (from 5.5/10,000 person-years in men who rarely drank milk to 8.7/10,000 person-years in men who consumed milk regularly;  $p = 0.047$ ), the association was not significant after adjustment for other factors. Nevertheless, there was a tendency for men who consumed the largest amounts of milk on a regular daily basis to also have the highest incidence of PD.

Although this may be the first published report to describe an association between milk intake and PD, presumably other investigators have also considered the possibility for such a relation, although with limited success. Studies of diet and PD, however, are often based on retrospective case-control designs where recall of food intake prior to the onset of PD (possibly by many years) can be subject to considerable variation and error. A strength of the current report and the HPFS and NHS is that both were based on prospective follow-up for incident PD.<sup>1</sup> As PD is a relatively uncommon disease, careful studies of the incidence of PD require long and costly periods of prospective follow-up.

Although the accurate collection of dietary intake data is known to be difficult, data in both the HPFS and NHS and the Honolulu Heart Program reflect intake at the time of questioning. Observations from the current report may also be less prone to the deficiencies of a 24-hour recall as only subjects who reported that consumption was "fairly typical" were considered for follow-up. Although in need of confirmation, this suggests that the effect of milk intake on the risk of PD could be through habitual rather than sporadic consumption of milk. Whether significant changes in milk intake occurred over long periods of time or whether the one-time measurement of milk intake in the current report reflects life-long consumption is not known.

Unfortunately, there are no clear explanations for the relation between milk intake and the risk of PD. As milk is a complex mixture of nutrients, any of its nutritional constituents could act as candidate mediators in the association between milk and PD. Calcium, however, is unlikely to be among these mediators because its intake from nondairy food items had no relation with PD. In the Honolulu Heart Program, total fat and protein also had no relation with the risk of PD. In addition, intakes of cheese, butter, and ice cream were unrelated to PD, although these food items are more likely to be consumed sporadically as compared with milk. Milk was also related to PD regardless of whether it was whole

or skim. Given the strong correlation between milk consumption and the intake of lactose and vitamin D in the Honolulu Heart Program, it was not possible to identify a distinct role for these nutrients in the development of PD. Although intakes of vitamins and supplements were not recorded, their routine use may have been minimal at the time when follow-up began (1965 to 1968). Nevertheless, an effect of dietary supplements on the risk of PD warrants consideration, particularly because supplements may be a less complex source of nutrients as compared with milk.

Effects of milk and dairy products could also have a role in altering the absorption of neuroprotective compounds associated with antioxidant capacity.<sup>17-19</sup> Rather than milk intake having an effect on PD, metabolic characteristics such as lactose intolerance could be protective. This is unlikely, however, as after removing men in the Honolulu Heart Program who consumed no milk (and presumably those most likely to be lactose intolerant), the association between milk intake and PD persisted.

Contamination of milk with neurotoxins may be of critical importance. High levels of organochlorine residues have been detected in milk,<sup>20-24</sup> and *substantia nigra* organochlorine levels have been found to be higher in cases of PD than in cases of Alzheimer disease and controls.<sup>25,26</sup> Other contaminants that have been found in milk include tetrahydroisoquinoline (used in the synthesis of pesticides),<sup>27,28</sup> which is known to induce parkinsonism in primates.<sup>29,30</sup>

A role for neurotoxins in Hawaii may be especially important, where, from 1981 to 1982, the milk supply on the island of Oahu was found to be contaminated with heptachlor (a chlorinated cyclodiene pesticide) from chopped pineapple leaves used as cattle feed.<sup>22-24</sup> At the time when follow-up began in the current report, much of the milk consumed in Hawaii came from local producers and local dairy farms. It is not clear, however, if this is true for other dairy products. Pesticides have also been shown to be a potent risk factor for PD in the Honolulu cohort.<sup>31,32</sup> Whether heptachlor contamination occurred prior to 1981 is uncertain.<sup>23,24</sup>

Whereas the findings from the Honolulu Heart Program are consistent with recent observations of an association between dairy products and PD,<sup>1</sup> additional confirmation is needed, particularly in terms of identifying the specific constituents of milk that contribute to the association between milk and PD. Whether observed effects are mediated through nutrients other than calcium or through neurotoxic contaminants warrants further study.

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# Excessive daytime sleepiness and subsequent development of Parkinson disease

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**Abstract—Objective:** To determine if excessive daytime sleepiness (EDS) can predate future Parkinson disease (PD). **Methods:** EDS was assessed in 3,078 men aged 71 to 93 years in the Honolulu-Asia Aging Study from 1991 to 1993. All were free of prevalent PD and dementia. Follow-up for incident PD was based on three repeat neurologic assessments from 1994 to 2001. **Results:** During the course of follow-up, 43 men developed PD (19.9/10,000 person-years). After age adjustment, there was more than a threefold excess in the risk of PD in men with EDS vs men without EDS (55.3 vs 17.0/10,000 person-years; odds ratio [OR] = 3.3; 95% CI = 1.4 to 7.0;  $p = 0.004$ ). Additional adjustment for insomnia, cognitive function, depressed mood, midlife cigarette smoking and coffee drinking, and other factors failed to alter the association between EDS and PD (OR = 2.8; 95% CI = 1.1 to 6.4;  $p = 0.014$ ). Other sleep related features such as insomnia, daytime napping, early morning grogginess, and frequent nocturnal awakening showed little relation with the risk of PD. **Conclusions:** Excessive daytime sleepiness may be associated with an increased risk of developing Parkinson disease.

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Excessive daytime sleepiness (EDS) is a major concomitant of Parkinson disease (PD). Although several studies describe an excess of EDS in patients with PD vs healthy adults,<sup>1–8</sup> it remains equivocal if EDS is related to the neuropathologic processes leading to PD, is a consequence of PD, or is secondary to dopaminergic agents that are used in the treatment of PD. There are no prospective follow-up studies of incident clinical PD in the presence vs the absence of EDS, and it is not known if EDS can predate PD.

The purpose of this report is to examine the association between EDS and the future risk of PD in a sample of elderly men enrolled in the Honolulu-Asia Aging Study. All men were free of PD and dementia when follow-up began. In the absence of pharmacologic therapy for PD, attention will focus on whether EDS can predict PD and the possibility that neurodegenerative processes are an important underlying component in the relation between EDS and the future risk of PD.

**Methods.** *Background and study sample.* From 1965 to 1968, the Honolulu Heart Program began following 8,006 men of Japanese ancestry living on the island of Oahu, HI for the development

of cardiovascular disease.<sup>9,10</sup> Beginning with examinations that were given from 1991 to 1993, the Honolulu-Asia Aging Study was created as an expansion of the Honolulu Heart Program for the study of neurodegenerative diseases and cognitive function in the elderly.<sup>11</sup> Subjects included 3,741 men aged 71 to 93 years (approximately 80% of the survivors in the original Honolulu Heart Program cohort). Procedures were in accordance with institutional guidelines and approved by an institutional review committee. Informed consent was obtained from the study participants.

For this report, follow-up began when sleep data were first collected at the beginning of the Honolulu-Asia Aging Study (1991 to 1993). Men with prevalent PD ( $n = 61$ ) were excluded from follow-up as were an additional 215 with prevalent dementia and 387 with missing EDS data. The remaining sample included 3,078 men with follow-up for incident PD based on three repeat neurologic examinations that occurred from 1994 to 2001.

*EDS and confounding information.* Features of usual daily sleep were reported through the use of a questionnaire that was administered by a trained research technician following a standardized protocol.<sup>12</sup> Men who reported being sleepy most of the day were defined as having EDS.<sup>13,14</sup> Similar questionnaires have been used elsewhere.<sup>3,4,15,16</sup> Additional sleep information included the average sleeping time at night and at napping. Insomnia was defined as having difficulty falling asleep or waking up far too early and not being able to go back to sleep. Men were reported as being groggy if they felt unrefreshed for >0.5 hours after awakening in the morning. The prevalence of loud snoring was based on complaints from a spouse or housemate, and frequent nocturnal

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awakening was defined as waking up several times during the night for reasons other than the need to use the bathroom.

To help isolate the independent association between EDS and the future risk of PD, several additional factors were considered as possible sources of confounding. They included age, midlife pack-years of cigarette smoking and coffee intake, daily bowel movement frequency, cognitive performance, depressed mood, and the use of antidepressants, antipsychotics, and sedatives. Midlife pack-years of cigarette smoking and coffee intake were measured at the time of initiation of the Honolulu Heart Program (1965 to 1968) as markers of typical lifetime exposures to these factors. Late-life coffee intake was not determined at the time when follow-up began (1991 to 1993) and current cigarette smoking was too uncommon to allow for its careful assessment. Determination of the other characteristics coincided with the measurement of the other sleep-related features (1991 to 1993).

For this report, cognitive performance is based on the Cognitive Abilities Screening Instrument, a comprehensive measure of intellectual function that has been developed and validated for use in crosscultural studies.<sup>17</sup> Performance scores range from 0 to 100, with high scores indicating better cognitive function than low scores. Depressive symptoms were recorded from a modified version of the Center for Epidemiologic Studies Depression Instrument.<sup>18</sup> Here, composite scores range from 0 to 33, with a score >8 defined as a depressed mood. Further description of the other factors is provided elsewhere.<sup>19-21</sup>

**PD case finding and diagnosis.** At the time when EDS was assessed (1991 to 1993) and during the course of follow-up (1994 to 2001), all subjects were questioned about a diagnosis of PD and the use of PD medications by a structured interview. Study participants received further screening by a technician trained in the recognition of the clinical symptoms of parkinsonism (including gait disturbance, tremor, and bradykinesia). Those with a history or sign of parkinsonism were referred to a study neurologist who administered standardized questions about the symptoms and onset of parkinsonism, previous diagnoses, and medication use, followed by a comprehensive and standardized neurologic examination. A diagnosis of PD was made by study neurologists according to published criteria without access to risk factor data examined in this report.<sup>22</sup> These required that the subject have the following: parkinsonism (e.g., bradykinesia or resting tremor combined with rigidity or postural reflex impairment); a progressive disorder; any two of a marked response to levodopa, asymmetry of signs, asymmetry at onset, or initial onset tremor; and absence of any etiology known to cause similar features. Cases of parkinsonism related to progressive supranuclear palsy, multiple system atrophy, cerebrovascular disease, drug-induced parkinsonism, postencephalitic parkinsonism, or posttraumatic parkinsonism were not included among the cases of PD.

**Statistical methods.** Crude and age-adjusted incidence rates of PD in person-years were estimated according to the presence and absence of EDS based on standard analysis of covariance methods.<sup>23</sup> Average sleep features and additional factors were also derived and age-adjusted in those with and without EDS.<sup>23</sup> Because the number of PD cases was small, logistic regression models were examined to assess the effect of EDS (and other factors) on the risk of PD based on exact testing methods.<sup>24</sup> The logistic regression was further adapted for a survival analysis where parameter estimates are known to be similar to those that appear in a proportional hazards regression model, particularly in the instance when event counts are low.<sup>25,26</sup> After adjustment for age and the other study characteristics, the odds ratio (OR) of PD (and 95% CI) was estimated comparing the risk of PD in men with EDS vs those without EDS. All reported *p* values were based on two-sided tests of significance.

**Results.** At the time when follow-up began (1991 to 1993), EDS was observed in 7.9% (244/3,078) of the men considered in this report. Table 1 describes the men with and without EDS in terms of common characteristics that have a putative or possible relation with PD. Between the two groups, men were of similar age. The age range in men with EDS was 71 to 92 years, and in men without EDS it was 71 to 93 years. Among the other characteristics, men with EDS had a midlife history of drinking less coffee than

**Table 1** Mean age and age-adjusted average and percents of selected study characteristics in men with and without EDS

Study characteristic	No EDS	EDS	<i>p</i> Value
n	2,834	244	
Age, y	77.1 ± 4.2	77.5 ± 4.4	
Midlife pack-years of smoking	25.7 ± 26.5	28.0 ± 30.2	
Midlife coffee intake, oz/d	13.9 ± 12.9	11.8 ± 13.0	0.012
Bowel movements/d	2.2 ± 0.5	2.3 ± 0.5	
Cognitive Abilities Screening Instrument	86.5 ± 8.4	84.7 ± 10.8	0.001
Depressed mood	8.9 (251)	18.5 (45)	<0.001
On antidepressants, antipsychotics, or sedatives	1.6 (45)	1.2 (3)	

Data are means ± SD or % (n).

EDS = excessive daytime sleepiness.

men without EDS (*p* = 0.012). Men with EDS also had poorer cognitive performance when follow-up began (*p* = 0.001) and were more likely to have a depressed mood than men without EDS (*p* < 0.001). Differences in the other characteristics were not significant, although men with EDS were exposed to more pack-years of smoking in midlife and reported having more frequent bowel movements. Treatment with antidepressants, antipsychotics, and sedatives was uncommon, with a small excess of use occurring in men without EDS.

Men with and without EDS were also compared in terms of the other sleep-related features in table 2. Although hours of nighttime sleeping were similar between the two groups, men with EDS reported napping longer than men without EDS (69 vs 42 minutes, *p* < 0.001). An excess of insomnia was also observed in men with EDS (39.0% with EDS and 28.7% without EDS, *p* < 0.001). In addition, grogginess was more likely in the presence of EDS (13%) vs its absence (2.5%, *p* < 0.001), and men with EDS were more likely to awaken frequently at night (13.8%) as compared to men without EDS (6.9%, *p* < 0.001). Although there was an excess of loud snoring in

**Table 2** Age-adjusted average and percents of sleep-related features in men with and without EDS

Sleep-related feature	No EDS	EDS
Hours of nighttime sleeping	7.0 ± 1.3	7.1 ± 1.7
Minutes of napping*	42 ± 44	69 ± 60
Insomnia*	28.7 (812)	39.0 (94)
Groggy for >0.5 hour after awakening*	2.5 (70)	13.0 (31)
Loud snoring	33.2 (877)	37.8 (84)
Frequent nocturnal awakening*	6.9 (196)	13.8 (34)

Data are means ± SD or % (n).

\* *p* < 0.001.

EDS = excessive daytime sleepiness.

**Table 3** Incidence of Parkinson disease in men with and without EDS

	Crude incidence per 10,000 person-years	Age-adjusted incidence per 10,000 person-years	Adjusted odds ratio (95% CI)*
No EDS	17.0 (34/2834)	17.0	Reference
EDS	54.9 (9/244)†	55.3†	2.8 (1.1, 6.4)‡

\* Adjusted for age, mid-life cigarette smoking and coffee drinking, bowel movement frequency, cognitive function, depressed mood, and insomnia.

†  $p = 0.004$ .

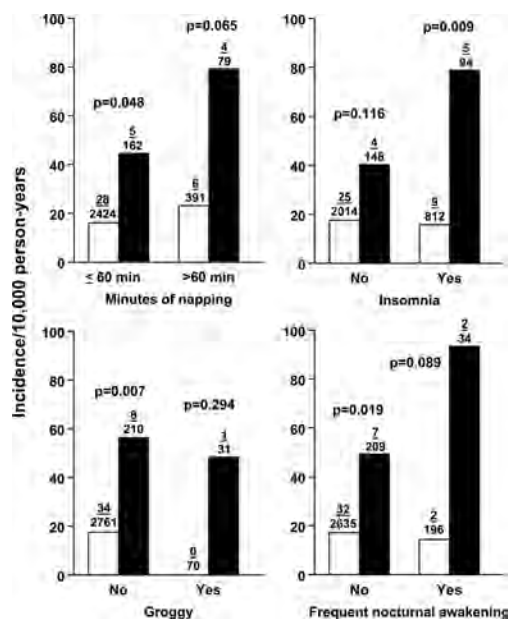
‡  $p = 0.014$ .

EDS = excessive daytime sleepiness.

men with EDS, it was not significantly more common than in men without EDS.

During the course of follow-up, 43 men developed PD (19.9/10,000 person-years). The average age at the time of diagnosis was 80 years (range 73 to 89). Among the sleep related features presented in table 2, only EDS was significantly related to the future risk of PD. Table 3 and the figure describe this latter finding in greater detail.

In the 244 men with EDS (table 3), nine were diagnosed with PD between 7 months and 4.9 years into follow-up (54.9/10,000 person-years). In those without EDS, PD was diagnosed in 34 of 2,834 men within 2 months to 7.3 years of follow-up (17.0/10,000 person-years). After age-adjustment, there was more than a threefold excess in the risk of PD in men with vs without EDS (55.3 vs 17.0/10,000 person-years; OR = 3.3; 95% CI = 1.4 to 7.0;  $p =$



**Figure.** Incidence of Parkinson disease (PD) in men with and without excessive daytime sleepiness (EDS) within strata of other sleep-related features. Solid bars represent the incidence of PD for men with EDS. Clear bars represent the incidence of PD for men without EDS.  $p$  values are for a test of an association between EDS and PD within a sleep-related feature. Numbers above the bars are the number of PD cases/sample at risk.

0.004). Further adjustment for mid-life cigarette smoking and coffee drinking, bowel movement frequency, cognitive function, depressed mood, and insomnia, failed to appreciably alter the association between EDS and PD (OR = 2.8; 95% CI = 1.1 to 6.4;  $p = 0.014$ ).

The excess of PD in men with EDS vs those without EDS also occurred consistently across risk factor strata. In particular, the figure describes the relation between EDS and the risk of PD in the absence and presence of the four sleep related disorders that had significant associations with EDS (table 2). Although sample size and statistical power are reduced, within each sleep-related strata, men with EDS consistently experienced an excess of PD as compared to when EDS was absent. An association between EDS and the risk of PD also persisted after the combined adjustment for all of the sleep-related features in table 2 (OR = 3.3; 95% CI = 1.4 to 7.3;  $p = 0.021$ ).

**Discussion.** Although sleep disturbances often coexist with PD, this report further suggests that EDS can predate clinical PD. The association between EDS and PD is also uninfluenced by medical intervention for PD and the effects of antidepressants, antipsychotics, and sedatives. This is especially important since the role of therapeutic agents that alter striatal dopaminergic transmission in EDS has been controversial.<sup>1-8,27</sup> In the absence of pharmacologic intervention for PD at the time when EDS was measured, findings support the hypothesis that EDS in PD involves the same pathophysiologic processes that lead to clinical PD and its motor symptoms.

This is further supported by evidence that REM sleep behavior disorder (RBD) is associated with alpha synuclein pathology and dopaminergic dysfunction.<sup>28</sup> Others report that RBD can predate clinical PD by an average of 10 to 12 years.<sup>29-33</sup> Although the current study was based on a shorter period of follow-up, PD ascertainment in the Honolulu-Asia Aging Study is currently ongoing. As with RBD, it will be interesting to determine if EDS can also predict clinical PD beyond 10 years of follow-up.

Undiagnosed RBD could also provide an explanation for the relation between EDS and incident PD. Although RBD is not known to be associated with EDS, it is not certain that RBD was absent in the sample of men with EDS who later developed PD. Other explanatory sleep disorders could include restless leg syndrome and periodic limb movements in sleep, both of which are common in PD and may precede its motor symptoms.<sup>34</sup> Although findings from polysomnography and other sleep-related disturbances were not available in the current sample of examined men, a thorough review of all neurologic records up to the time of PD diagnosis among the nine men with EDS made no mention of the classic signs of RBD, including abnormal sleep disturbances, vivid dreams, violent or injurious sleep behavior, and lower-limb restlessness. As noted in the figure, the relation between EDS and the risk of PD also persisted in the absence of other sleep-related features. In addition, the simple measurement of EDS<sup>3,4,15,16,35</sup> is a notably easier undertaking than is

polysomnography. As a predictor of incident PD, the administration of simple questions about EDS in elderly samples with suspected or early symptoms of PD could be a clinically useful adjunct in the follow-up of such individuals.<sup>1,3,4,15,16,28,35</sup> Others have also suggested that scales used in the diagnosis of PD should include more questions on sleep-related disorders.<sup>1</sup>

We are also not aware of a study that has conducted polysomnography in a general population-based setting that confirms that the future risk of PD in groups without RBD is different from those with RBD. To our knowledge, studies have been based on either a series of RBD patients<sup>29-31</sup> or in comparisons with controls without polysomnographic assessments.<sup>32,33</sup> The general population prevalence of RBD is also uncertain because polysomnography is rarely administered in the absence of an overt clinical indication. As noted by others, determining the prevalence of RBD would be costly but important.<sup>28</sup> Regardless, even if undiagnosed RBD explains the association between EDS and clinical PD, it seems noteworthy that the presence of EDS (as a nonspecific disorder with unknown and possibly many causes) is associated with more than a threefold excess in the risk of PD vs its absence.

Among the other sleep-related features considered in this report, only EDS had a significant association with the future risk of PD. In cross-sectional case-control studies, however, PD has been associated with frequent nocturnal awakenings, insomnia, nightmares, restless legs, snoring, and nocturnal vocalizations.<sup>3,4</sup> In one report, snoring and sleeping time were unrelated to PD.<sup>1</sup> Others observed that nighttime sleep disorders were similar in PD patients with and without EDS.<sup>2</sup>

The prevalence of EDS in the elderly may also be poorly underestimated because excessive somnolence is often unrecognized in subjective reporting.<sup>5</sup> Although the prevalence of EDS in the Honolulu sample (8%) is lower than the 17% prevalence in men enrolled in the Cardiovascular Health Study,<sup>15</sup> variation among study samples can be appreciable, ranging from 1% to 47% in healthy controls or in subjects without PD.<sup>2-5</sup> For those with PD, reported prevalence varies from 15% to 76%.<sup>2-5</sup> In Hawaii, in the 61 prevalent cases of PD who were excluded from follow-up, EDS data were available in 31 men. Among this group, 23% reported having EDS.

Unfortunately, the current study was not designed to identify the possible causes of EDS. In addition, whether similar associations between EDS and PD also occur in women is not known. The age when follow-up began for this report also tends to be older than in other longitudinal studies of PD. As a result, whether the association between EDS and PD persists in those who are younger warrants further consideration.

The pathophysiologic cause of EDS in individuals who later develop PD is equally unclear. Nevertheless, it is easy to speculate that the structural and

neurochemical defects in PD may be important. In particular, the pathogenesis of PD that includes neuron loss in the locus ceruleus, the hypothalamus, and the ascending reticular activating system, occurs in regions of the brain that are closely aligned with the coordination of sleep and wakefulness. Noradrenergic, serotonergic, and dopaminergic deficits known to occur in PD may also underlie the development of EDS, as well as motor and mood disturbances.<sup>36,37</sup> Recent work on the staging of Lewy pathology in PD further suggests that the disease process begins in the medulla and ascends to the caudal raphe nuclei and ceruleus-subceruleus complex in the pons with continued involvement of the substantia nigra.<sup>38</sup> Based on this progression, it is possible that the finding of EDS in men who later develop clinical PD corresponds with the timing of an early stage of PD where affected regions of the brain can alter sleep. Observations from the Honolulu-Asia Aging Study are consistent with the hypothesis that PD neurodegenerative processes underlie the relation between EDS and progression to clinical PD.<sup>1-6,27-33</sup>

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## RESIDENT AND FELLOW PAGE

Call for teaching videos

The *Neurology* Resident page is featured online at [www.neurology.org](http://www.neurology.org). The Editorial Team of this section is seeking teaching videos that will illustrate classic or uncommon findings on movement disorders. Such videos will aid in the recognition of such disorders. Instructions for formatting videos can be found in the Information for Authors at [www.neurology.org](http://www.neurology.org). Please contact the Editor, Karen Johnston ([kj4v@virginia.edu](mailto:kj4v@virginia.edu)), for more information or submit teaching videos online at <http://submit.neurology.org>.



time of discharge. The reasons for catheterisation included: urgency, precipitancy and incontinence in addition to poor mobility. 39/75 (52%) of noncatheterized patients were discharged home, 14/75 (19%) to residential homes and 18/75 (24%) to nursing homes, and 3/75 (4%) for further rehabilitation. 13/30 (43%) of catheterized patients were discharged home, 4/30 (13%) to residential homes and 12/30 (40%) to nursing homes and 1/30 (3%) for further rehabilitation.

A greater proportion of catheterized, compared to non-catheterized, patients were institutionalized. The likely explanation is that patients with more advanced disease are catheterized; however, presence of a catheter on its own might influence the destination of discharge. The number was not big enough to study the contribution of either.

Conclusion: The study emphasises the importance of urinary/continence issues in patients with parkinsonism

expedient short term urinary catheterization, whilst in hospital, might result in longer term catheterization.

Urinary catheterization could well be a marker of more advanced parkinsonism or co-existing co-morbidity. It is a marker for greater risk of institutionalization.

#### P1183

##### Depressive symptoms and Parkinson's disease: the Honolulu-Asia aging study

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Objective: To examine whether depressive symptoms predict incident Parkinson's disease (PD) and analyze the cross-sectional association between depressive symptoms and prevalent PD in elderly Japanese-American men in the Honolulu-Asia Aging Study (HAAS).

Background: Depression is the most common psychiatric abnormality in patients with PD, and is often undiagnosed.

Methods: The HAAS began in 1991 to study PD, dementia and other aging related diseases in the Honolulu Heart Program cohort, established in 1965. At the 1991-1993 examination, 3741 subjects ages 71-93 years underwent a comprehensive exam, including an 11-item version of the Center for Epidemiological Studies Depression Scale (CESD-11) and a neurological examination for PD. Presence of depressive symptoms was defined as a CESD-11 score  $\geq 9$ . Subjects with prevalent dementia or stroke at baseline were excluded from analysis.

Results: Rates of depressive symptoms were higher in both prevalent (17.9% vs. 9.3%;  $p=0.18$ ) and incident (14% vs. 9.3%;  $p=0.29$ ) PD groups, compared to those without PD. Using Cox proportional hazards models adjusting for age, hypertension, physical activity, pack-years smoking, BMI, cholesterol and marital status, presence of depressive symptoms was not a significant independent predictor for incident PD (RR = 1.51, 95% CI = 0.59-3.86). Among those with prevalent PD, rates of depressive symptoms were higher among those with cognitive impairment (50% vs. 15.4%,  $p=0.17$ ), functional impairment (33.3% vs. 7.1%,  $p=0.17$ ), and prevalent stroke (50% vs. 17.9%,  $p=0.37$ ), although none of these associations reached statistical significance likely due to small sample size.

Conclusions: Depressive symptoms are more common in PD patients. The lack of statistical significance in our study is probably related to the small number of PD cases. Among those with prevalent PD, those who had cognitive impairment, functional impairment, or stroke had higher rates of depressive symptoms. Physicians need to be aware of depressive symptoms in PD patients and should not always attribute these signs to physical constraints of the disease.

#### P1184

##### Patients with Parkinson's disease learn to control complex systems - An indication for intact implicit cognitive skill learning

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Implicit memory and learning mechanisms are composed of multiple processes and systems. Previous studies demonstrated a basal ganglia involvement in purely cognitive tasks that form stimulus response habits by reinforcement learning such as implicit classification learning. We will test the basal ganglia influence on two cognitive implicit tasks previously described by

Berry and Broadbent, the sugar production task and the personal interaction task. Furthermore, we will investigate the relationship between certain aspects of an executive dysfunction and implicit learning. To this end, we have tested 22 parkinsonian patients and 22 age-matched controls on two implicit cognitive tasks, in which participants learned to control a complex system. They interacted with the system by choosing an input value and obtaining an output that was related in a complex manner to the input. The objective was to reach and maintain a specific target value across trials (dynamic system learning). The two tasks followed the same underlying complex rule but had different surface appearances. Subsequently, participants performed an executive test battery including the Stroop Test, verbal fluency and the Wisconsin Card Sorting Test (WCST). The results demonstrate intact implicit learning in patients, despite an executive dysfunction in the parkinsonian group. They lead to the conclusion that the basal ganglia system affected in Parkinson's disease does not contribute to the implicit acquisition of a new cognitive skill. Furthermore, the parkinsonian patients were able to reach a specific goal in an implicit learning context despite impaired goal directed behavior in the WCST, a classic test of executive functions. These results demonstrate a functional independence of implicit cognitive skill learning and certain aspects of executive functions.

#### P1185

##### Does dopaminergic medication enhance deep sleep in Parkinson's disease? A polysomnographic study in 62 patients

N. J. Diederich, V. Paolini, M. Vaillant (Luxembourg City, Luxembourg)

Background: In Parkinson's disease (PD) excessive daytime sleepiness contrasts with fragmented nocturnal sleep. This sleep "destructuring" is progressive with disease duration. However, some PD patients maintain surprisingly high percentages of deep sleep. Based on pharmacological data, we hypothesized on an inverse relationship between dopaminergic medication and amount of deep sleep.

Methods: Retrospective analysis of polysomnography in 62 non-demented PD patients. All the patients received a stable dopaminergic medication. Daily dopaminergic dosage (DDD) was expressed in levodopa equivalents. The patients were divided in 3 groups. Group A: DDD  $\leq 300$ mg; group B: DDD = 300-600 mg; group C: DDD  $> 600$  mg. Increased deep sleep percentage was defined as  $> 25\%$ , and increased deep sleep time as  $> 75$  min. Statistical analysis included Chi<sup>2</sup>, Student t and Mann-Whitney tests.

Results: The 43 men and 19 women had a mean age of  $64.5 \pm 9.5$  years, a mean disease duration of  $6.1 \pm 4.4$  years and a mean Hoehn-Yahr stage of  $2.2 \pm 0.8$ . The mean DDD was  $567.5 \pm 369.9$  mg, with 16 patients in group A, 22 patients in group B and 24 patients in group C. Sleep efficiency was  $70 \pm 17\%$ , total sleep time  $313 \pm 84$  min, deep sleep time  $65 \pm 45$  min and deep sleep percentage  $16 \pm 11$ . Deep sleep was inversely correlated with age. The groups A and C showed less frequently increased deep sleep percentage than group B ( $p = 0.04$ ). In contrast, the patients of group B had a 10.7 higher risk of increased deep sleep percentage (95%CI = [1.2; 96.3]) and a 3.0 higher risk of increased deep sleep time (95%CI = [0.9; 10.4]) than group C.

Conclusions: While age decreases deep sleep, low or medium dosages of the dopaminergic medication increase absolute and relative amount of deep sleep. The effect seems to be most prominent at a medium dosage. Thus the hypothesis of a simple inverse relationship between dopaminergic dosage and amount of deep sleep in PD has to be refuted. In order to fully apprehend this potentially beneficial soporific effect of the dopaminergic medication at a medium dosage, the influence of the medication timetable on deep sleep amount has to be analyzed as well.

#### P1186

##### Construct validity of a computerized neuropsychological assessment (mindstreams) in patients with Movement Disorders

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Background: Movement Disorders are often accompanied by multi-domain cognitive impairment. Cognitive assessment of such individuals can be expensive, lengthy, and limited to centers with neuropsychologists.

Pre-clinical indicators of Parkinson's Disease:  
Recent Findings From the Honolulu-Asia Aging Study

Abstract

*Objective:* to describe constitutional factors found to pre-date the diagnosis of PD in the prospective Honolulu-Asia Aging Study.

*Background:* The neuropathological and chemical changes underlying Parkinson's disease are thought to begin years before the classic motor syndrome is recognized and diagnosed. As preventive and disease modifying therapies are developed it will be most useful to initiate these interventions before onset of the classic symptoms.

*Methods:* Beginning in 1965, environmental, life-style, and physical attributes were recorded at selected examinations over a 35 year period in a cohort of 8,006 Japanese-American men. Subjects were followed for clinical PD and incidental Lewy bodies. Men with clinical PD were identified in a variety of ways and the final diagnosis was made by consensus of two neurologists. Additionally, autopsies are available on 400 allowing for the use of incidental Lewy bodies as an additional endpoint for the PD process.

*Results:* In 35 years of follow-up, PD was observed in 137 men. Overall incidence (7.1/10,000 person-years) was generally higher than in Asia and similar to rates observed in Europe and the United States. Factors associated with increased PD risk were mid-life constipation, adiposity, and impaired olfaction. Deficits in olfaction and reaction time in later life were associated with an increased likelihood of Lewy bodies noted in the autopsy series. *Conclusions:* Olfactory deficits, mid-life obesity, constipation, and slow reaction time were measured in mid-life were indicators of future clinical PD and / or pathological changes associated with PD. These indicators may be useful for identifying a high risk group for participation in intervention trials aimed at preventing or slowing the progression of PD.

## Occupational Exposures and Movement Abnormalities among Japanese-American Men: The Honolulu-Asia Aging Study

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### Key Words

Occupational toxin exposures · Movement abnormalities · Neurological signs · Normal aging

### Abstract

**Objective:** The authors analyzed data on 1,049 men aged 71–93 years (excluding those with prevalent Parkinson's disease and stroke) from the Honolulu Heart Program (1965–1968) and the Honolulu-Asia Aging Study (1991–1999) to determine whether occupational exposures to pesticides, solvents, metals, manganese, and mercury during middle age were associated with 14 movement abnormalities 25 years later. **Methods:** Analyses of variance and multivariate logistic regression were used to assess associations of interest. **Results:** After adjustment for age, BMI, cognitive functioning, smoking, alcohol drinking, education, and physical activity, there was a positive association between abnormal 'facial expression' and the highest exposure to metals [odds ratio (OR) = 2.62; 95% confidence interval (CI) = 1.35–5.11; trend,  $p = 0.02$ ], and the highest exposure to mercury

(OR = 1.91; 95% CI = 1.04–3.49; trend,  $p = 0.03$ ). Age was positively associated with all movement abnormalities, and cognitive function, body mass index and physical activity were inversely associated with most movement abnormalities. **Conclusion:** Higher exposure to any metal, and specifically mercury, was associated with abnormal facial expression.

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### Introduction

Associations have been identified between occupational exposures and both the incidence (or prevalence) [1, 2] and severity [3–8] of neurological illness. Occupational exposure to mercury and manganese have been positively associated with movement abnormalities such as hand and arm tremors and mask-like facial appearance [4, 5], and exposure to pesticides and hydrocarbon solvents have been positively associated with Parkinson's disease (PD) [3, 6].

The objective of this study was to determine whether occupational exposures to pesticides, solvents and metals assessed prospectively are associated with the development of specific movement abnormalities associated with parkinsonism that may occur in normal aging as well. Such associations would provide evidence that the exposures may cause injury to the extrapyramidal motor system.

## Methods

### *Study Population*

The participants in this study were Japanese-American men who were participants in the Honolulu Heart Program (HHP) and the Honolulu-Asia Aging Study (HAAS). The HHP began in 1965 as a prospective cohort study of cardiovascular disease and stroke, and the HAAS was added in 1991 to investigate determinants of various health conditions in the elderly. Informed consent was obtained from the study participants and the study was approved by an institutional review committee. A detailed description of the methods of the HHP/HAAS has been previously published [9, 10].

Several exams have been conducted in the HHP and HAAS to date. Exam I took place during 1965–1968 and included 8,006 men who were between the ages of 45 and 68 years; exam II (1968–1970) included 7,498 men from the original cohort; exam III (1971–1974) included 6,860 men, and exam IV (1991–1993) included 3,845 men who were between the ages of 71 and 93 years. Participants who were diagnosed with PD ( $n = 61$ ) and stroke ( $n = 113$ ) prior to the first neurological exam (1991–1993) were excluded from the analysis; 2 men had both diagnoses. The neurological exams for the assessment of movement abnormalities took place during the fourth, fifth, and sixth exams. A total of 1,221 men received neurological evaluations at one or more of these exams, 426 during exam IV (1991–1993), 752 during exam V (1994–1996), and 294 during exam VI (1997–1999), and of these, 172 were excluded because of a diagnosis of PD and/or stroke. Therefore, the study sample included 1,049 men without PD or stroke.

### *Assessment of Occupational Exposures*

Information on occupational history was collected during exams I and III. No direct exposure measurements were made. Participants were asked questions about their present and usual occupation, and the age that they started and finished working in these occupations.

Industrial hygienists from the National Institute for Occupational Safety and Health (NIOSH) assessed the potential for pesticide, metal, and solvent exposure in each reported occupation [11]. They created four levels of exposure to the agent, indicating a score of 0 for no potential of exposure, 1 for low exposure, 2 for medium exposure and 3 for potential of high exposure. The ‘high’ classification was assigned to those occupation/industry pairings judged to have significant exposures that were frequently well above analytically detectable concentrations and were at least occasionally near or greater than the Occupational Safety and Health Administration (OSHA)-permissible exposure limits (PELs), if a PEL existed. A ‘high’ score meant that the industrial hygienists were confident that the industry/occupation pairing would frequently be exposed to the

agent. The ‘medium’ exposure classification was assigned to those occupation/industry pairings judged to involve tasks with detectable exposures to the selected agents, but which were considered to usually be below the OSHA-PELs. The ‘low’ exposure classification was assigned to those industry/occupation pairings judged to occasionally have undetectable exposures to the selected agents but which would rarely approach the OSHA-PEL. A ‘0’ score indicated that workers in the industry/occupation pairing were believed to have little potential exposure to the agent. The scores not only reflected the industrial hygienists’ view of the intensity of exposure, but also their confidence that jobs in these industries would mean exposure to these agents. Even though information was collected on ‘present’ and ‘usual’ jobs, ‘usual’ job was primarily used to determine to which industry-occupation group workers should be assigned to determine their exposure. However, if participants had none of the exposures in their usual job but had the exposures in their present job at exams I and/or III, then that information (i.e., in present job) was used in creating a measure of cumulative exposure. In addition, when exposures were present at both exams, the exposures closest to the outcome (i.e., exposures in exam III) were chosen. Exposure intensity scores (i.e., cumulative exposures) were obtained by multiplying the appropriate levels of exposure (0, 1, 2, 3) to usual or present job at exam I or III by the number of years exposed to the agent of interest. Exposure intensity scores were used as continuous variables or categorized into four levels.

### *Assessment of Movement Abnormalities*

A neurologist performed the motor examination section of the Unified Parkinson’s Disease Rating Scale (UPDRS) [12] to participants during exams IV, V and VI. The original coding for the movement abnormalities included five levels: 0 indicated an absence of the abnormality, and 1–4 described the presence of gradually more severe abnormalities. For example, posture was originally coded as follows: 0 = normal erect; 1 = not quite erect, slightly stooped posture, could be normal for older person; 2 = moderately stooped posture, definitely abnormal; 3 = severely stooped posture with kyphosis; 4 = marked flexion with extreme abnormality of posture. Each movement abnormality variable was recorded; if a subject received a score  $\geq 1$  on a UPDRS item, that movement abnormality was considered present (1), otherwise it was considered absent (0).

To examine movement abnormalities in relation to exposure variables, the disorder data that was closest in time to the exposure data (i.e., closest to exams I and III) was used. Thus, if a participant had a measured value of movement abnormality at exam IV, then that value was used. Similarly, if data at exam IV were missing, then the measured value from exam V was taken if available, otherwise the value was taken from exam VI. In addition to individual movement abnormalities, movement abnormalities were placed into groups for assessment with the occupational exposures. All 14 movement abnormalities were summed and the new variable was dichotomized into ‘none’ versus ‘any’ disorders. Also, an indicator variable was created that comprised increasing numbers (six levels) of five movement abnormalities: ‘facial expression’, ‘posture’, ‘gait’, ‘rapid alternating hand movements’, and ‘rigidity’. For example, level 1 of the new variable has zero, level 2 has one (‘facial expression’), and level 6 has all five movement abnormalities. These propensity scores were developed in an attempt to determine the association between the exposures and multiple movement abnormalities. These specific movement abnormalities, ‘facial expres-

**Table 1.** Prevalence of occupational exposure characteristics for study participants at exams I and III

	Pesticide		Solvent		Metal		Manganese		Mercury	
	n	%	n	%	n	%	n	%	n	%
Usual job exposure (exams I and III)										
None (0)	909	86.7	393	37.5	530	50.5	815	77.7	865	82.5
Low (1)	25	2.4	425	40.5	386	36.8	155	14.8	150	14.3
Medium (2)	3	0.3	99	9.4	59	5.6	10	0.9	34	3.24
High (3)	112	10.7	132	12.6	74	7.1	69	6.6	0	0.0
Years of exposure										
0	909	86.7	393	37.5	531	50.6	815	77.7	870	82.9
>0-15	40	3.8	117	11.2	83	7.9	44	4.2	28	2.7
16-30	72	6.9	294	28.0	219	20.9	106	10.1	72	6.9
31+	28	2.7	245	23.4	216	20.6	84	8.0	79	7.5
Intensity scores <sup>1</sup>										
None	909	86.7	393	37.5	531	50.6	815	77.7	870	82.9
Low	53	5.1	412	39.3	355	33.8	142	13.5	55	5.2
Medium	45	4.3	146	13.9	107	10.2	49	4.7	57	5.4
High	42	4.0	98	9.3	56	5.3	43	4.1	67	6.4

<sup>1</sup> Categories for exposure intensity scores (i.e., cumulative exposure) to mercury are 0, 1-25, 26-36,  $\geq 37$ ; categories for all other agents are 0, 1-39, 40-79,  $\geq 80$ . Total sample = 1,049 men.

sion', 'posture', 'gait', 'rapid alternating hand movements', and 'rigidity', were chosen because, in the multivariate analyses, they were more likely than the others to be related to at least one occupational exposure.

#### Assessment of Covariates

In exam I, a physical examination was performed and self-administered questionnaires were completed by each subject. Participants were re-examined and re-interviewed at subsequent exams. Body mass index (BMI) was calculated; a physical activity index was created by multiplying estimated oxygen consumption in liters per minute required for each activity by a weighting factor and summing those values for each level of physical activity [13]. From information obtained in the questionnaires, pack-years of smoking and ounces of alcoholic beverages consumed per month were created. Participants reported the number of years of education completed. Beginning at exam IV, the Cognitive Abilities Screening Index Instrument (CASI) was used to assess cognitive function [14]. CASI score was analyzed as a continuous and as a dichotomous variable - impaired cognition (CASI  $\leq 74$ ), and normal cognition (CASI  $>74$ ). Information on all covariates was assessed at exam IV.

#### Statistical Methods

All analyses were conducted using SAS version 8.02 [15]. Frequencies were obtained for all occupational exposure variables and covariates separately, and in association with each movement abnormality. Trend was assessed by using the Cochran-Armitage Trend Test and general linear models. Analysis of variance was used to obtain the mean levels of covariates by occupational ex-

posure category. Multivariate logistic regression models were used to assess associations of interest (using the first level as the referent) as well as confounding and effect modification. Age, BMI, smoking, alcohol consumption, CASI score, education, and physical activity were assessed for potential confounding and effect modification in associations of occupational exposures with movement abnormalities.

## Results

The prevalence of occupational exposure varied widely (table 1). Jobs with solvent or any metal exposure were most common. For usual job exposure, the prevalence ranged from 13.4% for any pesticide exposure to 62.5% for any solvent exposure. For mercury exposure, no participants had 'high exposure'. Approximately 10% of men had  $\geq 15$  years of pesticide exposure compared to 51% who had the same duration of solvent exposure. Exposure intensity scores also followed the above pattern for all of the exposures.

The five movement abnormalities with the highest prevalence in this population were abnormal 'hand movements' (63.8%), 'rapid alternating hand movements' (62.1%), 'rigidity' (59.6%), abnormal 'posture' (58.6%), and abnormal 'foot agility' (57.0%) (table 2). The major-

**Table 2.** Prevalence of movement abnormalities by age at exam IV, 1991–1993\*

Movement abnormalities	Age, years				All ages
	71–74	75–78	79–82	≥83	
Hand movements	45.9 (207)	57.5 (320)	68.8 (208)	80.6 (278)	63.8 (1,013)
Rapid alternating hand movements	39.3 (206)	56.5 (317)	68.9 (206)	81.0 (268)	62.1 (997)
Rigidity	42.5 (212)	56.5 (322)	64.8 (210)	71.8 (291)	59.6 (1,035)
Posture	33.2 (208)	51.4 (317)	64.1 (209)	81.4 (280)	58.6 (1,014)
Foot agility	36.4 (206)	56.5 (315)	55.9 (204)	74.6 (264)	57.0 (989)
Finger taps	38.9 (208)	51.4 (321)	62.0 (208)	71.5 (281)	56.6 (1,018)
Body bradykinesia	22.8 (211)	35.0 (323)	42.7 (211)	67.8 (292)	43.3 (1,037)
Gait	21.6 (213)	33.0 (318)	44.5 (209)	65.5 (287)	42.1 (1,027)
Postural stability	21.4 (210)	30.9 (311)	48.0 (204)	61.6 (268)	40.7 (993)
Facial expression	22.5 (213)	22.0 (323)	37.0 (211)	51.7 (296)	33.6 (1,043)
Speech	17.3 (214)	20.1 (324)	31.3 (211)	49.3 (296)	30.1 (1,045)
Hand tremor	25.8 (209)	24.5 (323)	29.1 (210)	34.6 (280)	28.5 (1,022)
Arising from chair	13.4 (209)	19.9 (317)	27.5 (207)	43.6 (275)	26.6 (1,008)

Prevalence in percentages; total number in parentheses.

Total sample = 1,049 men.

\* One-sided trend *p* value <0.01 for all movement abnormalities.

ity of participants (91%) had  $\geq 1$  abnormalities with this panel of tests (data not shown). Only 2.1% of participants reported having ‘tremor at rest’ (not shown in table 2). Due to the small numbers in this category, ‘tremor at rest’ was removed from further analyses. Prevalence of all movement abnormalities increased notably with increasing age (*p* for trend <0.01).

Study participants ranged in age from 71 to 93 years, with a mean age of 79 years (table 3). The mean BMI was 23 (99% of the men had a BMI  $\leq 31$ ) and the mean years of education was 9.6 years (range 2–24 years). Among participants who provided the information, 40% were never smokers, 54% were past smokers, and 6% were current smokers; 42% had never used alcohol, 24% had consumed alcohol in the past, and 34% currently drank alcohol (data not shown).

Significant trends were observed between several covariates and the exposure intensity scores (table 3). With increasing levels of all occupational exposures, mean levels of age increased while CASI scores and years of education decreased overall. In general, mean values of BMI, and smoking and alcohol consumption were not associated with the exposures; non-smokers and non-drinkers were excluded from these analyses. An exception involved mercury exposure, where increasing levels of alcohol consumption were associated with increasing levels of exposure (*p* = 0.033).

Cognitive ability (CASI score), BMI, and physical activity were inversely associated with increasing prevalence of most movement abnormalities (data not shown). BMI was not associated with prevalence of ‘arising from chair’ and ‘hand tremor’, and physical activity was not associated with ‘hand tremor’.

Logistic regression analyses revealed associations between exposure intensity scores to metals and one movement abnormality (table 4). There was a positive association between metal exposure in the highest category and abnormal (i.e., fixed or mask-like) ‘facial expression’ after full adjustment (OR = 2.62, 95% CI = 1.35–5.11, *p* for trend = 0.02). A significant positive association was also observed between persons in the highest level of mercury exposure and abnormal ‘facial expression’ (OR = 1.91, 95% CI = 1.04–3.49, *p* for trend = 0.03). The association between manganese exposure and abnormal ‘facial expression’ (OR = 1.71, 95% CI = 0.81–3.61, *p* for trend = 0.08) and abnormal ‘posture’ (OR = 1.99, 95% CI = 0.91–4.36, *p* for trend = 0.09) were elevated but statistical significance was borderline.

While heavy pesticide exposure more than doubled the likelihood of an abnormality in ‘rapid alternating hand movements’, after adjustment for age and other factors, the association was no longer significant (table 4). In contrast, there was an inverse association between heavy pesticide exposure and ‘gait’ after risk factor ad-

**Table 3.** Mean levels of participant characteristics at exam IV by exposure intensity at exams I and III

Participant characteristic	Intensity <sup>a</sup>	Pesticide		Solvent		Metal		Manganese		Mercury		Overall mean ± SD
		n	mean ± SD	n	mean ± SD	n	mean ± SD	n	mean ± SD	n	mean ± SD	
Age, years	None	907	79.2 ± 5.1	391	79.4 ± 5.1	529	79.5 ± 5.1	813	79.3 ± 5.1	867	79.4 ± 5.2	79.3 ± 5.1
	Low	52	78.0 ± 3.8	411	79.0 ± 5.2	354	78.5 ± 5.0	141	78.5 ± 5.2	55	77.5 ± 4.5	
	Medium	45	80.4 ± 5.2	146	79.7 ± 5.1	107	80.8 ± 5.3	49	81.3 ± 5.6	57	77.4 ± 4.1	
	High	42	83.5 ± 5.7	98	80.1 ± 5.1	56	80.3 ± 5.2	43	79.7 ± 4.9	67	81.3 ± 5.2	
	P <sub>trend</sub>		<0.0001		<0.0001		<0.0001		<0.0001		0.0049	
BMI, kg/m <sup>2</sup>	None	865	23.2 ± 3.3	374	23.0 ± 3.1	505	23.1 ± 3.1	776	23.1 ± 3.3	823	23.2 ± 3.3	23.2 ± 3.2
	Low	51	24.2 ± 3.5	392	23.5 ± 3.3	337	23.4 ± 3.5	132	23.7 ± 3.0	53	23.8 ± 3.4	
	Medium	40	23.2 ± 2.9	138	23.4 ± 3.6	100	23.1 ± 3.2	45	23.2 ± 3.2	57	23.5 ± 3.1	
	High	38	23.0 ± 2.9	90	22.8 ± 2.9	52	23.5 ± 2.9	41	23.5 ± 3.0	61	23.1 ± 3.2	
	P <sub>trend</sub>		0.7514		0.0559		0.6936		0.7760		0.6735	
Physical activity	None	806	30.5 ± 4.6	348	30.1 ± 4.3	472	30.1 ± 4.2	719	30.3 ± 4.4	770	30.4 ± 4.7	30.6 ± 4.7
	Low	48	32.1 ± 6.2	367	31.0 ± 5.2	314	31.0 ± 5.1	130	31.5 ± 6.2	50	31.0 ± 4.6	
	Medium	37	29.6 ± 4.4	132	30.4 ± 4.7	95	30.9 ± 5.5	44	31.1 ± 5.3	52	31.6 ± 5.4	
	High	39	31.2 ± 4.5	83	31.5 ± 4.5	49	32.6 ± 4.9	37	32.5 ± 4.5	58	31.7 ± 4.9	
	P <sub>trend</sub>		0.9710		0.0012		<0.0001		0.0008		0.0186	
CASI score	None	906	75.9 ± 19.3	391	76.3 ± 19.3	529	75.6 ± 19.5	812	75.3 ± 19.6	865	75.3 ± 19.8	75.6 ± 19.4
	Low	52	78.6 ± 13.1	410	75.6 ± 19.1	352	76.6 ± 17.9	140	79.2 ± 17.0	55	78.8 ± 18.2	
	Medium	44	70.1 ± 22.5	145	74.2 ± 20.4	107	72.5 ± 23.2	49	70.9 ± 20.9	57	79.8 ± 10.8	
	High	42	71.2 ± 22.6	98	74.2 ± 19.9	56	74.1 ± 18.7	43	74.4 ± 20.2	67	73.2 ± 19.9	
	P <sub>trend</sub>		<0.0001		<0.0001		<0.0001		0.0008		0.0443	
Education years	None	907	9.8 ± 3.1	391	10.6 ± 3.4	529	10.2 ± 3.2	813	9.8 ± 3.1	867	9.7 ± 3.1	9.6 ± 3.1
	Low	52	8.8 ± 2.8	411	9.2 ± 2.8	354	9.3 ± 3.0	141	9.5 ± 3.1	55	9.7 ± 3.1	
	Medium	45	7.8 ± 3.4	146	9.2 ± 2.7	107	8.8 ± 3.1	49	8.6 ± 3.1	57	9.2 ± 2.7	
	High	42	9.6 ± 3.6	98	8.3 ± 2.4	56	8.4 ± 2.2	43	8.7 ± 2.3	67	9.0 ± 3.9	
	P <sub>trend</sub>		0.0137		<0.0001		<0.0001		0.0004		0.1430	
Pack-years of smoking <sup>b</sup>	None	472	42.4 ± 36.0	186	40.7 ± 32.9	259	42.9 ± 35.3	415	43.2 ± 36.1	443	43.5 ± 35.5	42.4 ± 35.2
	Low	20	44.8 ± 29.9	228	44.0 ± 37.1	205	40.7 ± 34.1	75	42.1 ± 33.7	33	41.5 ± 37.1	
	Medium	20	48.0 ± 31.4	76	46.6 ± 35.9	44	54.8 ± 42.4	26	39.7 ± 31.9	34	35.1 ± 29.6	
	High	23	34.8 ± 24.7	45	34.4 ± 32.8	27	30.4 ± 23.8	19	30.3 ± 22.4	25	34.4 ± 34.2	
	P <sub>trend</sub>		0.7110		0.2741		0.3665		0.1279		0.1017	
Alcohol consumption <sup>c</sup> g/month	None	453	10.06 ± 15.77	181	10.80 ± 20.08	248	10.11 ± 16.50	386	9.98 ± 16.10	409	9.55 ± 13.95	10.02 ± 15.22
	Low	20	9.09 ± 10.23	198	8.99 ± 9.92	172	8.59 ± 10.20	72	9.63 ± 10.55	26	10.24 ± 11.05	
	Medium	18	10.23 ± 8.79	74	8.85 ± 9.84	58	13.05 ± 20.61	31	7.70 ± 9.55	38	10.98 ± 13.22	
	High	20	10.00 ± 11.33	58	12.59 ± 17.93	33	11.44 ± 15.71	22	15.16 ± 18.11	38	13.98 ± 27.74	
	P <sub>trend</sub>		0.5911		0.8712		0.6624		0.5221		0.0333	

<sup>a</sup> Categories for exposure intensity scores (i.e., cumulative exposure) to mercury are 0, 1–25, 26–36, ≥ 37; categories for all other agents are 0, 1–39, 40–79, ≥ 80.

<sup>b</sup> Pack-years of smoking include only current and former smokers.

<sup>c</sup> Alcohol consumption includes only current and former drinkers. Mean values of grams were divided by 100 for convenience.

justment,  $p$  for trend = 0.04. There was no association of exposure to solvents and movement abnormalities and there was no evidence of effect modification between the main exposures and the seven covariates presented in table 3 for any of the movement abnormalities.

No trend was observed between the occupational exposures and an indicator variable that comprised increasing numbers of these five movement abnormalities: ‘rapid alternating hand movements’, ‘rigidity’, ‘posture’,

‘gait’, and ‘facial expression’. This analysis was repeated with ‘body bradykinesia’ replacing ‘facial expression’; there was no change in the results. As the number of movement abnormalities increased, (a) the proportion of persons with low CASI scores (<74) increased ( $p < 0.001$ ), (b) the proportion of relatively younger persons (71–78 years) decreased ( $p < 0.001$ ), and (c) the proportion of persons who were less physically active (physical activity score 24–29.0) increased ( $p < 0.001$ ) (data not shown).

**Table 4.** Association between occupational exposure intensity<sup>a</sup> and selected movement abnormalities, unadjusted and adjusted odds ratios and 95% CI and p values from logistic regression models

	n	Unadjusted		Age adjusted		Risk factor adjusted <sup>b</sup>		P <sub>trend</sub>
		OR	95% CI	OR	95% CI	OR	95% CI	
<i>Pesticides</i>								
Rapid alternating hand movements								
None	868	1.00		1.00		1.00		0.35
Low	51	0.76	0.43–1.34	0.81	0.45–1.46	1.00	0.53–1.93	
Medium	44	1.09	0.58–2.04	0.93	0.48–1.80	0.87	0.40–1.91	
High	37	2.26	1.02–4.99	1.45	0.63–3.34	1.97	0.80–4.87	
Posture								
None	884	1.00		1.00		1.00		0.46
Low	50	0.72	0.41–1.27	0.77	0.42–1.40	0.82	0.43–1.59	
Medium	44	2.44	1.19–0.99	2.25	1.06–4.77	1.55	0.68–3.55	
High	39	0.93	0.49–1.77	0.45	0.22–0.93	0.59	0.27–1.27	
Gait								
None	895	1.00		1.00		1.00		0.04
Low	50	0.62	0.34–1.15	0.66	0.35–1.27	1.01	0.51–2.02	
Medium	45	0.97	0.53–1.78	0.79	0.41–1.51	0.73	0.33–1.60	
High	40	0.71	0.37–1.38	0.35	0.17–0.71	0.40	0.18–0.90	
Facial expression								
None	908	1.00		1.00		1.00		0.22
Low	50	0.56	0.28–1.11	0.63	0.32–1.27	0.80	0.37–1.74	
Medium	45	1.58	0.87–2.89	1.42	0.76–2.67	1.25	0.57–2.71	
High	42	1.10	0.58–2.10	0.67	0.34–1.32	0.62	0.28–1.39	
<i>Solvents</i>								
Rapid alternating hand movements								
None	372	1.00		1.00		1.00		0.77
Low	395	1.03	0.77–1.38	1.08	0.80–1.47	1.20	0.85–1.70	
Medium	138	1.03	0.69–1.55	0.99	0.65–1.51	0.97	0.61–1.53	
High	95	1.02	0.64–1.63	0.92	0.56–1.49	0.98	0.56–1.69	
Posture								
None	379	1.00		1.00		1.00		0.84
Low	400	1.16	0.87–1.54	1.25	0.92–1.69	1.21	0.86–1.70	
Medium	141	1.07	0.73–1.59	1.05	0.69–1.59	0.89	0.56–1.41	
High	97	1.25	0.79–1.98	1.12	0.69–1.82	1.18	0.68–2.06	
Gait								
None	384	1.00		1.00		1.00		0.28
Low	405	0.92	0.70–1.23	0.96	0.71–1.30	1.03	0.73–1.46	
Medium	144	0.95	0.65–1.40	0.91	0.60–1.37	0.93	0.58–1.48	
High	97	1.46	0.93–2.28	1.35	0.84–2.17	1.50	0.86–2.59	
Facial expression								
None	392	1.00		1.00		1.00		0.19
Low	409	1.11	0.83–1.49	1.18	0.87–1.60	1.32	0.91–1.92	
Medium	146	1.05	0.70–1.57	1.02	0.67–1.56	1.18	0.72–1.93	
High	98	1.35	0.86–2.14	1.29	0.80–2.07	1.57	0.89–2.79	
<i>Metals</i>								
Rapid alternating hand movements								
None	504	1.00		1.00		1.00		0.23
Low	340	0.93	0.70–1.24	1.04	0.77–1.40	1.18	0.85–1.65	
Medium	101	0.89	0.58–1.38	0.74	0.47–1.18	0.79	0.47–1.32	
High	55	0.81	0.46–1.43	0.70	0.39–1.28	0.66	0.33–1.29	
Posture								
None	512	1.00		1.00		1.00		0.28
Low	344	1.04	0.79–1.38	1.23	0.92–1.66	1.17	0.84–1.62	
Medium	105	1.03	0.67–1.57	0.84	0.53–1.34	0.78	0.47–1.31	
High	56	1.85	1.01–3.40	1.75	0.92–3.33	2.02	0.99–4.12	



**Table 4** (continued)

	n	Unadjusted		Age adjusted		Risk factor adjusted <sup>b</sup>		P <sub>trend</sub>
		OR	95% CI	OR	95% CI	OR	95% CI	
<i>Gait</i>								
None	520	1.00		1.00		1.00		0.22
Low	348	0.81	0.62–1.07	0.91	0.68–1.22	0.95	0.68–1.34	
Medium	106	1.19	0.78–1.81	1.01	0.65–1.58	1.07	0.64–1.79	
High	56	1.43	0.82–2.49	1.32	0.73–2.36	1.81	0.93–3.55	
<i>Facial expression</i>								
None	530	1.00		1.00		1.00		0.02
Low	352	0.96	0.72–1.27	1.07	0.79–1.45	1.26	0.88–1.80	
Medium	107	1.22	0.79–1.88	1.07	0.68–1.68	1.13	0.66–1.94	
High	56	1.65	0.95–2.88	1.57	0.88–2.80	2.62	1.35–5.11	
<i>Manganese</i>								
<i>Rapid alternating hand movements</i>								
None	774	1.00		1.00		1.00		0.54
Low	137	0.85	0.58–1.22	0.92	0.62–1.35	1.09	0.71–1.67	
Medium	47	1.29	0.68–2.41	1.01	0.52–1.98	0.92	0.44–1.92	
High	42	0.89	0.47–1.67	0.83	0.43–1.60	0.78	0.37–1.65	
<i>Posture</i>								
None	790	1.00		1.00		1.00		0.09
Low	136	0.89	0.62–1.29	1.01	0.68–1.49	1.12	0.73–1.72	
Medium	48	1.60	0.85–2.98	1.25	0.64–2.47	1.42	0.67–3.02	
High	43	1.67	0.86–3.26	1.64	0.82–3.31	1.99	0.91–4.36	
<i>Gait</i>								
None	800	1.00		1.00		1.00		0.38
Low	138	0.83	0.57–1.20	0.89	0.60–1.33	1.06	0.68–1.65	
Medium	49	1.55	0.87–2.76	1.20	0.65–2.23	1.29	0.63–2.62	
High	43	0.98	0.53–1.83	0.92	0.48–1.78	1.31	0.62–2.79	
<i>Facial expression</i>								
None	813	1.00		1.00		1.00		0.08
Low	140	0.88	0.60–1.30	0.96	0.64–1.43	1.26	0.79–1.99	
Medium	49	1.37	0.76–2.47	1.11	0.60–2.05	1.24	0.61–2.55	
High	43	1.18	0.62–2.26	1.15	0.60–2.21	1.71	0.81–3.61	
<i>Mercury</i>								
<i>Rapid alternating hand movements</i>								
None	826	1.00		1.00		1.00		0.27
Low	51	1.02	0.57–1.83	1.29	0.70–2.36	1.23	0.65–2.32	
Medium	57	0.77	0.45–1.33	0.95	0.54–1.66	1.01	0.55–1.86	
High	66	0.99	0.59–1.66	0.76	0.44–1.31	0.66	0.36–1.21	
<i>Posture</i>								
None	840	1.00		1.00		1.00		0.13
Low	53	1.54	0.85–2.79	2.16	1.17–4.01	2.09	1.09–4.01	
Medium	57	0.61	0.36–1.05	0.77	0.44–1.36	0.89	0.48–1.66	
High	67	1.84	1.06–3.18	1.46	0.81–2.63	1.64	0.85–3.14	
<i>Gait</i>								
None	853	1.00		1.00		1.00		0.04
Low	53	1.00	0.57–1.75	1.35	0.74–2.43	1.60	0.84–3.05	
Medium	57	0.76	0.43–1.33	0.99	0.55–1.77	1.07	0.56–2.04	
High	67	1.73	1.05–2.85	1.40	0.82–2.39	1.72	0.93–3.16	
<i>Facial expression</i>								
None	867	1.00		1.00		1.00		0.03
Low	54	0.92	0.51–1.67	1.16	0.63–2.14	1.61	0.82–3.16	
Medium	57	0.79	0.43–1.42	0.99	0.54–1.82	1.05	0.52–2.09	
High	67	1.63	0.99–2.69	1.37	0.82–2.32	1.91	1.04–3.49	

<sup>a</sup> Categories for exposure intensity scores (i.e., cumulative exposure) to mercury are 0, 1–25, 26–36, ≥ 37; categories for all other agents are 0, 1–39, 40–79, ≥ 80.

<sup>b</sup> Results adjusted for age, BMI, physical activity, CASI score, education, smoking, and alcohol consumption.

p value for trend is obtained from using the continuous form of the exposure variable in the risk-factor-adjusted logistic model.

## Discussion

Our findings were based on men who were free of PD and stroke. We found that exposures to both metal and mercury were positively and independently associated with fixed 'facial expression'. The literature supports the association between exposure to metals and certain movement abnormalities. We also observed very strong associations for age and CASI score with all movement abnormalities. The results regarding age and movement abnormalities are consistent with the results of previous studies [16, 17].

A few studies have investigated the effects of occupational exposure, mostly manganese and mercury exposures, on movement abnormalities. A study conducted in Brazil investigated the health effects of the fungicide Maneb (a pesticide that contains manganese) in 50 male rural workers [18]. There was a significantly higher prevalence of several neurological symptoms in the exposed group (e.g., plastic rigidity with cogwheel phenomenon, bradykinesia) compared to the unexposed group. In another study, 5 patients who experienced chronic manganese intoxication for 10 years showed significant gradual deterioration in neurologic features, the most prominent of which were gait, rigidity, and speed of foot tapping [19]. Case reports of persons exposed to manganese intoxication showed associations with several neurological symptoms including paraplegia, slowness and difficulty in speech, shaking of the arms and legs, and mask-like facial appearance [5]. Occupational exposure to mercury compounds was associated with hand and arm tremors [4, 8, 20, 21]. The biologic mechanisms involved in the neurotoxicity of mercury include increase of intracellular  $Ca^{2+}$  with disturbance of neurotransmitter function, oxidative stress, and inhibition of protein synthesis [22].

The literature provides ample evidence that movement abnormalities that occur with PD are caused by selective degeneration of dopaminergic neurons of the substantia nigra, oxidative damage, and mitochondrial impairment [23, 24]. The lipophilic pesticide rotenone has been shown to cause degeneration of the dopaminergic neurons in substantia nigra in rats [25]. The rotenone-treated animals developed motor and postural deficits characteristic of PD, such as hypokinesia, unsteady movement, hunched posture, rigidity, and rest tremor. Manganese is known to increase the oxidation rate of dopamine [26].

The association between several occupational and environmental agents and PD is well documented. A French case-control study, after adjustment for confounders, re-

ported a positive association between PD and occupational exposure to pesticides (OR = 2.2, 95% CI = 1.1–4.3) [3]. Their results, however, did not show a clear exposure-response relationship. Pezzoli et al. [6] reported that the intensity of occupational exposure to hydrocarbon solvents was directly proportional to the severity of PD symptoms. They identified nine blue-collar occupations that experienced a preponderance of hydrocarbon exposure, including 'farmers'. In a case-control study [27], investigators found an increased risk for PD with occupational exposure to some metals, and a protective relationship with exposure to mercury. Persons who were exposed to manganese for more than 20 years had an elevated risk for PD (OR = 10.61, 95% CI = 1.06–105.83), and this was also true for exposure to copper (OR = 2.49, 95% CI = 1.06–5.89).

There are a few limitations of this study. Information on short part-time jobs, non-occupational sources of exposures to these specific compounds, and biological markers of the exposures of interests were not included in our assessment of exposure. Misclassification of environmental exposures is likely to have resulted in non-differential bias, producing weaker associations. We based our exposure estimates on reported usual or last job held. Therefore, this may not have accurately reflected all the jobs that the participants held over their exposure history. Although the intensity values of exposure (0, 1, 2, 3) failed to take into account the variability in use of personal protective equipment, local exhaust ventilation, etc., we do not believe that this failure seriously biased our exposure classification. First, the workers were all assessed during the same time period and would have had roughly the same access to personal protective equipment and other engineering controls regardless of job type or industry. Second, the industrial hygienists from NIOSH assigned intensity levels based not only on the job titles, but also on the industry and their knowledge of the specific job duties. Even so, some inter-worker exposure variability would still be present, but it is often not feasible, in occupational epidemiological studies, to capture and analyze individual exposures.

This study has several strengths. The information for this study was collected prospectively, removing any possibility of recall bias. The sample size is large, thus allowing for adequate power even after stratification. A unique approach was implemented in the design of the study by excluding PD and stroke cases, thereby removing the confounding effects of these medical conditions on the associations with movement abnormalities. Several potential effect modifiers and confounders were available for as-

assessment in this study. Assessment of the movement abnormalities was carried out using the UPDRS, a validated, diagnostic instrument for diagnosis of PD, by an experienced neurologist. Industrial hygienists utilized their professional expertise to assign levels of likely exposure to all reported usual jobs. This study used the best possible method for assessing chronic exposure in an occupational epidemiologic study and it is likely that this process contributed to a reduction in exposure misclassification bias [28].

Movement abnormalities eventually result in decreased independence in activities of daily living and increased mortality [16, 29, 30]. In a study of disability as related to movement abnormalities, 48% of persons who reported tremors had difficulty with household tasks and 18% had difficulty dressing themselves [29]. In a population of elderly persons in East Boston, Massachusetts, who were 65 years of age or older, the prevalence of parkinsonian signs were 14.9% for people 64–74 years of age, 29.5% for those 75–84 years of age, and 52.4% for those 85 and older [16]. After adjustment for age and sex, the overall risk of death among people with parkinsonism was twice that among people without. Another cohort study demonstrated that the severity of gait disorders and the rate of progression were related to increased mortality [30]. Movement abnormalities might be expected to have caused the men in our study to have some difficulties with activities of daily living. Whether these abnormalities are associated with increased mortality is worthy of future study.

Studies investigating risk factors for movement abnormalities are important because these outcomes are be-

coming more of a public health problem as the population ages. The number of elderly persons ( $\geq 65$  years) living in the US in 2000 was 35 million, and this number has been predicted to grow dramatically over the next few decades [31]. Knickman and Snell [31] surmise that the most important challenge related to aging populations may be that of keeping seniors disability-free. Identifying and reducing exposures in the environment and workplace has significant implications for influencing overall health and promoting healthy aging. By investigating occupational risk factors that could increase movement abnormalities, this study plays a role in potentially reducing disability in old age.

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# Association of Olfactory Dysfunction with Risk for Future Parkinson's Disease

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**Objective:** Although olfactory dysfunction is commonly associated with Parkinson's disease (PD), it is not known whether such dysfunction can predate the onset of clinical PD in a community-based population. This study examines the association of olfactory dysfunction with future development of PD in Honolulu-Asia Aging Study cohort members.

**Methods:** Olfaction was assessed from 1991 to 1996 in 2,267 men in the Honolulu-Asia Aging Study aged 71 to 95 years who were free of clinical PD and dementia at the time of olfaction testing. Participants were followed for up to 8 years for incident PD.

**Results:** In the course of follow-up, 35 men were diagnosed with PD (24.6/10,000 person-years). The average age at the time of diagnosis was  $82.9 \pm 3.8$  (range, 76–93) years, and the average time to a diagnosis was  $4.0 \pm 1.9$  (range, 1–8) years. During the first 4 years of follow-up, age-adjusted incidence of PD declined from 54.5/10,000 person-years in the lowest quartile of odor identification to 26.6, 8.2, and 8.4/10,000 person-years in the second, third, and fourth quartiles, respectively ( $p < 0.001$  for trend). After adjustment for age and other potential confounders, the odds ratios for PD in the lowest quartile was 5.2 (95% confidence interval, 1.5–25.6) compared with the top two quartiles. This relation was not evident beyond 4 years of follow-up.

**Interpretation:** Impaired olfaction can predate clinical PD in men by at least 4 years and may be a useful screening tool to detect those at high risk for development of PD in later life.

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Olfactory dysfunction is associated with Parkinson's disease (PD), whether measured by odor identification, recognition, or threshold.<sup>1,2</sup> Evidence is accumulating that impaired olfaction may precede the classical motor manifestations by several years; however, definitive affirmation that this occurs in a general population is lacking. Olfactory deficits occur in the earliest stages of clinical PD.<sup>3,4</sup> Asymptomatic first-degree relatives of patients with PD are more likely than those without a family history to have impaired olfaction.<sup>5</sup> In an important study but one using a selected sample of relatives of PD patients, olfactory deficits were shown to precede PD.<sup>6</sup> Recent neuropathological advances suggest that the olfactory system is among the earliest brain regions involved in PD,<sup>7</sup> and olfactory deficits are associated with the presence of incidental Lewy bodies in the brains of decedents without parkinsonism or dementia during life.<sup>8</sup>

Despite these findings, it is not known whether ol-

factory deficits can precede the cardinal motor features of PD in a general population-based setting. The aim of this study was to longitudinally examine the association of impaired odor identification with future risk for PD in the population-based, longitudinal Honolulu-Asia Aging Study (HAAS).

## Subjects and Methods

### Study Sample

From 1965 to 1968, the Honolulu Heart Program began following 8,006 men of Japanese ancestry for development of cardiovascular disease.<sup>9</sup> All the men were born 1900 to 1919 and living on the island of Oahu, Hawaii, at study inception. The HAAS was created as an expansion of the Honolulu Heart Program to study dementia and PD beginning with the 1991–1993 full cohort examination and continuing with follow-up examinations: 1994–1996, 1997–1999, and 1999–2000.<sup>10,11</sup> Procedures for all examinations were in accordance with institutional guidelines and approved by an

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institutional review committee. Informed consent was obtained from all study participants.

### *Olfaction and Potential Confounding Variables*

Olfaction was tested using the Brief Smell Identification Test (B-SIT; also known as the Cross-Cultural Smell Identification Test), which contains 12 of the 40 items of the University of Pennsylvania Smell Identification Test.<sup>12,13</sup> Participants were asked by trained research technicians in face-to-face interviews to identify the correct odor from four possible choices for each item. The odor identification score was the number correct (range, 0–12). A higher score reflects better odor identification. Testing was performed during the 1991–1993 and 1994–1996 HAAS examinations. During the 1991–1993 examination, a subgroup of 948 men received the Brief Smell Identification Test as a component of the second phase of this examination. These men were selected for phase 2 based on cognitive screening scores with sampling from high, intermediate, and low scoring groups, as described previously.<sup>11</sup> During the 1994–1996 examination, 2,705 men were examined and all received the olfactory testing. For those who had testing at both examinations, the earliest available odor identification score was used. Overall, 2,906 men received olfactory testing at least once.

Additional factors were considered as possible sources of confounding to help isolate the independent association between impaired olfaction and risk for future PD. These included age at the time of olfactory assessment, midlife cigarette smoking and coffee intake, bowel movement frequency, excessive daytime sleepiness, and cognitive function. Midlife pack-years of cigarette smoking and coffee intake were measured during the baseline Honolulu Heart Program examination (1965–1968) as typical lifetime exposures to these factors. Late-life coffee intake was not assessed at the time of olfactory testing, and current cigarette smoking was too uncommon to allow for careful assessment. Cognitive function was assessed at the time of olfactory testing using the Cognitive Abilities Screening Instrument (CASI)<sup>11,14</sup> a comprehensive measure of intellectual function that has been developed and validated for use in cross-cultural studies. Scores range from 0 to 100, with higher scores indicating better cognitive function. Bowel movement frequency and excessive daytime sleepiness were assessed from 1991 to 1993.

### *Parkinson's Disease Case Finding and Diagnosis*

Efforts to identify all PD cases in the cohort began in 1991 and have continued through all subsequent examinations. Detailed case finding methods have been previously published.<sup>10,15,16</sup> During each examination all participants were questioned about a diagnosis of PD, symptoms of parkinsonism, and the use of PD medications by structured interview. They also received an examination by research technicians trained to recognize the clinical signs of parkinsonism (including gait disturbance, tremor, and bradykinesia). Those with a history of PD, use of PD medications, or symptoms or signs of parkinsonism were referred to a study neurologist who administered standardized questions about symptoms and the onset of parkinsonism, previous diagnoses, and medication use, followed by a comprehensive and standardized neurological examination that included the Unified Parkin-

son's Disease Rating Scale.<sup>17</sup> Videotaping was added to the standardized neurologist examination in 1999. Final diagnosis was by consensus of at least two neurologists using published diagnostic criteria without access to risk factor data examined in this report. Inclusion criteria were: (1) parkinsonism (eg, bradykinesia or resting tremor combined with rigidity or postural reflex impairment); (2) a progressive disorder; (3) any two of a marked response to L-dopa, asymmetry of signs, asymmetry at onset, or initial onset tremor; and (4) absence of any causative factor known to cause similar features.<sup>18</sup> Cases of parkinsonism related to progressive supranuclear palsy, multiple system atrophy, cerebrovascular disease, drug-induced parkinsonism, postencephalitic parkinsonism, or posttraumatic parkinsonism were not included among the cases of PD. Up to 8 years of follow-up data were available for each participant.

### *Statistical Methods*

Crude and age-adjusted incidence rates of PD per 10,000 person-years of follow-up were estimated across approximate quartiles of odor identification scores (based on the best balance of sample size) using standard analysis of covariance procedures.<sup>19</sup> Scores in the first, second, third, and fourth quartiles were 0 to 5, 6 to 7, 8 to 9, and 10 to 12, respectively. Based on the possibility that olfactory impairment precedes the motor symptoms of PD by a limited period,<sup>6,20</sup> separate analyses were performed for the first 4 years and second 4 years of follow-up. Average values and percentages of potential confounders were also derived and age-adjusted across the odor identification quartiles. Because the number of PD cases was small, logistic regression models were examined to assess the association of the odor identification score with the risk for PD based on exact testing methods.<sup>21</sup> Here, a test for trend was provided by modeling the odor identification score as an independent variable in its original format with scores ranging from 0 to 12. The logistic regression was further adapted for a survival analysis where parameter estimates are known to be similar to those that appear in a proportional hazards regression model, particularly in the instance when event counts are low.<sup>22,23</sup> After adjustment for age and the other study characteristics, odds ratios for PD and 95% confidence intervals were estimated comparing the risk for PD in men in each of the bottom two odor identification quartiles with men in the top two quartiles (reference group). All *p* values were based on two-sided tests of significance.

### **Results**

Among the 2,906 men who received olfaction testing at baseline, 58 with prevalent PD (ie, had PD at the time of olfactory testing), 234 with dementia, and 347 with nasal congestion on the day of olfactory testing were excluded from follow-up, leaving 2,267 men in the study sample. The average age at the beginning of follow-up was  $79.7 \pm 4.1$  (range, 71–95) years. During the course of follow-up, 35 men were diagnosed with PD (24.6/10,000 person-years). The average age at the time of diagnosis was  $82.9 \pm 3.8$  (range, 76–93)

years, and the average time to a diagnosis was  $4.0 \pm 1.9$  (range, 1–8) years.

Table 1 displays the study characteristics across quartiles of odor identification. Decreased odor identification was associated with older age, greater pack-years of smoking, more midlife coffee intake, less frequent bowel movements, excessive daytime sleepiness, and lower CASI score. In all instances, there was a significant test for trend ( $p < 0.03$ ).

Table 2 shows the incidence of PD within quartiles of odor identification for the first 4 years and second 4 years of follow-up. During the first 4 years of follow-up, age-adjusted incidence of PD, expressed as number of cases per 10,000 person-years, decreased from 54.5 in the lowest quartile of odor identification, to 26.6 in the second quartile, to 8.2 in the third quartile, and to 8.4 in the highest quartile ( $p < 0.001$  for trend). After adjusting for age, midlife cigarette smoking and coffee drinking, bowel movement frequency, excessive daytime sleepiness, and CASI score, the relative odds of development of PD using the highest two quartiles of odor identification as the reference group were 3.1 (95% confidence interval [CI], 0.6–16.1) for the second quartile and 5.2 (95% CI, 1.5–25.6) for the lowest quartile ( $p = 0.001$  for trend).

For the second 4 years of follow-up, there was no apparent relation between olfaction and incident PD (see Table 2). Age-adjusted incidence of PD was 18.0 in the lowest quartile of odor identification, 42.1 in the second quartile, 23.9 in the third quartile, and 28.6 in the highest quartile ( $p = 0.694$  for trend). PD cases diagnosed in the first 4 years of follow-up were similar to those diagnosed in the second 4 years with respect to the clinical characteristics described in Table 1 with the exception of coffee consumption. On average, participants with PD diagnosed in the first 4 years of follow-up consumed more than twice the amount of coffee compared with those diagnosed in the second 4 years of follow-up (13.6 vs 6.4 oz/day;  $p = 0.034$ ).

Because of the possibility that some men may have performed poorly on the olfaction test because of cognitive impairment or early undiagnosed dementia, we repeated the analysis for the first 4 years of follow-up after excluding 280 men who scored less than 74 on the CASI (equivalent to a Mini-Mental State Examination score of 22<sup>24</sup>). This cutoff score corresponds to the 16th percentile of CASI scores and has been used in previous analyses involving the CASI in this cohort.<sup>25</sup> Age-adjusted incidence per 10,000 person-years was 54.6 (8 PD cases/418 at risk) for the first quartile, the lowest odor identification score quartile; 29.8 (5/455) for the second quartile; 9.1 (2/563) for the third quartile; and 4.5 (1/551) for the fourth quartile ( $p = 0.001$  for trend). In a model adjusting for the same factors as in Table 2 and using the highest two quartiles as the reference group, the odds ratios for incident PD were 4.0 (95% CI, 0.7–26.4) for the second quartile and 6.1 (95% CI, 1.4–40.9) for the first quartile. The corresponding test for trend yielded a  $p$  value of 0.001. When a similar analysis was performed for the second 4 years of follow-up removing those with a CASI score of less than 74, there was still no association between olfactory identification and PD incidence.

The average time from olfaction testing to PD diagnosis was examined for each quartile of odor identification. The average time to diagnosis in the lowest quartile was 3.1 years, followed by 4.1 years in the second quartile, 4.8 years in the third quartile, and 4.7 years in the highest quartile. The time to PD diagnosis increased significantly with higher odor identification after adjustment for age ( $p = 0.005$ ).

## Discussion

Results from the HAAS presented here are unique in that this is the first population-based prospective study to demonstrate that odor identification deficits can

**Table 1. Mean Age and Age-Adjusted Average and Percentage of Characteristics by Quartile of Odor Identification Score**

Study Characteristics	Quartile of Odor Identification Score			
	1st (0–5) <sup>a</sup> (n = 549)	2nd (6–7) <sup>a</sup> (n = 515)	3rd (8–9) <sup>a</sup> (n = 622)	4th (10–12) <sup>a</sup> (n = 581)
Mean age $\pm$ SD, yr <sup>b</sup>	81.2 $\pm$ 4.5	80.2 $\pm$ 4.2	79.2 $\pm$ 3.7	78.4 $\pm$ 3.3
Mean midlife pack-years of smoking $\pm$ SD <sup>b</sup>	28.3 $\pm$ 28.4	27.0 $\pm$ 28.4	25.3 $\pm$ 26.4	20.7 $\pm$ 23.6
Mean midlife coffee intake $\pm$ SD, oz/day <sup>b</sup>	14.6 $\pm$ 12.9	13.8 $\pm$ 13.3	13.4 $\pm$ 12.8	12.6 $\pm$ 12.2
Mean bowel movements/day $\pm$ SD <sup>c</sup>	2.1 $\pm$ 0.6	2.2 $\pm$ 0.5	2.3 $\pm$ 0.5	2.3 $\pm$ 0.5
Excessive daytime sleepiness, % <sup>d</sup>	8.4	7.6	6.0	5.3
Mean CASI score $\pm$ SD <sup>c</sup>	80.4 $\pm$ 11.5	83.6 $\pm$ 8.7	84.9 $\pm$ 7.9	86.6 $\pm$ 7.0

<sup>a</sup>Number of odors recognized. <sup>b</sup>Significant decline with increased olfaction ( $p < 0.001$ ). <sup>c</sup>Significant increase with increased olfaction ( $p < 0.001$ ). <sup>d</sup>Significant decline with increased olfaction ( $p = 0.029$ ).  
SD = standard deviation; CASI = Cognitive Abilities Screening Instrument.

**Table 2. Incidence of Parkinson's Disease by Quartile of Odor Identification Score**

Quartile of Odor Identification Score	Incidence/10,000 Person-Years (PD cases/sample at risk)		Adjusted OR (95% CI) <sup>a</sup>
	Crude	Age Adjusted	
<i>First 4 Years of Follow-up</i>			
1st (0–5 odors identified)	51.1 <sup>b</sup> (10/549)	54.5 <sup>b</sup>	5.2 <sup>c</sup> (1.5–25.6)
2nd (6–7 odors identified)	25.9 (5/515)	26.6	3.1 (0.6–16.1)
3rd (8–9 odors identified)	8.4 (2/622)	8.2	Reference
4th (10–12 odors identified)	8.9 (2/581)	8.4	Reference
Overall	22.3 (19/2267)		
Test for trend, <i>p</i>	<0.001	<0.001	0.001
<i>Second 4 Years of Follow-up</i>			
1st (0–5 odors identified)	16.7 (2/389)	18.0	0.3 (0.0–2.7)
2nd (6–7 odors identified)	40.2 (5/409)	42.1	2.2 (0.5–4.1)
3rd (8–9 odors identified)	24.5 (4/526)	23.9	Reference
4th (10–12 odors identified)	30.4 (5/522)	28.6	Reference
Overall	28.0 (16/1846)		
Test for trend, <i>p</i>	0.550	0.694	0.646

<sup>a</sup>Adjusted for age, midlife cigarette smoking and coffee drinking, bowel movement frequency, excessive daytime sleepiness, and the Cognitive Abilities Screening Instrument. <sup>b</sup>Significant excess risk for Parkinson's disease versus the reference (*p* = 0.001). <sup>c</sup>Significant excess risk for Parkinson's disease versus the reference (*p* = 0.007).  
 PD = Parkinson's disease; OR = odds ratio; CI = confidence interval.

predate the development of clinical PD in men by at least 4 years. These results remained significant when restricting the at-risk population to those without cognitive impairment.

It is well established that olfactory deficits are common in PD, occurring at about the same frequency as resting tremor,<sup>2,26,27</sup> and previous evidence suggests that impaired olfaction may precede the cardinal motor features of PD. In cross-sectional studies, PD patients report subjective problems with smell before diagnosis,<sup>4</sup> and olfactory deficits have been found in untreated patients with early PD.<sup>3,28</sup> In one study, up to 90% of PD patients tested had lower odor identification scores than healthy matched control subjects, and olfactory deficits were unrelated to severity or duration of disease or use of medications.<sup>26</sup> One explanation for the lack of association between olfactory impairment and severity of cardinal motor features is that olfactory deficits reach a maximum early in the course of PD whereas motor signs continue to worsen through the later stages.<sup>29</sup> Consistent with this idea is a recent imaging study using a group of PD patients early in their disease that found a significant positive correlation between odor identification and dopamine transporter binding on [<sup>99m</sup>Tc] TRODAT-1 single-photon emission tomography imaging in the putamen, but no correlation between dopamine transporter binding and motor function or symptom duration.<sup>29</sup>

Asymptomatic first-degree relatives of PD patients have also been reported to have significantly lower

odor identification scores than similarly aged control subjects without a family history of PD.<sup>5</sup> Hyposmic, nonparkinsonian relatives of PD patients are reported to have lower striatal dopamine transporter binding as measured by [<sup>123</sup>I] β-CIT single-photon emission tomography imaging compared with normosmic relatives, suggesting subclinical striatonigral degeneration in the hyposmic patients.<sup>30</sup> In a follow-up report from the same prospective study, 4 of 40 hyposmic patients with decreased [<sup>123</sup>I] β-CIT binding ratios on single-photon emission tomography at baseline were diagnosed with PD 2 years after the baseline examination. None of the 38 normosmic patients were diagnosed with PD. Among the patients who underwent a second scan, mean decline in β-CIT binding was greater in the hyposmic patients than in those who were normosmic.<sup>6</sup>

A prospective study of World War II veteran twins tested olfactory identification in 19 unaffected brothers who had a twin with PD. Two of these had newly developed PD after 7 years of follow-up. In these men, repeat olfactory testing demonstrated that the average decline in olfactory identification scores was greater compared with the decline among those who did not experience development of PD.<sup>20</sup> Taken together, these studies provide strong evidence that impaired olfaction typically occurs before the classical motor features of PD.

Findings from the HAAS presented here demonstrate that impaired olfaction was not a strong predic-



tor of PD when follow-up time from olfaction testing to development of PD was beyond 4 years. Although small sample size limits definitive conclusions, this finding cannot be attributed to one or two PD cases happening to fall in a high olfaction quartile. One interpretation of this finding is that the relation of olfactory deficits to greater risk for future PD begins to weaken beyond a threshold of approximately 4 years between testing and diagnosis. This idea is supported by three lines of evidence. First is our finding that time from olfactory testing to diagnosis is shortest among those in the lowest quartile of odor identification. Second are the findings of the two prior prospective studies examining olfaction and PD. Although sample size issues limit firm conclusions, in the study of olfaction and PD in World War II veteran twins discussed earlier, olfaction was not a sensitive indicator of incident PD when measured 7 or more years before onset of motor signs.<sup>20</sup> Follow-up was only 2 years from olfactory testing to diagnosis in the only other prospective study demonstrating impaired olfaction in unaffected family members of PD patients who were destined to experience development of PD.<sup>6</sup> Therefore, findings from these two studies suggest that olfactory impairment begins between 2 and 7 years before PD diagnosis. Lastly, although the exact time between disease onset and appearance of classical motor features of PD is not known, estimates from functional neuroimaging and pathological studies suggest a preclinical period between the onset of neuronal loss in the substantia nigra and PD diagnosis of approximately 5 to 7 years.<sup>31–33</sup>

The pathological substrate of olfactory deficits in PD is unknown. Neuronal loss and Lewy body formation are well documented in the olfactory structures in PD.<sup>1,7</sup> One study of seven PD cases and seven control subjects found a strong correlation between neuron loss in the anterior olfactory nucleus and duration of disease.<sup>34</sup> Another study of 10 cases and control subjects used tyrosine hydroxylase immunohistochemistry to identify dopaminergic cells specifically and found that the number of these cells in the olfactory bulb of PD patients was increased relative to age- and sex-matched control subjects. Noting the neuroinhibitory role of dopamine in olfactory transmission, it was speculated that higher dopamine function suppresses olfaction.<sup>35</sup>

The work of Braak and colleagues,<sup>36</sup> who meticulously examined the brains of deceased persons without neurological disease, suggests that the olfactory structures together with the dorsal motor nucleus of the vagus nerve are the earliest brain regions to be affected by Lewy degeneration,<sup>7,36</sup> supporting the expectation that impaired olfaction could be one of the earliest signs of disease. Additional support for this hypothesis comes from a recent HAAS publication that demonstrates an association of impaired olfactory identification during late life with the presence of incidental Lewy bodies in

the substantia nigra or locus ceruleus of deceased cohort members without clinical PD or dementia during life.<sup>8</sup>

Another possible explanation for the olfactory deficits in PD is related to impaired olfactory neurogenesis. The olfactory bulb is one of two regions in the brain that receive new neurons throughout life. The neural stem or precursor cells originate in the subventricular zone between the striatum and lateral ventricle, and migrate along the rostral migratory stream to the olfactory bulb where they mature into functioning interneurons.<sup>37,38</sup> Diminished olfactory neurogenesis in mice is associated with impaired fine olfactory discrimination.<sup>39</sup> Dopamine depletion impairs precursor cell proliferation in rodents, and reduced numbers of these cells have been documented in the subventricular zone in the brains of PD cases.<sup>40</sup>

Olfactory deficits in PD may not entirely be related to pathology in the olfactory structures. Recent pathological studies have documented diminished volume and number of neurons and Lewy pathology in the corticomедial nuclear complex of the amygdala in PD patients without dementia. The cortical nucleus of the amygdala has olfactory connections and is known to be involved in olfactory function, suggesting the possibility that neurodegeneration in the amygdala may also contribute to the olfactory deficits in PD.<sup>41</sup>

Motoric aspects of sniffing affect odor detection, and PD patients have been shown to exhibit significant impairment in sniff airflow rate and volume. Furthermore, olfactory function improves with increased sniff vigor and is significantly correlated with a subset of measures on the Unified Parkinson's Disease Rating Scale related to axial function, prompting speculation that impaired sniffing may be another motor symptom of PD.<sup>42</sup>

There are potential limitations to this study. First, it is important to note that the HAAS population consists entirely of men and the results of this analysis may not be applicable to women. Second, although the Brief Smell Identification Test was designed to be free from cultural bias, there are still issues related to the HAAS Japanese American men that limit the validity of applying published norms to this population. However, by using quartiles of odor identification that demonstrate a dose–effect relation with incident PD, a true biological mechanism is strongly suggested that likely applies to all populations. Third, the average age at onset of PD in this study is older than usually reported. This is related to the age range of the cohort at the beginning of follow-up. However, there is no evidence that the relation between olfactory dysfunction and the onset of PD changes with age. Lastly, as in any large prospective study, it is possible that some cases of PD were missed. The fact that our reported incidence rates

are similar to other populations suggests that this is not a major factor.<sup>43</sup>

Strengths of this study include the longitudinal design, large sample size with excellent follow-up, and use of well-validated test instruments to prospectively assess olfaction and potential confounders such as cognitive function. The study also benefited from rigorous case finding methods that utilized standardized neurological examinations. Final diagnosis was by consensus of neurologists with movement disorders expertise using published diagnostic criteria.

In conclusion, we found that impaired olfaction is associated with an increased risk for development of PD within 4 years. This relation appears to weaken beyond that time. Olfactory testing together with screening for other potential early indicators of PD such as constipation or sleep disturbances could provide a simple and relatively economic means of identifying individuals at high risk for development of PD who could participate in trials of medications designed to prevent or slow disease progression.<sup>44,45</sup> More expensive but conceivably more specific tests such as transcranial echosonography or dopamine transporter imaging might narrow this at-risk population even further.

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## Low LDL Cholesterol and Increased Risk of Parkinson's Disease: Prospective Results from Honolulu-Asia Aging Study

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**Abstract:** Low-density lipoprotein cholesterol (LDL-C) levels are suggested to be associated inversely with Parkinson's disease (PD). To test the hypothesis that LDL-C levels may increase PD risk, we studied a prospective cohort of 3,233 men (Honolulu-Asia Aging Study) for whom the LDL-C from fasting lipid profiles was obtained during 1991 to 1993. The cohort was followed longitudinally until 2001 for incident Parkinson's cases. During follow-up, 41 men developed PD (18.4/10,000 person-years). Although the incidence of PD increased with decreasing LDL-C in a dose-dependent manner, the association was only significant for men aged 71 to 75 years. In the latter group, risk of PD declined from 38.5/10,000 person-

years in men with LDL-C levels <80 mg/dl to less than 9/10,000 person-years for concentrations that were ≥140 mg/dl. After adjustment for age, smoking, coffee intake, and other factors, the relative odds of PD for men at the 80th versus the 20th percentile of LDL-C (135 vs. 85 mg/dl) was 0.4 (95% confidence interval: 0.2, 0.9). This prospective study supports the hypothesis that low LDL-C is associated with an increased risk of PD. Although confirmation is required, the underlying mechanisms may be useful in understanding key aspects of PD. © 2008 Movement Disorder Society

**Key words:** Parkinson's disease; LDL cholesterol; apolipoprotein E, statin, prospective study

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As an age-related neurodegenerative disorder, Parkinson's disease (PD) currently affects up to 1 million Americans, and these numbers are expected to grow as the population of the United States ages. Although the etiology is known in a small percentage of genetically related cases, the disorder is largely idiopathic, and likely involves interactions of the genome and the environment.<sup>1</sup> There is no treatment that prevents the

disease or slows progression and there are few factors identified that may alter disease risk.

A recent systematic review demonstrated that the apolipoprotein E (APOE) ε2 allele is positively associated with higher prevalence of sporadic PD.<sup>2</sup> Furthermore, the APOE ε2 allele has been consistently associated with lower plasma low-density lipoprotein cholesterol (LDL-C).<sup>3,4</sup> Interestingly, there has been one published abstract reporting lower plasma cholesterol concentrations in patients with PD than in controls.<sup>5</sup> Further, there has been a report of dramatically lower cholesterol biosynthesis in patients with PD than in controls.<sup>6</sup> There has, however, been, no subsequent follow-up on this association until recently. We reported a case-control study that associated lower LDL-C levels with higher prevalence of PD.<sup>7,8</sup> Independently, a

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prospective study using the Rotterdam cohort reported lower total cholesterol and increased PD risk in women.<sup>9</sup> The purpose of this report is to examine the association of LDL-C with incidence of PD in the longitudinal community-based Honolulu Heart Program/Honolulu-Asia Aging Study in men.

## METHODS

### Study Design

The Honolulu Heart Program began in 1965 with examination of 8,006 men of Japanese ancestry, 45 to 68 years old, living on the island of Oahu, Hawaii. The initial examination consisted of face-to-face interview and physical evaluation. Demographic, dietary, and health status data were obtained.<sup>10,11</sup> Follow-up examinations were performed in 1968 to 1970, 1971 to 1974, 1991 to 1993, 1994 to 1996, 1997 to 1998, and 1999 to 2001. An institutional review committee approved the procedures, and informed consent was obtained from all participants. Details regarding study design have been previously published.<sup>12–14</sup>

### Study Population

Research on neurodegenerative diseases of aging in this cohort began in 1991 with establishment of the Honolulu-Asia Aging Study. This analysis for this study was based on the 3,233 subjects who were free of prevalent neurodegenerative disease such as PD and dementia during the 1991 to 1993 follow-up examination.

### PD Case-finding and Diagnosis

Cases of PD were identified in several ways. Before 1991, cases of PD were identified through a review of all hospital records of study participants for new and preexisting diagnoses of PD, an ongoing review of all Hawaii death certificates, and a review of medical records at the offices of local neurologists for all cohort members suspected of having PD.

During subsequent examinations (1991–1993, 1994–1996, 1997–1998, 1999–2001) all participants were questioned about a diagnosis of PD and the use of PD medications by a structured interview at each examination. A symptom questionnaire also was administered to study participants by a technician trained in the recognition of the clinical symptoms of parkinsonism. Those subjects with the self-reported history or signs of parkinsonism were referred to a study neurologist who administered standardized questions about the symptoms and onset of parkinsonism, previous diagno-

ses, and medication use, followed by a comprehensive and standardized neurologic examination that included the Unified PD Rating Scale.<sup>15</sup> A diagnosis of PD was made by consensus of two study neurologists according to published criteria.<sup>16</sup> These required that the subject have the following: (1) parkinsonism (e.g., bradykinesia or resting tremor combined with rigidity or postural reflex impairment); (2) a progressive disorder; (3) any two or more of four signs (marked response to levodopa, asymmetry of signs, asymmetry at onset, or initial onset tremor); and (4) absence of any etiology known to cause similar features. Cases of parkinsonism related to progressive supranuclear palsy, multiple system atrophy, cerebrovascular disease, drug-induced parkinsonism, postencephalitic parkinsonism, or post-traumatic parkinsonism were not included among the cases of PD.

Age at diagnosis was used instead of age at onset to avoid inaccuracies associated with recall of symptom onset for a chronic disease with gradual onset. Age at initial diagnosis was used regardless of who made the diagnosis.

### Determination of Fasting LDL-Cholesterol Levels

Blood specimens were obtained (1991–1993) after a 12-hour overnight fast, and separated into mixed plasma and buffy coats for storage at  $-70^{\circ}\text{C}$  until shipment on dry ice to the University of Vermont for lipid determination.<sup>17</sup> LDL-C was calculated from the Friedewald formula ( $\text{LDL} = \text{total} - \text{HDL} - \text{TG}/5$ ) in men with triglyceride concentrations  $<400$  mg/dl.<sup>18</sup>

### Determination of Confounders

Information on other potentially confounding variables included age; pack-years of cigarette smoking; intake of coffee assessed at the 1965 examination; bowel movement frequency; and the Cognitive Abilities Screening Instrument (CASI) assessed at the 1991 examination. Mid-life pack-years of cigarette smoking and coffee intake were measured during the baseline Honolulu Heart Program examination (1965–1968) as typical lifetime exposures to these factors. Late life coffee intake was not assessed at the time of LDL-C assessment and current cigarette smoking was too uncommon by this time to allow for its careful assessment. The CASI is an instrument designed for use in cross-cultural settings combining features of the Folstein Mini-Mental State Examination, the Modified Mini-Mental State Examination, and the Hasegawa Dementia Rating Scale.<sup>19</sup> Performance scores range from 0 to 100 with high scores indicating better

**TABLE 1.** Average age and age-adjusted features across ranges of LDL-C

Feature	Range in LDL-C (mg/dl)					
	<80 (513) <sup>a</sup>	80 to <100 (667)	100 to <120 (837)	120 to <140 (689)	140 to <160 (325)	≥160 (202)
Age <sup>b</sup> (yrs)	78.1 ± 4.9 <sup>c</sup>	77.9 ± 4.6	77.3 ± 4.4	77.0 ± 4.1	76.6 ± 3.9	77.0 ± 4.1
Pack-years of smoking	27.0 ± 27.7	27.2 ± 28.9	26.8 ± 27.3	25.5 ± 25.5	28.5 ± 27.0	23.6 ± 26.7
Coffee intake (oz/month)	13.2 ± 12.5	13.5 ± 12.4	14.1 ± 13.6	13.8 ± 12.5	13.7 ± 13.8	14.6 ± 13.6
Bowel movements/day	2.3 ± 0.6	2.2 ± 0.5	2.3 ± 0.6	2.2 ± 0.6	2.2 ± 0.6	2.2 ± 0.5
HDL-C (mg/dl)	51.4 ± 16.9	52.0 ± 14.2	51.7 ± 12.7	51.3 ± 11.9	50.6 ± 10.8	49.7 ± 10.1
Alcohol intake <sup>b</sup> (mL/d)	25.1 ± 49.2	18.6 ± 41.3	17.2 ± 33.8	18.3 ± 38.5	15.8 ± 45.1	15.4 ± 30.0
Apo ε2 alleles <sup>b</sup> %	15.3 (79) <sup>d</sup>	11.0 (74)	9.8 (82)	7.2 (49)	4.1 (13)	3.5 (7)
CASI <sup>c</sup>	83.6 ± 14.4	85.4 ± 11.9	84.7 ± 11.6	86.1 ± 8.7	86.4 ± 8.0	85.8 ± 7.8

<sup>a</sup>Sample size.

<sup>b</sup>Significant decrease with increasing LDL-C (*P* < 0.001).

<sup>c</sup>Mean ± standard deviation.

<sup>d</sup>Number of men with an ε2 allele.

<sup>e</sup>Significant increase with increasing LDL-C (*P* < 0.001).

cognitive function than low scores. We use <74 on the CASI as a cutoff for referral for further dementia evaluation. This corresponds to approximately the lower 15th percentile and corresponds closely to a score of 22 on the Mini-Mental State Examination. For men eventually diagnosed with prevalent dementia, 96% had a CASI <74. For men without dementia, 11% had a CASI <74. Because cigarette smoking, intake of coffee and caffeine, and bowel movement frequency have previously been shown to modify the risk of PD in this cohort of men,<sup>12,20–22</sup> the analyses of LDL-C association with PD were adjusted for these covariates. Alcohol consumption (assessed in 1991), CASI, and APOE genotypes also were included for relative odds analysis because they are suspected of being associated with PD.<sup>2,23–27</sup>

### Statistical Analysis

Crude- and age-adjusted incidence rates of PD in person-years were estimated within ranges of LDL-C based on standard analysis of covariance methods.<sup>28</sup> Age-adjusted average levels of possible or putative confounders were also compared across the LDL-C ranges using similar techniques.<sup>28</sup> Because the number of PD cases was expected to be small, logistic regression models were examined to assess the effect of LDL-C (and other factors) on the risk of PD based on exact testing methods.<sup>29</sup> Here, LDL-C was modeled as a continuous variable. The logistic regression was further adapted for a survival analysis where parameter estimates are known to be similar to those that appear in a proportional hazards regression model, particularly in the instance when event counts are low.<sup>30,31</sup> After adjustment for age and the other characteristics, the relative odds of PD [and 95% confidence intervals

(CI)] was estimated comparing the risk of PD for men with LDL-C levels at the 80th percentile (135 mg/dl) versus men with concentrations at the 20th percentile (85 mg/dl). All reported *P*-values were based on two-sided tests of significance.

### RESULTS

The mean age of men at the time of LDL-C collection (1991–1993) was 77 years (age range, 71–93 years). In the course of follow-up, 41 men developed PD (18.4/10,000 person-years). The average age at onset was 79.3 years (range, 73–89), and the average time from collection of LDL-C to diagnosis was 3.3 years (range, 1 month–7.3 years). Average age and age-adjusted features across ranges of LDL-C are listed in Table 1. There is an association between lower LDL-C level with APOE ε2 allele, older age, and higher alcohol intake (*P* < 0.001) (Table 1). CASI is significantly associated with increasing LDL-C (Table 1).

There is an increase in incidence of PD with decreasing LDL-C in a “dose-dependent” manner after adjusting for age (*P* = 0.044, test for trend, see Table 2). The relative odds of PD comparing men at the 80th versus 20th percentile of LDL-C is 0.6 (95% CI: 0.4, 1.1, Table 3) after adjusting for all confounders. The association between LDL-C and PD is more clear for the men who were aged ≤75 years at the beginning of follow-up [*P* = 0.014, and relative odds of 0.4 (95% CI: 0.2, 0.9), Tables 2 and 3], but is not observed for those who were older [*P* = 0.824, and relative odds of 0.9 (95% CI: 0.4, 2.0), Tables 2 and 3]. In the younger age group, risk of PD declined from 38.5/10,000 person-years in men with LDL-C levels <80 mg/dl to less than 9/10,000 person-years for concentrations that were 140 mg/dl and higher. After adjustment for age, smok-

**TABLE 2.** Incidence of Parkinson's disease across ranges of LDL-C

LDL-C range (mg/dl)	Incidence/10,000 person-years	
	Unadjusted	Age-adjusted
Aged 71 to 75 years (23/1359)*		
<80	38.6 (5/188)	38.5
80 to <100	39.2 (7/251)	39.3
100 to <120	18.4 (5/366)	18.5
120 to <140	21.7 (5/313)	21.7
140 to <160	8.8 (1/153)	8.8
≥160	0.0 (0/88)	0.0
Test for trend	<i>P</i> = 0.015	<i>P</i> = 0.014
Aged 76 to 93 years (18/1874)		
<80	20.4 (4/325)	20.7
80 to <100	14.6 (4/416)	14.9
100 to <120	9.3 (3/471)	9.3
120 to <140	15.6 (4/376)	15.4
140 to <160	17.0 (2/172)	16.4
≥160	12.9 (1/114)	12.7
Test for trend	<i>P</i> = 0.862	<i>P</i> = 0.824
All ages (41/3233)		
<80	27.7 (9/513)	28.4
80 to <100	24.3 (11/667)	25.2
100 to <120	13.5 (8/837)	13.4
120 to <140	18.5 (9/689)	18.1
140 to <160	13.0 (3/325)	12.5
≥160	7.0 (1/202)	6.9
Test for trend	<i>P</i> = 0.062	<i>P</i> = 0.044

\*PD cases/sample at risk.

ing, coffee intake, and other factors, the relative odds of PD for men at the 80th versus the 20th percentile of LDL-C (135 vs. 85 mg/dl) was 0.4 (95% CI: 0.2, 0.9). Excluding a single case of PD that was diagnosed within 6 months of follow-up in those with a baseline age ≤75 years also failed to alter the relationship between LDL-C and PD. HDL-C was not associated with PD. Although the association between LDL-C and PD was weaker in those who were older (76–93 years), a test for an interaction effect between age and LDL-C concentration on the risk of PD could not be properly assessed in the current sample because of limited statistical power and the small number of PD cases.

### COMMENTS

Our finding that lower LDL-C is associated with increased risk of PD in Japanese–American men after age-adjustment is consistent with recent reports in the literature.<sup>7,8</sup> Lower LDL-C also has been associated with higher mortality in the elderly<sup>32</sup> and higher risk of Alzheimer's disease.<sup>33</sup> Interestingly, a recent study of the Rotterdam cohort also found an association of lower total cholesterol and increased PD risk, but only in women.<sup>9</sup> Conversely, in the current study, as well as

a recent case–control report,<sup>8</sup> an association also was found in men, possibly related to the fact that fasting LDL-C rather than total cholesterol was assessed.

Our findings should be interpreted with caution. The relationship of lower LDL-C with PD was markedly stronger in the younger-aged men (age ≤75) (Tables 2 and 3). Although the risk of PD seemed to decline with rising LDL-C levels in those who were older (Table 2), the dose–response relationship was not significant for the older age group. The small sample size in each subgroup analysis may contribute to this unstable estimation of association. It is also possible that there may be greater diagnostic uncertainty among the older Parkinson's cases. Heterogeneity in clinically diagnosed PD, however, has also been documented both in hospital-based studies and several population studies,<sup>34,35</sup> and may be governed by distinct neuropathologic processes with competing and independent etiologic influences.<sup>36</sup> For example, genetic factors play a more important role in young-onset PD.<sup>37</sup> The finding of weaker association among the older-aged men is consistent with increasing etiological heterogeneity with age. Additional research in different and larger populations is needed to confirm these findings.

As noted earlier, excluding a single case diagnosed in the first 6 months did not affect the relationship between LDL-C and PD, suggesting that lower LDL-C predated the diagnosis of PD. Even so, it is possible that the neurodegenerative processes, known to begin years before diagnosis, may have influenced LDL-C levels directly or indirectly.<sup>38,39</sup> For example, a subtle prodromal syndrome could cause lifestyle changes that result in lower LDL-C, making low cholesterol a marker of PD risk rather than a cause. Alternatively, processes causing nigral neuronal loss in PD also may lead to decreased biosynthesis of cholesterol.

Medications or other management strategies for hypercholesterolemia may also be responsible for the

**TABLE 3.** Relative odds of PD in men at the 80th versus the 20th percentile of LDL-C (135 vs. 85 mg/dl) by age at the beginning of follow-up (1991–1993)

Age group	Relative odds of PD	
	Age-adjusted	Risk factor adjusted*
Aged 71–75 yrs	0.4 (0.2, 0.9)**	0.4 (0.2, 0.9)
Aged 76–93 yrs	0.9 (0.4, 2.0)	1.0 (0.5, 2.1)
All ages	0.6 (0.4, 1.1)	0.6 (0.4, 1.1)

\*Adjusted for age, smoking, coffee intake, bowel movement frequency, HDL-C, alcohol intake, the presence of Apo ε2 alleles, and CASI.

\*\*95% confidence interval.

association of low cholesterol and increased PD risk. In two recently published studies,<sup>7,8,40</sup> the potential mechanistic role of statins was noted. Controls had higher degree of statin use, raising the possibility that statin use was protective.<sup>8,40,41</sup> Unfortunately, statin use in the current sample could not be carefully assessed because it was extremely uncommon when follow-up began (1991–1993). Among the 3,233 men, only 107 used statins. It may be, however, that men began to use statins in the course of follow-up and that this could be important. In the Honolulu sample, it is difficult to evaluate the effects of intervening statin use on the risk of PD, because the number of PD cases becomes increasingly small as follow-up is delayed to later exams when treatment with statins first occurred. Based on follow-up from the original 1991 to 1993 baseline, statin use was neither related to incident PD, nor did it alter the relationship of LDL-C and PD. Although our data are unable to address the association between statin use and the risk of PD, future cohort studies where statin use is more frequent would be of great interest. Regardless of the findings from such studies, no result would contradict the well-recognized health benefits of statin use in individuals with elevated LDL-C.

The question of whether low LDL-C contributes to the cause of PD or is an early marker of the neurodegenerative process cannot be answered definitively from this study. First, only the age at PD diagnosis is available, and it is presently impossible to know the actual age at disease onset. This complicates determining causal relationships in a disease that likely begins years before obvious symptoms or diagnosis. Furthermore, some early cases of PD could have been missed because of limited sensitivity of the screening questions; however, this would tend to dilute any association of low LDL-C and PD rather than strengthen it.

Thus, understanding the underlying mechanisms (biological and/or etiological) for this association between lower LDL-C and increased PD risk could have a profound influence in understanding key aspects of sporadic PD. In addition, it may also have public health implications if the association turns out to be causal.

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## Appendix 2

**Background:** Over the past decade, researchers have provided considerable evidence to suggest that, even in the absence of dementia, people with PD exhibit impaired performance on tasks such as sentence comprehension which rely heavily on the access and manipulation of morphosyntactic linguistic knowledge. The behavioral and neurochemical bases for these impairments, however, remain unclear.

**Methods:** Twenty-six nondemented, medicated people with PD and 24 age, sex, and education matched controls participated in the research which involved three testing phases. Firstly, the morphosyntactic language abilities of the two groups were defined using a battery of off-line morphosyntactic language-oriented tasks. Next, a series of on-line (or automatic) word recognition tasks within the morphosyntactic priming paradigm were employed to chart the time course of lexical access in 19 members of each group. Participants with PD completed Stages 1 and 2 whilst experiencing maximum clinical benefit from their dopaminergic medication (i.e., when on medication). Finally, 7 participants with PD also completed on-line priming procedures after an extended period of 12 hours medication withdrawal (i.e., when off medication).

**Results:** When on medication, participants with PD exhibited impaired performance on a range of off-line morphosyntactic tasks such as grammaticality judgement and sentence construction. Whilst the experimental participants demonstrated morphosyntactic priming, priming was observed over an abnormally brief time course. Priming performance was unaffected, however, by medication withdrawal.

**Conclusions:** The present data suggest that, regardless of dopamine availability, nondemented people with PD are able to access grammatical information in a normal manner. The time frame in which this information remains available for processing, however, appears to be reduced relative to normal. Taken together, these results support the notion that changes in the temporal availability of morphosyntactic information may contribute to impaired performance on off-line language-oriented tasks such as sentence construction and grammaticality judgement.

#### P425

##### Performance on a computerized reaction time test predicts incidental Lewy bodies

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**Objective:** To examine the association of performance on the 3RT Test, a computer administered test of simple and choice reaction times, with the presence of incidental Lewy bodies (LB) in the substantia nigra (SN) or locus ceruleus (LC) of decedent participants from the Honolulu-Asia Aging Study (HAAS) who did not have clinical Parkinson's disease (PD).

**Background:** In early, untreated Parkinson's disease, simple reaction time is impaired while choice reaction time is affected later. Accrued evidence supports the idea that the pathogenic process leading to PD is commonly active well before the clinical illness is diagnosed. Simple reaction time might be useful for the identification of persons in the preclinical phase when the neuropathologic lesions are established but have not progressed sufficiently to cause recognizable signs and symptoms. We hypothesized that persons with slow simple reaction time would be more likely to have incidental LB in the SN or LC at autopsy. **Methods:** The 3RT Test was administered to all subjects at the 1994-96 examination of the HAAS, a prospective study of neurodegenerative diseases of aging in a cohort of Japanese-American men living in Hawaii and born 1900-1919. The brains of 96 men without PD who underwent RT testing were examined for LB in the LC or SN.

**Results:** Among the 96 brains examined, 8 had incidental LB. The mean interval from RT testing to death was 2.2 years (median 2.3 years, range 0.01-4.6). The percent of brains with incidental LB increased consistently from 0% (0/24, fastest quartile) to 16.7% (4/24, slowest quartile) among subjects classified into four subsets according to simple reaction time ( $P = 0.037$  for age adjusted test for trend). This relationship was not significant for choice reaction time.

**Conclusions:** Slow reaction time predicted incidental LB. The 3RT might help to identify preclinical PD and to monitor progression to clinical illness in selected subjects.

#### P426

##### Associations of fatigue in Parkinson's disease

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**Objective:** To evaluate the presence or absence of fatigue in patients with Parkinson's disease.

**Background:** Fatigue is a common symptom in PD with estimates ranging from 2 to 42%. Although correlated with depression in 2 studies, it has been reported to be present in non-depressed and non-sleep disturbed patients. Furthermore, PD patients report that it differs from the fatigue present before the onset of their illness.

**Methods:** We have studied the presence or absence of fatigue in 100 consecutive PD patients attending the Movement Disorder clinic. Full demographic data was collected with patients completing an hourly on-off diary in which they were asked to report the presence or absence of fatigue. PD severity was assessed by Hoehn and Yahr scale (H&Y), UPDRS (II to V), the Schwab and England Activities of Living Index (S&E), Hospital Anxiety and Depression scale, SF-36, PDQ 39, A-B Neurotoxicity Scale (with a control population) in order to fully assess the well being of the patient.

**Results:** Fatigue was reported in 17% of patients in the on state, and 30% in the off state. 34% reported fatigue in both on and off states. Fatigue was significantly associated with greater disease severity, S&E, and higher levels of anxiety and depression. It was significantly more common in the off state.

**Conclusions:** The study identified factors contributing to fatigue in PD, particularly the off state of motor impairment. The next stage of the project is to attempt to improve factors associated with fatigue in the same patients and to reassess the presence of fatigue.

#### P427

##### Kinematics analysis of the movements the hand (tapping test) by Hilbert transformed in patient with Parkinson's disease and controls

P. Chana, H. Gonzalez, A. Guierrez, N. Barrnetos (Santiago, Chile)

Patients with Parkinson's disease present dysfunction of sequential movements with a loss of harmony in movement. It is evaluated with Hilbert transformed the movement of the tapping.

**Method:** Six patients with PD and 5 controls were studied. The mean age was  $63.4 \pm 2.4$  years old in PD and  $61.8 \pm 4$  years in controls (mean disease duration,  $6.9 \pm 3.4$  years). All the patients were in treatment with levodopa, and were evaluated in on state. All the participants were told to touch sequentially with their fingers two separate points (30 cm). They were instructed to perform this "as quickly as possible". The movement was recorded with a standard camera perpendicular to a referential xy system. The mean finger is placed a refractory mark to the light. The video was digitized at 30 Hz and was attached to a computational program. ROC curves were used for statistical analysis.

**Results:** Data were analyzed Hilbert transformed graphic, phase angle inclination and phase angle derived. The curve ROC has an area under the curve of 0.864 for the phase angle derived. Whose sensibility is of 72% and specificity of 100%.

**Conclusion:** The phase angle derived was a very good discriminator between the patients and controls. The phase angle derived was better discriminator to speed of movement.

#### P428

##### Validation of a screening questionnaire for Parkinson's disease in Singapore

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**Objective:** To evaluate the sensitivity and specificity of a previously developed and validated screening questionnaire for Parkinson's disease (PD) amongst patients attending a neurology clinic in Singapore.

**Background:** The questionnaire developed by Tanner, et al. (1990) has been validated in the USA and Spain [Duarte, et al. 1995] and found to have an excellent sensitivity and specificity for PD.

# Parkinsonian Signs and Substantia Nigra Neuron Density in Decedents Elders without PD<sup>†</sup>

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Substantia nigra (SN) neurons were counted on single, transverse caudal midbrain sections from 217 male participants in the Honolulu-Asia Aging Study, aged 74–97 years at death. Quadrants areas within the SN were determined with a planimeter and neuronal density was expressed as neurons/mm<sup>2</sup> for 10 Parkinson's disease (PD) cases, 29 incidental Lewy body cases, and 178 controls with neither condition. Mean densities in all quadrants were significantly lower in the PD group compared with the other groups ( $p = 0.006$ ). This relationship was strongest in the ventrolateral quadrant. In a subgroup of 50 controls who were examined with the Unified Parkinson's Disease Rating Scale an average of 2.1 years prior to death, there was an association of stooped posture ( $p = 0.009$ ), postural instability ( $p = 0.013$ ), body bradykinesia ( $p = 0.048$ ), and gait disturbance ( $p = 0.05$ ) with neuron density in the dorsolateral quadrant; and impaired speech ( $p = 0.014$ ), abnormal facial expression ( $p = 0.022$ ), and difficulty rising from a chair ( $p = 0.032$ ) with neuron density in the dorsomedial quadrant. There was a significant association of increasing number of signs present with decreasing neuron density in both quadrants ( $p = 0.001$  for trend). Low SN neuron density may be the basis for parkinsonian signs in the elderly without PD.

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Loss of pigmented neurons in the substantia nigra (SN) pars compacta is always found in Parkinson's disease (PD). Neuropathological studies have reported the most severe neuronal loss in the ventrolateral tier of the pars compacta. Nigral neuron counts and striatal dopamine levels are estimated to be diminished by 50% and 80%, respectively, before parkinsonian symptoms and motor signs appear,<sup>1,2</sup> suggesting that the process underlying this chronic degenerative condition begins long before clinical signs develop. The presence of incidental Lewy bodies (ILBs), Lewy bodies found in deceased patients without clinical PD, is associated with intermediate SN neuron loss between PD cases and controls, prompting speculation that ILB represent preclinical PD.<sup>2</sup>

Autopsy studies of neuronal loss in the SN and clinical studies of parkinsonian signs in elderly populations demonstrate that both occur with aging.<sup>2,3</sup> However,

opportunities to evaluate both in persons without PD have been lacking.

The prospective Honolulu Heart Program/Honolulu-Asia Aging Study (HHP/HAAS) has sought autopsies on cohort members since 1991. In this report, a single-section counting method is used to estimate density of neurons in the SN in PD cases, ILB cases, and individuals with neither condition. Among individuals without PD, parkinsonian signs, measured during a standardized neurological examination, are evaluated for correlation with neuron density in the SN.

## Methods

### Study Population

The HHP began in 1965 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 cardiovascu-

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<sup>†</sup>This article is a US Government work and, as such, is in the public domain in the United States of America.

lar disease.<sup>4–6</sup> Research on dementia and PD, including autopsy research, began during the 1991 to 1993 examination. Subsequent examinations took place 1994 to 1996 and 1997 to 1999.<sup>7,8</sup>

### *Autopsy Methods and Determination of Neuron Density*

Autopsy consent was obtained from the authorized legal representative. HAAS neuropathologists were shielded from clinical information to avoid bias. Autopsy procedures have been published.<sup>9</sup>

Neuron counts in the SN were performed on a  $\times 30$ , scaled, microprojector tracing of a single, transverse hematoxylin and eosin–stained,  $10\mu$ - thick section of the nucleus at the level of the roots of the oculomotor nerve. Dorsal and ventral borders of the nucleus were formed by the midbrain tegmentum and the crus cerebri, respectively. The margin of the cerebral peduncle adjacent to the emerging roots of the third nerve and the lateral mesencephalic sulcus, respectively, marked the medial and lateral extent of the nucleus. Maximum transverse dimension of the traced nucleus was measured with a ruler. The midpoint of this measurement was 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 were 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 were connected dividing the tracing into dorsal and ventral halves forming four quadrants: dorsomedial, ventromedial, dorsolateral, and ventrolateral. Neurons were counted in each quadrant. The area of each quadrant was determined from the known magnification of the tracing and the planimetric measurement of the traced quadrant. Neuron density was calculated and expressed as neurons/mm<sup>2</sup> (Fig 1).

### *Parkinson's Disease Case Finding*

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 the study neurologist. Final diagnosis was determined by consensus of two neurologists using published criteria.<sup>10</sup> Case finding methods have been published.<sup>11</sup> PD cases were confirmed pathologically by Lewy bodies present in either the SN or locus ceruleus (LC).

### *Incidental Lewy Body Cases*

Single hematoxylin and eosin–stained sections from both midbrain and pons were examined for Lewy bodies in neurons of the SN and LC. Individuals who had Lewy bodies in either the SN or LC without history of PD or dementia with Lewy bodies were defined as having ILB.

### *Unified Parkinson's Disease Rating Scale*

During the 1991 examination, 3,734 men underwent examinations including screening with the Cognitive Abilities Screening Instrument.<sup>12</sup> More comprehensive testing, including the Unified Parkinson's Disease Rating Scale (UPDRS),<sup>13</sup> was administered to 426 participants either cognitively impaired or randomly selected from cognitively normal men.

Parkinson's disease was not considered in the sampling strategy.<sup>8</sup> Similar strategies were used during subsequent examinations when 752 and 294 men, respectively, received the UPDRS. Examinations were performed by a neurologist or geriatrician experienced in parkinsonism. The UPDRS performed closest to death was used.

Informed consent was obtained and procedures were approved by an institutional review committee.

### *Statistical Methods*

Features of the study sample are described as age-adjusted means in the men with PD, ILB, and in those with neither condition based on standard analysis of covariance techniques.<sup>14</sup> Plots are provided to describe the distributions of neuron densities (counts/mm<sup>2</sup>) for each quadrant. To examine differences in neuron density according to age, clinical status, and UPDRS signs, we modeled neuron density as an overdispersed integer response after a negative binomial distribution.<sup>15</sup> Here, generalized linear models were used with the other characteristics serving as independent variables. All reported *p* values were based on two-sided tests of significance.

## **Results**

### *Mean Neuron Density by Age and Diagnosis*

Neuron counts were performed on 220 brains obtained between 1991 and 1998. For the analysis comparing neuron density among PD, ILB, and control groups, we removed atypical parkinsonism cases including two having dementia with Lewy bodies and one with clinical parkinsonism without LB. There were 10 with neuropathologically confirmed PD and 29 ILB cases. The remaining 178 control brains had neither condition. Table 1 shows clinical characteristics for the three groups. Age at death ranged from 74 to 97 years. Mean death age was significantly higher for the ILB group (85.8 years) compared with the controls (83.7 years; *p* = 0.049). As expected, mean UPDRS score for PD cases was significantly higher than controls (*p* = 0.005) and ILB cases (*p* = 0.022). Mean UPDRS score for the ILB cases was similar to controls.

Neuron densities by 5-year age groups within each quadrant are shown in Figure 2 for controls. Only density in the dorsolateral quadrant was associated with age at death.

Table 1 shows mean quadrant specific densities for all three groups. Mean densities in all four quadrants in PD brains were significantly lower than control brains (*p* < 0.006). Densities in PD cases were also lower than in ILB brains for all quadrants, and differences were statistically significant in ventrolateral (*p* = 0.001), dorsolateral (*p* = 0.049), and ventromedial (*p* = 0.036) quadrants. The greatest difference in density between controls and PD cases occurred in the ventrolateral quadrant (15.4 neurons/mm<sup>2</sup>). Mean densities in the ILB brains were consistently intermediate between control and PD groups. In Figure 3,

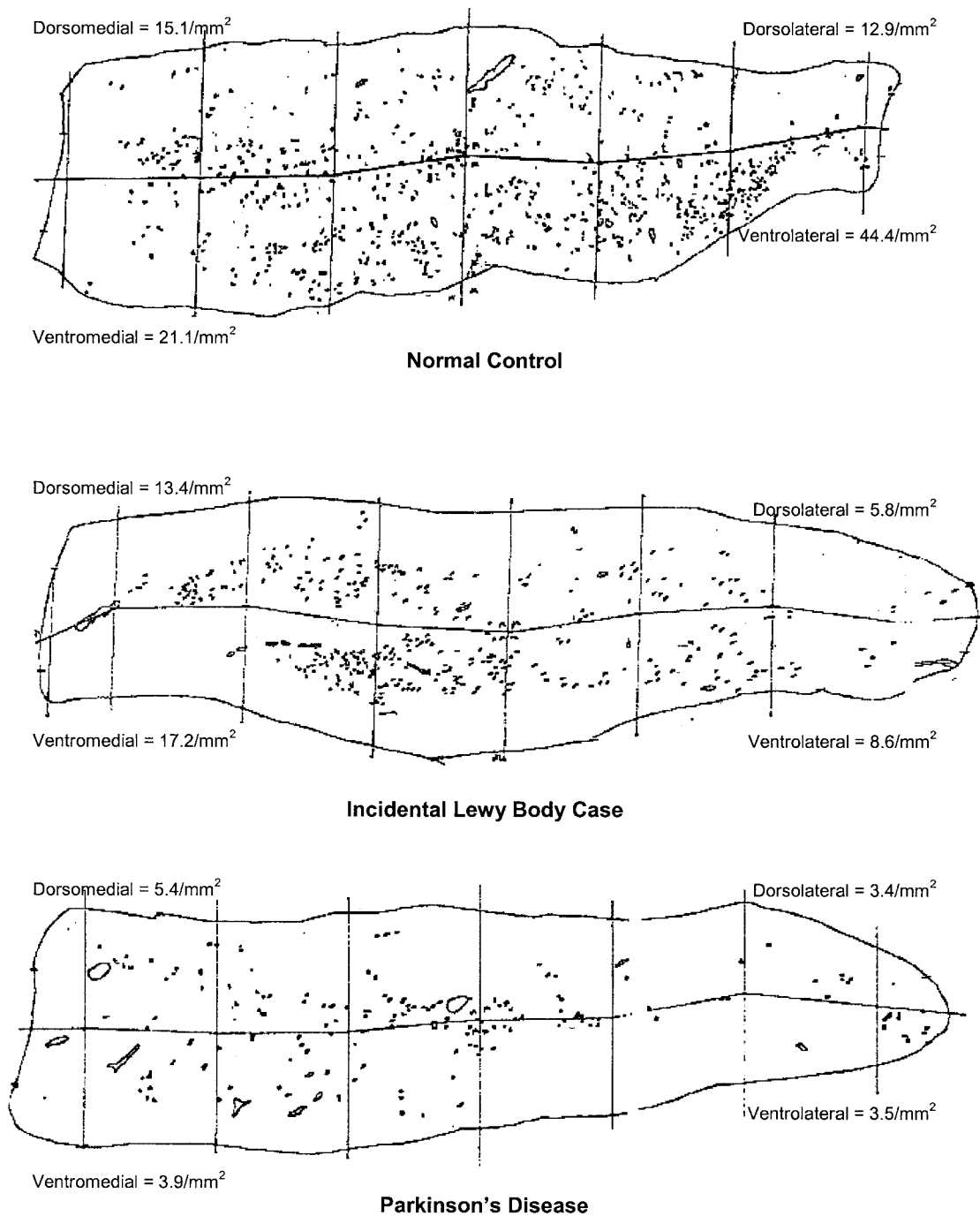


Fig 1. Selected scaled images of the substantia nigra.

data are presented as scatterplots of neuron density by diagnostic group in each of the four quadrants.

#### Mean Neuron Density and Presence of Parkinsonian Signs

Fifty-four of 178 subjects in the control group had a UPDRS examination. To avoid including cases with false-positive UPDRS signs related to stroke, four cases with stroke by HHP surveillance criteria were ex-

cluded. The 50 remaining comprised the group for analysis of the relationship between UPDRS signs and neuron density in the SN in decedents without LB in the LC or SN. There were 25 subjects with no neurological diagnosis and 25 subjects with dementia meeting the Diagnostic and Statistical Manual, 3rd edition, revised<sup>16</sup> criteria. Eight met neuropathological criteria for Alzheimer's disease, whereas the remaining had vascular dementia (7 subjects), mixed cause dementia (8

Table 1. Group Characteristics; Mean (Standard deviation)

Feature	PD (n = 10)	ILB (n = 29)	Control (n = 178)
Mean death age (yrs)	85.5 (3.1)	85.8 (6.1) <sup>a</sup>	83.7 (5.3)
CASI <sup>b</sup>	55.3 (35.1)	58.6 (35.9)	63.1 (28.9)
Pack years smoke <sup>c</sup>	24.3 (27.1)	28.1 (24.5)	35.0 (33.4)
Coffee (ounces/day) <sup>c</sup>	10.1 (5.4)	11.9 (12.9)	14.0 (12.3)
Education (yr) <sup>d</sup>	3.1 (1.2)	2.7 (1.0)	2.7 (1.0)
Mean density-ventrolateral quadrant <sup>d</sup>	6.4 (3.4) <sup>e</sup>	18.1 (7.4)	21.8 (10.1)
Mean density-dorsolateral quadrant <sup>d</sup>	7.7 (4.8) <sup>f</sup>	13.7 (5.8)	15.3 (8.7)
Mean density-ventromedial quadrant <sup>d</sup>	11.1 (7.5) <sup>g</sup>	19.1 (9.2)	20.4 (10.7)
Mean density-dorsomedial quadrant <sup>d</sup>	11.7 (9.1) <sup>h</sup>	19.1 (8.9)	22.3 (10.7)
Mean UPDRS score <sup>i</sup>	36.3 (11.7) <sup>j</sup>	17.0 (7.3)	17.4 (10.1)

<sup>a</sup>Significantly older than controls ( $p = 0.049$ ).

<sup>b</sup>Adjusted for age at most recent CASI.

<sup>c</sup>Adjusted for age at baseline.

<sup>d</sup>Adjusted for age at death.

<sup>e</sup>Significantly less than ILB ( $p = 0.001$ ) and controls ( $p < 0.001$ ).

<sup>f</sup>Significantly less than ILB ( $p = 0.049$ ) and controls ( $p < 0.005$ ).

<sup>g</sup>Significantly less than ILB ( $p = 0.036$ ) and controls ( $p < 0.006$ ).

<sup>h</sup>Significantly less than controls ( $p < 0.002$ ).

<sup>i</sup>Adjusted for age at UPDRS examination.

<sup>j</sup>Significantly higher than ILB ( $p = 0.022$ ) and controls ( $p = 0.005$ ).

PD = Parkinson's disease; ILB = incidental Lewy body; CASI = cognitive Abilities Screening Instrument; UPDRS = Unified Parkinson's Disease Rating Scale.

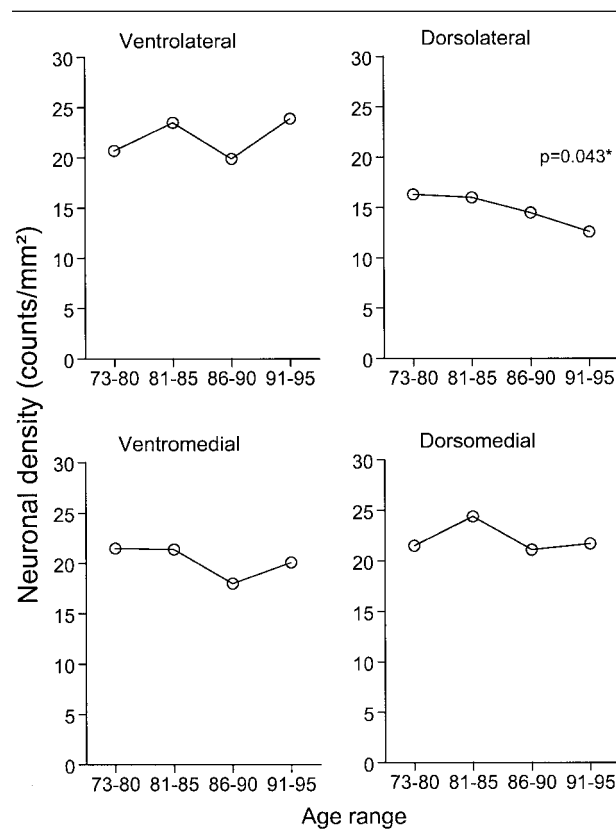


Fig 2. Mean substantia nigra neuron density by 5-year age groups for the four quadrants, 178 decedents without Parkinson's disease or incidental Lewy bodies. \*Significant decline with age.

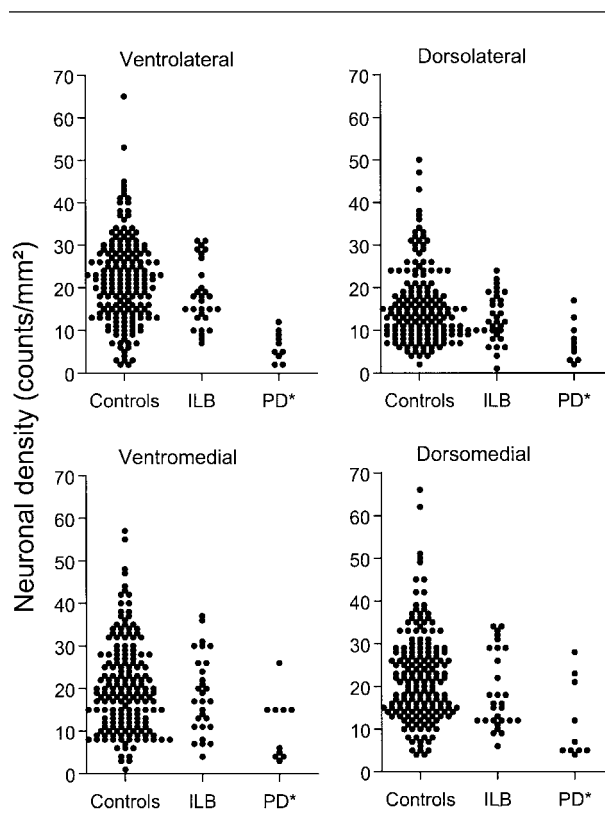


Fig 3. Scatterplots of substantia nigra neuron density for Parkinson's disease (PD) cases, incidental Lewy body (ILB) cases, and controls in the four quadrants. \*Average neuronal density is significantly lower versus controls and cases of ILB ( $p < 0.01$ ).

Table 2. Mean Neuronal Counts/mm<sup>2</sup> according to the Absence and Presence of Key UPDRS\*\* Parkinsonian Signs among 50 men without Parkinson's Disease or Lewy Bodies, Mean ± SD (no. of subjects)

Quadrant	UPDRS Sign	Absent	Present	Difference	<i>p</i>
Dorsolateral	Stooped posture	15.6 ± 7.4 (37)	10.5 ± 5.2 (13)	5.1 (1.4–7.8)*	0.009
	Postural instability	15.9 ± 7.5 (32)	11.3 ± 5.6 (18)	4.6 (1.1–7.3)	0.013
	Body bradykinesia	15.0 ± 7.3 (41)	10.6 ± 5.5 (9)	4.5 (0.4–7.6)	0.048
	Gait disturbance	15.4 ± 7.5 (35)	11.5 ± 5.9 (15)	3.9 (0.0–6.8)	0.050
Dorsomedial	Impaired speech	22.4 ± 10.1 (46)	12.0 ± 6.4 (4)	10.4 (2.7–15.1)	0.014
	Abnormal facial expression	22.3 ± 10.1 (46)	12.5 ± 7.2 (4)	9.8 (1.8–14.7)	0.022
	Difficulty rising from chair	23.0 ± 10.6 (39)	16.4 ± 7.3 (11)	6.6 (0.7–11.0)	0.032
	Impaired leg agility	23.1 ± 11.1 (35)	17.9 ± 7.1 (15)	5.2 (–0.7–9.5)	0.078
Ventrolateral	Abnormal facial expression	20.2 ± 8.6 (46)	12.8 ± 7.6 (4)	7.4 (–0.6–12.4)	0.066
	Postural instability	20.3 ± 8.3 (32)	18.4 ± 9.5 (18)	1.8 (–3.9–6.2)	0.496
	Difficulty rising from chair	20.0 ± 6.3 (39)	18.2 ± 8.5 (11)	1.8 (–4.9–6.7)	0.551
	Body bradykinesia	19.9 ± 8.6 (41)	18.3 ± 9.2 (9)	1.5 (–5.8–6.8)	0.639
Ventromedial	Postural instability	18.9 ± 8.9 (32)	16.8 ± 10.9 (18)	2.1 (–4.2–6.7)	0.474
	Abnormal facial expression	18.3 ± 8.8 (46)	16.0 ± 18.0 (4)	2.3 (–9.9–9.3)	0.637
	Stooped posture	18.5 ± 10.1 (37)	17.1 ± 8.2 (13)	1.5 (–5.7–6.5)	0.644
	Impaired leg agility	18.4 ± 9.6 (35)	17.7 ± 9.9 (15)	0.7 (–6.3–5.7)	0.818

UPDRS = Unified Parkinson's Disease Rating Scale.  
\*95% confidence interval.

subjects), or no identifiable pathological cause (2 subjects). Mean interval between UPDRS examination and death was 2.1 years (range, <1–5 years).

Within this group with no history of PD and no Lewy bodies in the SN or LC, 13 UPDRS motor signs were analyzed. No individuals had tremor at rest. All other signs were observed in at least one person. Each sign on the motor portion of the UPDRS was regarded as present if the individual scored two or higher. This cutoff was chosen because a score of one is considered normal for elderly individuals for many signs according to UPDRS guidelines. Signs were considered absent if the UPDRS score was zero or one. Table 2 compares mean neuron densities in the four quadrants between individuals with a particular sign present and individuals with the sign absent. Only the top four signs with the greatest difference between groups are shown for each quadrant. Statistically significant differences were found only in dorsolateral and dorsomedial quadrants. In the dorsolateral quadrant mean densities were significantly lower among those with the sign present for stooped posture ( $p = 0.009$ ), postural instability ( $p = 0.013$ ), body bradykinesia ( $p = 0.048$ ), and gait disturbance ( $p = 0.05$ ). In the dorsomedial quadrant, there were significant differences for impaired speech ( $p = 0.014$ ), abnormal facial expression ( $p = 0.022$ ), and difficulty rising from a chair ( $p = 0.032$ ).

Figures 4 and 5 address whether a greater number of UPDRS signs were correlated with decreasing neuron density. Figure 4A shows neuron density in the dorsolateral quadrant by number of UPDRS signs present of the three signs that were significantly associated with density individually (stooped posture, postural instability, and body bradykinesia). There was a significant

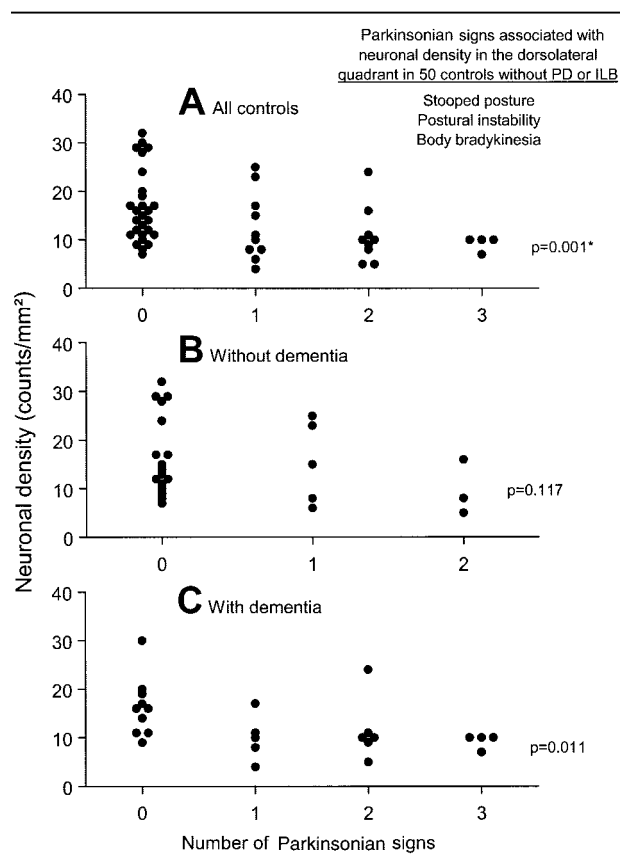


Fig 4. Neuron density in the dorsolateral quadrant of the substantia nigra by number of Unified Parkinson's Disease Rating Scale signs (stooped posture, postural instability, body bradykinesia) in 50 controls without Parkinson's disease or incidental Lewy bodies (LBs), in the subset of 25 controls with no dementia, and the subset of 25 controls with dementia. \**p* value for a test for trend.



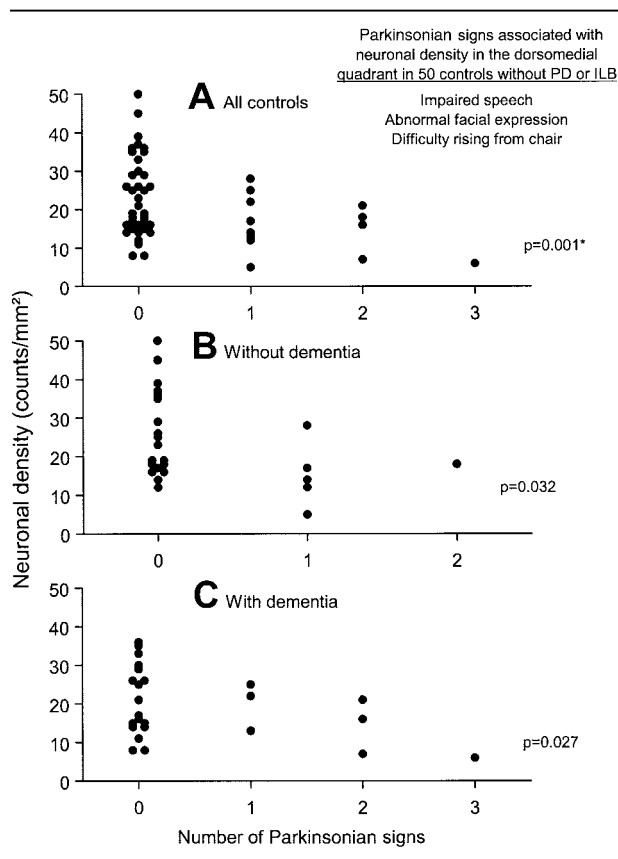


Fig 5. Neuron density in the dorsomedial quadrant of the substantia nigra by number of Unified Parkinson's Disease Rating Scale signs (impaired speech, abnormal facial expression, difficulty rising from chair) in 50 controls without Parkinson's disease or incidental Lewy bodies, in the subset of 25 controls without dementia and the subset of 25 controls with dementia. \**p* value for a test for trend.

trend for lower densities as the number of signs increased from zero to three ( $p = 0.001$ ). Findings were similar when analyzing separately the subsets without ( $n = 25$ ;  $p = 0.117$ ) and with dementia ( $n = 25$ ,  $p = 0.011$ ; see Fig 4B, C).

In the dorsomedial quadrant using the three UPDRS signs (impaired speech, abnormal facial expression, and difficulty rising from a chair) significantly associated with lower densities, there was a significant correlation between lower neuron density and higher number of UPDRS signs ( $p = 0.001$ ; see Fig 5A). Similar significant trends for lower densities occurred with increasing numbers of signs in the dorsomedial quadrant when separate analyses were performed on the subsets without ( $p = 0.032$ ) and with dementia ( $p = 0.027$ ; see Fig 5B, C). Adjusting for age did not alter findings.

## Discussion

In this elderly population, both PD and ILB cases had lower neuron densities in the SN on average compared with control brains. The ventrolateral quadrant was

most severely affected in PD cases where there was a 70% reduction in neuron density in PD cases compared with controls. These findings are in agreement with others using a similar counting method but analyzing absolute neuron counts rather than densities.<sup>2</sup> Although our original hypotheses were based on neuron density, use of absolute counts resulted in similar findings.

There was no consistent decline in neuron density in the SN with advancing age; only a modest decline was found in the dorsolateral quadrant. This is likely secondary to the narrow age range in our sample. In the report by Fearnley and Lees, age range extended 70 years from 21 to 91 years.<sup>2</sup> Functional neuroimaging studies have likewise shown age-related decreases in striatal dopamine transporters, a marker of dopaminergic neuron number, when ages spanned several decades.<sup>17</sup>

Most interestingly, among brains from elderly individuals without PD or ILB, several UPDRS signs were associated with neuron density in the dorsomedial and dorsolateral quadrants. Signs that were independently associated with low neuron density were stooped posture, postural instability, and body bradykinesia in the dorsolateral quadrant and, impaired speech, abnormal facial expression, and difficulty rising from a chair in the dorsomedial quadrant. When considering the three UPDRS signs independently associated with neuron density in each of the above quadrants, the more signs present during life, the lower the neuron density.

Parkinsonian signs are common in the elderly. In one community study, more than one third of people older than age 65 years had parkinsonism, defined as presence of two or more of gait disturbance, rigidity, bradykinesia, or tremor.<sup>3</sup> Greater than 50% of cognitively intact participants in a study of aging who were aged 80 years or older were found to have at least one parkinsonian sign from the UPDRS.<sup>18</sup> Parkinsonian signs are reported to occur in nearly 30% of elderly with cognitive decline.<sup>19</sup> Finally, parkinsonism occurs in approximately one third of persons with Alzheimer's disease, and it has been reported that parkinsonian signs in AD are associated with neurofibrillary tangle pathology in the SN.<sup>20</sup> Autopsy studies also have reported variable declines in SN neuron counts in AD brains.<sup>21,22</sup> To test whether the association of UPDRS signs with low SN neuron density was present only in dementia cases, we separated the control group into those with dementia and those without dementia. Relationships persisted in both subsets (see Figs 4 and 5).

To our knowledge, this is the first study to systematically investigate anatomical correlates of parkinsonian signs in elderly persons without neurodegenerative disease. Studies using unbiased stereological counting methods have documented lower numbers of tyrosine hydroxylase-positive neurons in the SN of aged mon-

keys and an inverse association between the number of these neurons and time taken to complete a motor task.<sup>23</sup> A strong association between slow motor activity and diminished level of putaminal dopamine has been observed in aged squirrel monkeys, although an age-related loss of tyrosine hydroxylase-immunoreactive nigral cells was not apparent.<sup>24</sup>

Measures of PD severity have been linked with dopamine neuron decline in humans. Nigral neuron loss is correlated with PD duration,<sup>2,22,25</sup> and higher scores on the motor section of the UPDRS have been correlated with loss of dopamine transporters in PD subjects undergoing [<sup>123</sup>I] FP-CIT SPECT.<sup>26,27</sup> Because there seems to be a correlation of disease severity and degree of dopaminergic neuron loss in PD, it is reasonable to speculate that there might be mild symptoms not sufficient to make a diagnosis of PD in some individuals found at autopsy to have low numbers of neurons in the SN. Our findings are consistent with this, although it is impossible to say whether the process(es) leading to SN neuron loss and parkinsonian signs in the deceased men without PD or ILB in this study were linked to PD. However, because the strongest association between neuron density and UPDRS signs in Lewy body-negative men occurred in dorsomedial and dorsolateral quadrants rather than the ventrolateral quadrant where PD pathology is greatest, an entirely different age-related mechanism is likely. Projections from the dorsal tier of the SN extend primarily to caudate nucleus where dopamine levels are reported to be decreased in normal aging but preserved, relative to the profound losses in the putamen, in PD.<sup>1,28,29</sup> Other candidate mechanisms include striatal small vessel cerebrovascular disease, neurotoxins, viruses, or genetic factors.

The question arises whether some aged individuals with parkinsonian signs may benefit from dopaminergic medication. Interestingly, the signs most associated with low neuron density in the non-PD, non-ILB group were primarily axial. These signs are known to be less responsive to L-dopa in PD and may be equally unresponsive in elderly parkinsonian patients without PD. In one trial of 10 healthy elderly volunteers, L-dopa had no effect on parkinsonian impairment,<sup>30</sup> although it is possible that individuals selected did not have severe enough signs at baseline to allow detection of improvement. Dopaminergic therapy has been reported to improve upper limb motor movements in aged rhesus monkeys.<sup>31</sup>

Although on average, individuals with PD had lower SN neuron densities than LB-negative controls, it is clear from the scatterplot in Figure 3 that there are individuals with very low densities who do not have PD. A similar pattern has been noted in imaging studies that show a decline in dopamine transporters with age to the point that there is overlap between PD pa-

tients and unaffected elderly.<sup>17,27,32</sup> Pathological studies using single-section counting methods<sup>2,22</sup> and unbiased stereological methods<sup>25</sup> also have found this overlap, although to a lesser degree than in our population. These studies had fewer and younger controls.

Although many LB-negative individuals with low SN neuron densities had UPDRS signs, there were some that did not. There may be mechanisms that allow persons to function well with fewer neurons. It is also possible that some of these individuals developed parkinsonian signs between their last examination with the UPDRS and death.

Alternatively, this overlap in SN neuron density between PD cases and controls could, in part, be related to our single-section counting method that may not accurately reflect density throughout the nigra in all cases. Vulnerability of dopamine-producing neurons in PD is known to be heterogeneous. Depletion seems to be highest in caudal and middle regions as opposed to rostral.<sup>33,34</sup> Our sections were from upper caudal midbrain.

Unbiased stereological counting methods would have allowed accurate estimates of neuron number and density for the entire SN. We were unable to use this technique for several reasons. First, it would have been technically difficult to obtain quadrant specific counts because of lack of precise boundaries for these regions. In addition, because the entire structure of interest is required for sampling and serial sectioning, ability to use that structure for other studies is limited.<sup>35</sup> Ma and colleagues<sup>36</sup> have demonstrated that under proper conditions (well-defined cutting level; constant section thickness), neuron counts on single sections of the SN accurately reflect values obtained with stereological procedures involving the dissector method. For this study, we believe that a single section taken from midbrain in an anatomically standardized way gives an ordinal measure that allows relative comparisons with similarly sampled brains.

In summary, we found, like others, lowest neuron densities in the ventrolateral quadrant of the SN in PD cases with intermediate levels found in ILB cases compared with controls without LB or clinical PD. Among a subgroup of decedents without LB, there was an association of UPDRS signs assessed during late life with lower neuron density that was most prominent in dorsolateral and dorsomedial quadrants. Future efforts will focus on risk factors for diminished neuron density in the SN and on relationships of neuron density with  $\alpha$ -synuclein pathology in other brain regions.

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## Association of Olfactory Dysfunction With Incidental Lewy Bodies

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**Abstract:** Olfactory dysfunction is found in early Parkinson's disease (PD) and in asymptomatic relatives of PD patients. Incidental Lewy bodies (ILB), the presence of Lewy bodies in the brains of deceased individuals without a history of PD or dementia during life, are thought to represent a presymptomatic stage of PD. If olfactory dysfunction were associated with the presence of ILB, this would suggest that olfactory deficits may precede clinical PD. The purpose of this study was to determine the association of olfactory dysfunction during late life with ILB in the substantia nigra or locus ceruleus. Olfaction was assessed during the 1991–1994 and 1994–1996 examinations of elderly Japanese–American men participating in the longitudinal Honolulu–Asia Aging Study. Among those who later died and underwent a standardized postmortem examination, brains were examined for Lewy bodies in the substantia nigra and the locus ceruleus with hematoxylin and eosin stain. Lewy

bodies in the brains of individuals without clinical PD or dementia were classified as ILB. There were 164 autopsied men without clinical PD or dementia who had olfaction testing during one of the examinations. Seventeen had ILB. The age-adjusted percent of brains with ILB increased from 1.8% in the highest tertile of odor identification to 11.9% in the mid-tertile to 17.4% in the lowest tertile ( $P = 0.019$  in test for trend). Age-adjusted relative odds of ILB for the lowest versus the highest tertile was 11.0 ( $P = 0.02$ ). Olfactory dysfunction is associated with ILB. If incidental Lewy bodies represent presymptomatic stage of PD, olfactory testing may be a useful screening tool to identify those at high risk for developing PD.  
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**Key words:** olfaction; Lewy bodies; epidemiology; Parkinson's disease

Olfactory dysfunction, whether measured by odor identification, recognition, or threshold, is associated with Par-

kinson's disease (PD)<sup>1,2</sup> and may be one of the earliest signs. Recent pathological studies have found that the olfactory bulb and tract are among the earliest brain regions affected by Lewy pathology.<sup>3</sup> An important question is whether olfactory dysfunction precedes the onset of the classic motor signs. If olfaction were impaired in individuals without PD or dementia whose brains were later found to have incidental Lewy bodies (ILB) in the brainstem pigmented nuclei, this would provide evidence that olfaction is affected before motor signs are obvious.

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The goal of this study was to examine the association of olfactory dysfunction measured up to 4 years prior to death with the presence of ILB in brains of deceased men without clinical PD or dementia who were participants in the Honolulu–Asia Aging Study (HAAS). Impaired olfactory function in persons found to have ILB would provide evidence that this sign may predate the typical motor signs of PD. If so, screening tests of olfactory function might be useful to identify persons at high risk for developing clinical PD.

### PATIENTS AND METHODS

Started in 1965, the Honolulu Heart Program (HHP) is a longitudinal study of heart disease and stroke in a cohort of Japanese–American men living on the island of Oahu, Hawaii. The 8,006 men participating in the baseline examination were born between 1900 and 1919. Follow-up has continued through reexaminations of the cohort and surveillance of hospital and death records.<sup>4</sup> With establishment of the HAAS, research on diseases of aging including PD was initiated at the 1991–1993 examination,<sup>5</sup> and all cases of PD in the cohort were identified.<sup>6,7</sup> Additional cases of PD were identified during follow-up examinations in 1994–1996, 1997–1999, and 2000–2001 and by examination of medical records.<sup>7</sup> The study was approved by the Kuakini Medical Center Institutional Review Board and participants signed informed consents at all examinations.

Olfaction was tested with the 12-odor Cross-Cultural Smell Identification Test (CC-SIT) adapted from the 40-odor University of Pennsylvania Smell Identification Test (UPSIT-40) during the 1991–1993 and 1994–1996 examinations.<sup>8,9</sup> Participants were asked to identify the correct odor from four possible choices for each item. The odor identification score was the number correct (range, 0–12). For this analysis, the most recent available odor identification score was used.

Covariates were chosen based on their association with olfactory function or PD risk. Cognitive function was assessed at the same examination as olfaction using the Cognitive Abilities Screening Instrument (CASI).<sup>5,10</sup> The score range is 0 to 100, with 100 being a perfect score. Cigarette smoking measured in pack–years and coffee consumption measured in ounces per day were assessed at the 1965 examination as markers of midlife exposure. History of head injury severe enough to lose consciousness was assessed during the 1991–1993 examination.

An autopsy component of the HHP/HAAS was initiated in 1991.<sup>11</sup> Autopsy was discussed with all participants prior to death and consent for autopsy was given by the closest living family member according to Hawaii

state law. This analysis was performed with 164 brains from autopsies performed during the years 1991–2001 from individuals without a clinical diagnosis of PD or dementia according to the *Diagnostic and Statistical Manual of Mental Disorders* third revised edition criteria.<sup>12</sup>

Standardized gross and microscopic examinations of multiple brain regions are performed routinely. Details have been previously described.<sup>13,14</sup> Relevant to this report, formalin-fixed sections of midbrain from the level of the exit of the third cranial nerve and the mid pons at the level of the locus ceruleus are stained with hematoxylin and eosin. One of three neuropathologists shielded from the clinical diagnosis performs a standardized microscopic evaluation of single sections through the substantia nigra and locus ceruleus for Lewy bodies.

### Statistical Analysis

Average characteristics are described for ILB cases and controls with statistical comparisons based on exact methods using Wilcoxon rank-sum tests.<sup>15</sup> Crude and age-adjusted prevalence of ILB were also estimated across ranges of odor identification with standard analysis of covariance models.<sup>16</sup> To display our findings, the ranges of olfaction scores were selected to form approximate tertiles with the best balance of sample size. Scores in the first, second, and third tertiles ranged from 0 to 5 ( $n = 55$ ), 6 to 7 ( $n = 51$ ), and 8 to 12 ( $n = 58$ ), respectively. Because the number of ILB cases was small, tests of significance were based on exact permutation tests for logistic regression models.<sup>17</sup> After adjustment for age and the other characteristics, relative odds (and 95% confidence intervals) were estimated comparing the odds of ILB in the bottom and middle tertiles of odor identification with the odds of ILB in the top tertile. All reported  $P$  values were based on two-sided tests of significance.

### RESULTS

Characteristics of the population are described in Table 1. There were 17 brains with ILB and 147 control brains without Lewy bodies. Mean age at olfaction testing for those with ILB (83.5 years) was higher than those without ILB (81.5 years), although the difference was not statistically significant. The mean interval from the most recent examination when olfaction was tested to death was similar for ILB (3.7 years) and controls (3.4 years). Mean odor identification score was significantly lower in the ILB cases (4.6) versus the controls (6.3;  $P = 0.011$ ). Mean CASI score was essentially the same for those with ILB and those without (79.3 and 79.8, respectively). Differences in the other characteristics between

**TABLE 1.** Characteristics of autopsied men with and without ILB

Characteristic	ILB (17)*	Controls (147)
Number of identified odors (among 12)	4.6 ± 2.5 <sup>a</sup>	6.3 ± 3.1 <sup>b</sup>
Age at olfaction testing (yr)	83.5 ± 6.1 (77–93) <sup>c</sup>	81.5 ± 5.1 (73–94)
Time from olfaction testing to death (yr)	3.7 ± 1.6	3.4 ± 2.0
Education level (yr)	9.6 ± 3.6	10.6 ± 3.3
Family history of Parkinson's disease, % (n)	5.9 (1) <sup>d</sup>	3.4 (5)
CASI at time of olfaction testing	79.3 ± 14.8	79.8 ± 13.1
Midlife pack-years of smoking	28.7 ± 26.7	28.6 ± 29.0
Midlife coffee intake (oz/day)	12.7 ± 13.4	15.2 ± 12.4
History of head injury, % (n)	5.9 (1)	1.4 (2)
Lewy bodies in the substantia nigra, % (n)	35.3 (6)	
Lewy bodies in the locus ceruleus, % (n)	94.1 (16)	

\*Number of autopsied men.

<sup>a</sup>Mean ± standard deviation.<sup>b</sup>Significant difference from cases ( $P = 0.011$ ).<sup>c</sup>Range.<sup>d</sup>Number of cases.

the ILB cases and controls were not statistically significant.

Table 2 shows the unadjusted and age-adjusted percent of autopsied men with ILB within the tertiles of odor identification score. In the lowest tertile, 10 of 55 (18.2%) had ILB, compared to 6 of 51 (11.8%) for the middle tertile and 1 of 58 (1.7%) for the highest tertile of olfactory score. Both the unadjusted and age-adjusted percent of brains with ILB decreased significantly with increasing tertile of odor identification score ( $P = 0.006$  for unadjusted and 0.019 for age-adjusted test for trend). The age-adjusted relative odds of ILB in the lowest versus the highest tertile of odor identification was 11.0 (95% confidence interval = 1.3–526;  $P = 0.02$ ).

Family history of PD and a history of head injury occurred too infrequently to allow for adjustment of these factors. Adjustment for the remaining study characteristics in Table 1 had little effect on the association between olfaction and ILB. Effects of interaction between the other characteristics and olfaction were also

negligible. Figure 1 illustrates these observations for two of the study characteristics. As noted in the top of Figure 1, regardless of the time from olfaction testing to death, the percent of brains with ILB declined significantly with increasing olfaction test scores for deaths that occurred within 3.4 years ( $P = 0.035$ ) and for deaths 3.4 years or more ( $P = 0.031$ ) after olfaction testing. Here, 3.4 years corresponds to the median time from olfaction testing to death. Similarly, when participants were divided into high and low CASI score groups (Fig. 1B), the percent of brains with ILB remained less in men with higher olfaction test scores than in men whose olfaction testing was poor.

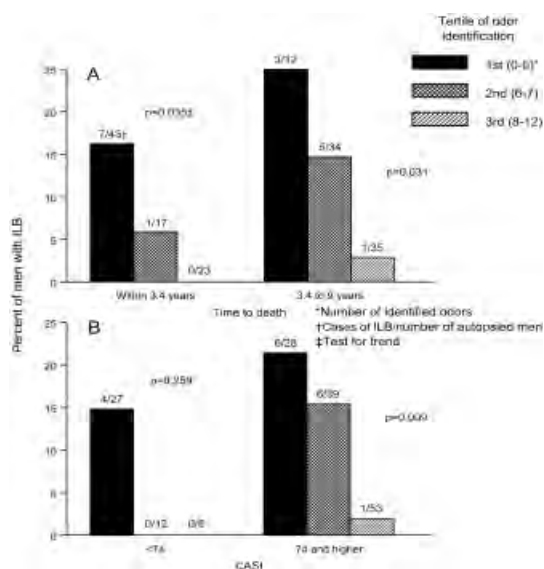
## DISCUSSION

To our knowledge, this is the first report of olfactory deficits in individuals without parkinsonism or dementia during life who were found at autopsy to have ILB. The significance of this finding relies on the premise that the presence of ILB represents presymptomatic stage of PD

**TABLE 2.** Percent of autopsied men with ILB within tertile ranges of odor identification

Tertile of odor identification	Percent with ILB		Age-adjusted relative odds of ILB versus the top tertile of odor identification
	Unadjusted	Age-adjusted	
First (0–5) <sup>a</sup>	18.2 <sup>b</sup> (10/55) <sup>c</sup>	17.4 <sup>d</sup>	11.0 (1.3–526) <sup>e</sup>
Second (6–7)	11.8 (6/51)	11.9	7.4 (0.8–351)
Third (8–12)	1.7 (1/58)	1.8	Reference
Test for trend	$P = 0.006$	$P = 0.019$	

<sup>a</sup>Number of identified odors.<sup>b</sup>Significant excess versus the top tertile of odor identification ( $P = 0.006$ ).<sup>c</sup>Cases of ILB/number of autopsied men.<sup>d</sup>Significant excess versus the top tertile of odor identification ( $P = 0.020$ ).<sup>e</sup>95% confidence interval.



**FIG. 1.** Percent of autopsied men with ILB within tertiles of odor identification, according to time from olfaction testing to death (A) and to CASI score (B).

in persons who died prior to developing motor signs of the disease. This is supported by several lines of evidence. First, pathological and imaging studies indicate that the process underlying neuronal loss in the substantia nigra begins at least 4 years before overt motor signs of the disease develop<sup>18,19</sup> and that 80% of striatal dopamine is lost before symptoms of PD become apparent.<sup>20</sup> Additionally, the frequency of Lewy bodies in elderly persons without clinical PD or dementia (ILB) is 5 to 20 times that of overt clinical PD.<sup>21</sup> Finally, in a recent pathological study from the HAAS, substantia nigra neuron counts from brains with ILB were found to fall between those of unaffected controls and PD brains,<sup>14</sup> validating the earlier work of Fearnley and Lees.<sup>18</sup> Therefore, olfactory deficits in individuals with ILB suggest that impaired olfaction may occur as one of the earliest signs of PD and that olfactory testing may be useful to detect individuals at high risk for developing PD.

Studies of PD patients also indicate that impaired olfaction occurs early in the disease process and may precede the onset of motor signs of PD. In one study, up to 90% of PD patients tested had lower UPSIT-40 scores than normal matched controls, and olfactory deficits were unrelated to severity or duration of disease or use of medications.<sup>22</sup> Olfactory deficits have been found in untreated patients with early PD.<sup>23,24</sup> We determined the mean olfaction identification score for prevalent nondemented PD cases in the HAAS cohort and compared it to the mean score for nondemented non-PD participants. Consistent with previous reports, the mean score for 36

PD cases who were assessed at the 1991–1993 and 1994–1996 examinations was 3.3 versus 7.2 in 2,218 non-PD controls ( $P < 0.001$ ).

Odor identification scores are lower in asymptomatic first-degree relatives of PD patients compared to control subjects without a family history of PD.<sup>25</sup> Declines in striatal dopamine transporter binding as measured by [<sup>123</sup>I] beta-CIT single photon emission tomography (SPECT) have been reported in hyposmic relatives of PD patients while normosmic relatives had normal binding.<sup>26</sup> In a recent prospective study of first-degree relatives of PD patients by the same group of investigators, 4 of 40 hyposmic subjects were diagnosed with PD over 2 years of follow-up compared to none of the 38 normosmic subjects. All four of the subjects who developed PD had strongly reduced [<sup>123</sup>I] beta-CIT binding ratios at the baseline examination.<sup>27</sup> Lastly, 7 of 10 non-PD subjects with idiopathic impaired olfaction and hyperechogenicity of the substantia nigra on transcranial sonography were found to have evidence of loss of dopamine transporters on SPECT with <sup>123</sup>I-FP-CIT. Together, these studies provide strong evidence that impaired olfaction is a preclinical marker of PD.<sup>28</sup>

Although the cause of impaired sense of smell in PD is unknown, the association of impaired olfaction with ILB suggests that the cause of the deficits may be linked to the processes leading to Lewy body formation. It is speculated that the olfactory tract may act as a conduit for environmental toxins that gain access to the brain through the rootlets of the olfactory nerve in the olfactory epithelium of the nose.<sup>29</sup> Autopsy studies have demonstrated Lewy bodies, Lewy neurites, and neuronal loss in the olfactory bulb, olfactory tract, and the anterior olfactory nucleus of PD patients and the degree of neuronal loss has been correlated with duration of PD.<sup>29</sup> Furthermore, pathological studies of ILB cases using  $\alpha$ -synuclein immunostaining to examine multiple brainstem and cortical regions have found that the olfactory structures are among the earliest to be affected by Lewy pathology in addition to the dorsal glossopharyngeal-vagus nuclear complex.<sup>3,30</sup> Impaired olfactory identification has been found to correlate significantly with dopamine transporter binding in the putamen on [<sup>99m</sup>Tc] TRODAT-1 SPECT imaging but not with motor scores on the Unified Parkinson's Disease Rating Scale (UPDRS) among persons with early PD. The authors speculate that olfactory loss may be a marker for but not causally related to nigrostriatal dopaminergic cell loss and subsequent motor signs of PD.<sup>31</sup>

Mechanical aspects of sniffing also play a role in the odor sensory deficits in PD. In a recent study comparing 20 PD patients to 20 controls, the PD patients exhibited

significant impairment in sniff airflow rate and volume. Furthermore, olfactory function improved with increased sniff vigor.<sup>32</sup> Olfactory function was significantly correlated with a subset of measures on the UPDRS related to axial function, prompting speculation that impaired sniffing may be another motor symptom of PD.

The role of dopamine in the olfactory deficits in PD is not clear. Reduced levels of dopamine may underlie the olfactory deficits in PD. The olfactory tract projects to the piriform cortex and this region receives dopaminergic input from the ventral tegmental area and the substantia nigra. Additionally, there is evidence that dopamine D2 receptors are expressed in the olfactory bulb and that dopamine may modulate olfactory input to the bulb.<sup>33</sup> In contrast, a recent pathological study indicated that the number of dopaminergic cells in the olfactory bulb of PD patients was increased relative to age- and gender-matched controls. The authors, noting the neuroinhibitory role of dopamine in olfactory transmission, speculated that the increased number of dopamine neurons leads to higher dopamine function that actually suppresses olfaction.<sup>34</sup> This may explain why levodopa does not reverse olfactory deficits in PD patients.<sup>23,35,36</sup>

There are potential limitations to this study. First, some of the men could have been diagnosed with PD by their private physician between the time of their last HAAS examination and death. Such cases would have been misclassified as ILB cases. The HHP maintains a rigorous system of follow-up that tracks participants through hospital and death records. A review of these records on all ILB cases in this study revealed none with a diagnosis of PD prior to death in the physicians' notes or problem list. The mean time between the last documented contact with a physician and death was 11 months. The overall percent and age distribution of ILB in our study is similar to others.<sup>21</sup> Regarding generalizability of these findings to the U.S. population, a direct comparison of CC-SIT scores might not be possible. However, the finding of an association of impaired olfaction with the presence of ILB is unlikely to be affected by ethnicity or gender. Lastly, the substantia nigra and locus ceruleus were the only brainstem regions examined for Lewy bodies and sections were stained with hematoxylin and eosin. Recent pathological studies suggest that Lewy bodies may occur in the dorsal motor nucleus of the glossopharyngeal-vagus nerve complex and olfactory bulb in the early stages of the disease process without involvement of the substantia nigra or locus ceruleus.<sup>3</sup> Use of  $\alpha$ -synuclein immunostaining to look for Lewy pathology in multiple brainstem regions may have identified more ILB cases. Efforts are now underway in the HAAS for a comprehensive survey of relevant

brainstem regions and olfactory bulbs using state-of-the-art  $\alpha$ -synuclein immunostaining.

A strength of the study is the longitudinal design with excellent follow-up and characterization of cognitive and motor function of participants during life. This provides a high level of certainty that participants were free of PD and dementia at the time of olfactory testing. Additionally, all brains were examined using the same standardized methods and neuropathologists were shielded from clinical data.

Impaired olfaction is one of the earliest signs of PD, often predating the diagnosis by 2 years or more. Olfactory testing may be a simple and economical way to identify an age-appropriate population at high risk for developing PD that could be enrolled in pharmaceutical trials aimed at preventing or slowing progression of PD. Combining olfaction testing with cognitive testing (to rule out early Alzheimer's disease), functional neuroimaging, or susceptibility genetic testing could refine this population further.<sup>37</sup> Conversely, given the finding that high scorers on the olfaction test (highest tertile) have very low probability of ILB, olfactory testing may be a useful screen to identify control subjects for PD case-control studies.

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## Bowel Movement Frequency in Late-Life and Incidental Lewy Bodies

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**Abstract:** It is not known if constipation is associated with the preclinical phase of Parkinson's disease (PD), often characterized by the presence of incidental Lewy bodies (ILB). Such an association could provide evidence that constipation is an early symptom of PD. The purpose of this report is to examine the association between late-life bowel movement frequency and ILB. Bowel movement frequency was assessed from 1991 to 1993 in 245 men aged 71 to 93 years in the Honolulu-Asia Aging Study who later received postmortem examinations. All were without clinical PD and dementia. Brains were examined for ILB in the substantia nigra and locus ceruleus. Among the decedents, 30 men had ILB (12.2%). After age-adjustment, the percent of brains with ILB declined with increasing bowel

movement frequency ( $P = 0.013$ ). For men with  $<1$ ,  $1$ , and  $>1$  bowel movement/day, corresponding percents were 24.1, 13.5, and 6.5%. Findings persisted after additional adjustment for time to death, mid-life pack-years of smoking and coffee intake, physical activity, and cognitive function. Infrequent bowel movements are associated with ILB. Findings provide evidence that constipation can predate the extrapyramidal signs of PD. Constipation could be one of the earliest markers of the beginning of PD processes. © 2007 Movement Disorder Society

**Key words:** bowel movement; constipation; lewy body; parkinson's disease; preclinical.

Careful studies using alpha synuclein staining indicate that the earliest appearance of Lewy pathology in Parkinson's disease (PD) starts in the myenteric plexus of the gut and the dorsal motor nucleus in the lower medulla.<sup>1,2</sup> It is speculated that when these regions are affected, impairments can occur in colonic transit.<sup>1,2</sup>

Based on these reports, Braak and colleagues further propose a staging system that follows the sequence of disease progression in the brain from the dorsal motor nucleus of the vagal nerve (stage I) to the locus ceruleus (stage II), the substantia nigra (stage III), and finally to the cerebral cortex (stages IV–VI).<sup>3</sup> To reach a higher stage, it is thought that Lewy pathology must first appear lower in the brain stem. It is in stage III where the classic motor symptoms of PD begin to be evident, but only after considerable loss of dopamine producing neurons has taken place in the substantia nigra.<sup>4–7</sup> It is the presence of incidental Lewy bodies (ILB) in regions of the brain corresponding to stages I, II, and III in individuals without clinical PD and dementia that is thought to be

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associated with a pre-extrapyramidal phase of PD.<sup>6-8</sup> If constipation is associated with ILB in these regions, this could provide evidence that constipation is one of the earliest symptoms of PD, predating the development of its typical motor features. To date, studies examining the association of constipation with brainstem Lewy pathology in the absence of clinical PD are lacking. The purpose of this report is to assess the association between late-life bowel movement frequency and ILB in the substantia nigra and locus ceruleus. Findings are from a sample of autopsied men without clinical PD and dementia who were enrolled in the Honolulu-Asia Aging Study.

## PATIENTS AND METHODS

### Background and Study Sample

From 1965 to 1968, the Honolulu Heart Program began following 8,006 men of Japanese ancestry living on the island of Oahu, Hawaii for development of cardiovascular disease.<sup>9,10</sup> Beginning with examinations that were given from 1991 to 1993, the Honolulu-Asia Aging Study was launched as an expansion of the Honolulu Heart Program for the study of neurodegenerative diseases and cognitive function in the elderly.<sup>11</sup> Subjects included 3,734 men aged 71 to 93 years (approximately 80% of the survivors in the original Honolulu Heart Program cohort). Procedures were in accordance with institutional guidelines and approved by an institutional review committee. Informed consent was obtained from the study participants.

For this report, frequency of bowel movements was assessed at clinical examinations at the initiation of the Honolulu-Asia Aging Study (1991–1993). Participants were aged 71 to 93 years. As part of an ongoing autopsy component of the Honolulu-Asia Aging Study,<sup>12</sup> autopsy considerations were discussed with a cohort member prior to death, and consent was given by the closest living family member according to the laws of the State of Hawaii. Autopsies were performed from 1991 to 2002 following a rigid study protocol. During this time, there were 1,986 deaths, among which, 430 had an autopsy (21.7%). In the latter group, microscopic evaluation is pending in 20 men.

Men with clinical PD and dementia, including dementia with Lewy bodies, were excluded based on diagnoses made by study neurologists according to published criteria without access to risk factor data.<sup>13-16</sup> There were 25 with a diagnosis of PD, 119 with dementia, and 2 with dementia with Lewy bodies. Data on bowel movement frequency were missing in 19 men. The final sample includes 245 men.

### Frequency of Bowel Movements and Confounding Information

Information on the frequency of bowel movements was collected through a questionnaire administered by a trained research technician. Each participant was asked, “how often do you have a bowel movement”? Answers included, (1) less often than once each week, (2) approximately one time each week, (3) approximately two times each week, (4) approximately every other day, (5) once each day, (6) approximately two to three times each day, and (7) more often than three times each day. Additional confounding information was selected based on possible or putative associations with constipation or PD. The information included age, time to death, mid-life pack-years of smoking and coffee intake, physical activity, and cognitive function. Mid-life pack-years of cigarette smoking and coffee intake were measured at the time of initiation of the Honolulu Heart Program (1965–1968) as markers of typical lifetime exposures to these factors. Late-life coffee intake was not determined at the time of bowel movement assessment (1991–1993) and current cigarette smoking occurred too infrequently in this elderly sample to allow for its careful consideration. Determination of the other characteristics coincided with the time of collection of data on the frequency of bowel movements (1991–1993).

In this report, assessment of physical activity was based on the use of the physical activity index, a common measure used to quantify overall metabolic output in a typical 24-hour period and shown to be inversely associated with the risk of stroke and coronary heart disease.<sup>17,18</sup> High scores indicate greater physical activity. Cognitive performance was based on the Cognitive Abilities Screening Instrument, a comprehensive measure of intellectual function that has been developed and validated for use in cross-cultural studies.<sup>19</sup> Performance scores range from 0 to 100 with high scores indicating better cognitive function than low scores.

### Determination of Incidental Lewy Bodies

Standardized gross and microscopic examinations of multiple brain regions were performed routinely. Details have been previously described.<sup>7,20</sup> Relevant to this report, single sections of formalin fixed midbrain from the level of the exit of the third cranial nerve and the midpons at the level of the locus ceruleus were stained with hematoxylin and eosin. Sections were examined for Lewy bodies as part of the standardized microscopic examination by a study neuropathologist without access to clinical diagnoses and risk factor status.

**TABLE 1.** Distribution of bowel movement frequencies and the number and percent of men with incidental Lewy bodies (ILB) in the autopsied sample of men

Bowel movement frequency	Number of men	Number with ILB	Percent with ILB
Less often than once each week	2	1	50.0
Approximately one time each week	1	0	0.0
Approximately two times each week	9	3	33.3
Approximately every other day	9	1	11.1
Once each day	148	20	13.5
Approximately two to three times each day	68	5	7.4
More often than three times each day	8	0	0.0
Total	245	30	12.2

### Statistical Methods

Average age-adjusted characteristics were estimated across ranges of bowel movement frequency based on standard analysis of covariance techniques.<sup>21</sup> Similar methods were used to derive the crude and age-adjusted percent prevalence of ILB. After adjustment for age and the other characteristics, relative odds (and 95% confidence intervals) were estimated using bootstrap methods comparing the odds of ILB between the categories of bowel movement frequency.<sup>22</sup> In a test for trend, the odds of ILB was also modeled across the seven strata of bowel movement frequency with bowel movement frequency included as a single independent covariate that increased from 1 (less often than once each week) to 7 (more than three times each day). Because of the small sample size, tests of significance of the association between bowel movement frequency and ILB were derived from exact permutation tests for logistic regression models.<sup>23</sup> All reported *P*-values were based on two-sided tests of significance.

### RESULTS

Table 1 gives the observed distribution of bowel movement frequencies in the autopsied sample and the number and percent of men with ILB. Because of the small number of men in the extreme ranges of bowel movement frequency, bowel movement frequencies were categorized as <1, 1, and >1 bowel movement/day in subsequent tables and for comparison between bowel movement strata.

Table 2 provides a comparison between the 245 autopsied men considered in this report and the decedents who did not receive an autopsy. As with those who received an autopsy, the latter group was also without a clinical diagnosis of PD and dementia. After removing 50 cases of PD, an additional 241 cases of dementia, and 130 with missing bowel movement data from the 1,556 deaths that occurred without an autopsy, 1,135 decedents remained for comparison.

As noted in Table 2, frequency of bowel movements was similar between the decedents with and without an autopsy. About 8 to 9% reported having <1 bowel movement/day, while most (nearly two-thirds) had 1 bowel movement/day. There were no marked differences between the two groups in any of the other study characteristics.

Among the confounding variables in the 245 men with autopsies, there was a slight increase in the average age with increasing bowel movement frequency. Although younger, the average time to death in those with <1 bowel movement/day was more than a year shorter than in the other bowel movement groups. Neither age nor time to death was significantly associated with bowel movement frequency.

Mid-life pack-years of smoking declined with increased bowel movement frequency. Men with <1 bowel movement/day averaged 43.1 pack-years of smoking versus 26.7 in men with >1 bowel movement/day (*P* = 0.021). Men with <1 bowel movement/day also

**TABLE 2.** Age-adjusted percents of bowel movement frequency, average age, and age-adjusted average levels of study characteristics in decedents with and without an autopsy

Characteristic	Autopsy	
	Yes (245)*	No (1,135)
Percent with >1 bowel movement/day	8.4 (21)	9.1 (103)
Percent with 1 bowel movement/day	60.2 (148)	64.1 (727)
Percent with <1 bowel movement/day	31.3 (76)	26.8 (305)
Age (yr)	78.3 ± 4.5 <sup>a</sup>	78.8 ± 4.9
Time to death (yr)	5.9 ± 3.0	5.8 ± 2.8
Mid-life pack-years of smoking	30.8 ± 28.5	30.1 ± 29.3
Mid-life coffee intake (oz/d)	14.0 ± 13.4	13.9 ± 12.5
Physical activity index	30.5 ± 4.3	30.6 ± 4.8
Cognitive abilities screening instrument score	84.9 ± 9.1	85.2 ± 10.7

There are no significant differences between decedents with and without an autopsy.

All men were without PD and dementia.

\*Numbers in parentheses are sample sizes.

<sup>a</sup>Mean ± standard deviation.

**TABLE 3.** Percent of men with incidental Lewy bodies according to frequency of bowel movements

Bowel movements/day	Percent with Lewy bodies	Relative odds
Unadjusted		
<1	23.8 (5/21) <sup>a</sup>	4.3 <sup>b</sup> (1.2, 17.1) <sup>c</sup>
1	13.5 (20/148)	2.2 (1.0, 7.2)
>1	6.6 (5/76)	Reference
Test for trend	$P = 0.010$	
Age adjusted		
<1	24.1	4.5 <sup>d</sup> (1.2, 17.5)
1	13.5	2.2 (1.0, 7.2)
>1	6.5	Reference
Test for trend	$P = 0.013$	
Risk factor adjusted <sup>e</sup>		
<1	23.7	4.5 <sup>f</sup> (1.1, 23.4)
1	14.0	2.3 (0.9, 6.9)
>1	6.6	Reference
Test for trend	$P = 0.016$	

<sup>a</sup>Number with Lewy bodies/sample size.

<sup>b</sup>Excess percent with Lewy bodies versus men with >1 bowel movement/day ( $P = 0.036$ ).

<sup>c</sup>95% confidence interval.

<sup>d</sup>Excess percent with Lewy bodies versus men with >1 bowel movement/day ( $P = 0.034$ ).

<sup>e</sup>Adjusted for age, time to death, mid-life pack-years of smoking and coffee intake, physical activity, and the cognitive abilities screening instrument score.

<sup>f</sup>Excess percent with Lewy bodies versus men with >1 bowel movement/day ( $P = 0.036$ ).

consumed the most coffee during mid-life, and their cognitive function was the highest among the bowel movement groups. As physical activity increased, bowel movements became more frequent. None of the latter differences were statistically significant.

Among the 245 autopsied men, 30 had ILB (12.2%). Table 3 provides details on the relationship between ILB and bowel movement frequency. After age-adjustment, the percent of brains with ILB declined with increasing bowel movement frequency ( $P = 0.013$ ). For men with <1, 1, and >1 bowel movement/day, corresponding percents were 24.1, 13.5, and 6.5%. Findings persisted after additional adjustment for time to death, mid-life pack-years of smoking and coffee intake, physical activity, and cognitive function.

## DISCUSSION

In an earlier report from the Honolulu-Asia Aging Study, infrequent bowel movements in mid-life were shown to be associated with an increased risk of PD.<sup>24</sup> Although constipation is a common symptom of PD and can predate its clinical onset by many years,<sup>24,25</sup> its role in the pre-extrapyramidal phase of PD is uncertain. In the current report, bowel movement frequency was ascertained in a sample of elderly men in the Honolulu-Asia Aging Study who later received autopsies following a standardized protocol. Men with <1 bowel movement/day had a near 4-fold excess of

ILB as compared to those with >1 bowel movement/day. Assuming that ILB represents an early phase of PD, an association between ILB and constipation provides evidence that constipation could be one of the earliest markers of the beginning of the PD process.

The existence of early recognizable non-motor symptoms such as constipation is plausible based on reports that 50% of nigral neurons and 80% of striatal dopamine are lost by the time PD is diagnosed.<sup>4,6</sup> Pathologic and neuroimaging studies have also shown that neuronal loss in the substantia nigra begins at least four years prior to the onset of the extrapyramidal signs of PD.<sup>5,6</sup> In the Honolulu-Asia Aging Study and elsewhere, it has been shown that there is a greater loss in neuron density in the substantia nigra in the presence versus the absence of ILB. In the presence of ILB, neuronal loss is also less extensive than when PD is present.<sup>6,7</sup> Braak et al. offer an important rationale for describing the progression of the PD process with the development of incidental Lewy pathology in Meissner's and Auerbach's plexi in the gastrointestinal tract, ascending to the dorsal motor nucleus of the vagus nerve, the caudal raphe nuclei, the locus ceruleus, and eventually to the substantia nigra.<sup>2,3</sup> These pathologic studies have shown that Lewy bodies and Lewy neurites often occur in the dorsal motor nucleus of the vagus nerve without involvement of the locus ceruleus and the substantia nigra.<sup>1</sup> Although the current report focused on the classic definition of ILB in the substantia nigra and locus ceruleus, efforts are now underway for a comprehensive survey of the brainstem and basal ganglia. Once complete, it may be possible to characterize associations between constipation and Lewy pathology within other affected regions or between the neuropathologic stages of PD.<sup>3</sup>

There are several potential explanations that further support a neuropathologic link between constipation and ILB. Loss of dopamine producing neurons in the colon and the presence of Lewy bodies in the myenteric plexus of the gut are known to occur.<sup>26,27</sup> Lewy bodies and Lewy neurites may occur in Meissner's and Auerbach's plexi in the gastrointestinal tract of non-parkinsonian individuals.<sup>2</sup> In addition, control of defecation may be altered by abnormalities in skeletal muscle of the pelvic floor and anal sphincter through central nervous system derangements.<sup>27-30</sup> As a result, both autonomic and central nervous system abnormalities could have a role in the manifestation of constipation as a PD process that occurs before the appearance of extrapyramidal signs. Although the cause of the neuropathologic process in PD remains unknown, it is possible that environmental toxins or pathogens first affect vulnerable neuron popula-

tions in the enteric nervous system and gain access to the central nervous system via retrograde axonal transport.<sup>2</sup>

Other explanations for the link between constipation and ILB are less apparent. In the current study, the association between bowel movement frequency in late-life and ILB were unexplained by age, time to death, mid-life pack-years of smoking and coffee intake, physical activity, and cognitive function. It seems interesting that the excess of ILB in men with <1 bowel movement/day persisted in spite of an elevated exposure to the protective effects of cigarette smoking. Unfortunately, there are two obstacles that prevent a careful assessment of the importance of this finding. First, although smoking failed to alter the association between bowel movement frequency and ILB, the smoking data used in this report was measured 23 to 28 years prior to the determination of bowel movement frequency. Second, smoking was rare when the men were aged 71 to 93 years at the time when bowel movement frequency was assessed.

In the earlier report from the Honolulu-Asia Aging Study, the inverse relationship between mid-life bowel movement frequency and the future risk of PD was also independent of laxative use and the intake of fruits, vegetables, and grains.<sup>24</sup> While the latter were not available for the current study at the time when late-life bowel movement frequency was assessed, the use of laxatives in mid-life and the dietary intake of fruits, grains, and vegetables from a mailed questionnaire that was administered 3 to 5 years earlier failed to offer additional explanation for the association between bowel movement frequency and ILB. Although mid-life bowel movement frequency from the earlier report from the Honolulu Asia-Aging Study was not significantly related to ILB in the current autopsied sample, associations could have been diminished by the 17 to 31 year lag between the mid-life measurement and the time of autopsy. In spite of this lag, in 238 men with mid-life bowel movement data, there was more than a 2-fold excess of ILB in those with <1 bowel movement/day (25% <sup>3</sup>/<sub>12</sub>) versus men whose bowel movements were more frequent (11.9% <sup>27</sup>/<sub>226</sub>).

As in any long-term follow-up study, there are several limitations in the current report. Perhaps most important is the possibility of the misclassification of a case as ILB when there was a prior diagnosis of PD by a private physician. The Honolulu-Asia Aging Study, however, has maintained a rigorous and comprehensive system of follow-up that was first initiated by the Honolulu Heart Program in 1965. In addition to complete physical and neurologic examinations, there is access to hospital admissions, medical records from private physicians, and death reports. After a thorough review of all available resources, including

physician notes and problem lists prior to death, there was no evidence of ILB misclassification.

The 12.2% prevalence of ILB in the current sample of men whose age at death ranged from 74 to 97 years also corresponds reasonably well with the prevalence reported by others.<sup>31</sup> In the latter, prevalence of Lewy bodies in the absence of PD increased from 3.8 to 12.8% between the 6th and 9th decades of life. Others have noted that Lewy body disease is 5 to 20 times more common than PD.<sup>32</sup> Unfortunately, this suggests that there could be a substantial rise in the prevalence of PD as life expectancy increases, allowing for Lewy pathology to progress to regions of the brain associated with the typical signs of PD.<sup>32</sup>

There may also be inaccuracies in the reporting of bowel movement frequencies among the study participants. Nevertheless, while bowel movement and constipation questionnaires vary among study samples, frequency of bowel movements in the sample of men in the current report are similar to those described elsewhere.<sup>33-37</sup> In the National Health and Nutrition Examination Survey, 64 to 74% recorded daily defecation compared to 60.4% in the current sample.<sup>33</sup> In an industrial community, 5.1% reported having <5 bowel movements/week, 68% reported having 5 to 7/week, and 26% reported having 2/day.<sup>34</sup> The latter corresponds well with the 27.8% of men in the current cohort who reported having approximately two to three movements/day. In one report in which bowel movement frequency was recorded in a similar fashion as in the current sample, 58.9% reported having 1 bowel movement/day, approximately 30% had 2/day, with the remaining sample being evenly divided between those with <1 and >2/day.<sup>35</sup>

Whether findings in the current study apply to other population segments and to women are also uncertain. In general, however, risk factor associations for cardiovascular and dementia outcomes in the sample from Hawaii are comparable to those that have been described elsewhere. Strengths of the study include its longitudinal design and the careful and comprehensive efforts by study neurologists and neuropathologists to characterize cognition, motor function, and brain morphology while adhering to a standardized protocol.

While constipation can predate PD by many years, the finding of an association with ILB suggests that impairments in colonic transit may occur as one of the earliest symptoms of an evolving PD process. Findings are consistent with the theory that the gastrointestinal tract serves as a possible port of entry for neurotoxicants that are the cause of PD.<sup>2,3</sup> Although in need of further confirmation (and clarification of its use in clinical applications), information

on bowel movement frequency could become a useful adjunct in detecting individuals at high risk for future PD.

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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/mm<sup>2</sup>) 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.

Key Words: Parkinson's disease, constipation, Lewy body, substantia nigra, neuron density.

## Introduction

Constipation is frequent in patients with Parkinson's disease (PD),<sup>1-18</sup> and may precede the extrapyramidal symptoms of clinical PD by many years.<sup>1,2,11,12</sup> 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.<sup>1</sup> Constipation was also associated with the presence of incidental Lewy bodies (LB) in decedents who did not have PD during life.<sup>19</sup> 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.<sup>20,21</sup> We have previously reported that parkinsonian signs in elderly without diagnosed PD during life are associated with lower neuron density in the SN.<sup>22</sup> 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.<sup>23-25</sup> 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.<sup>1</sup> 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,<sup>26</sup> mid-life coffee intake,<sup>27</sup> triceps skinfold thickness,<sup>28</sup> excessive daytime sleepiness,<sup>29</sup> 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).<sup>30</sup>

### *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.<sup>1,31</sup>

### *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.<sup>32</sup> 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 3<sup>rd</sup> 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/mm<sup>2</sup>.

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 PD<sup>26</sup> and a positive relationship with neuron density in the SN that is independent of clinical PD and LB.<sup>33</sup> 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/mm<sup>2</sup>. 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.<sup>34</sup> 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/mm<sup>2</sup> 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  $78.3 \pm 4.7$  years (range: 71-93). The average time from assessment of bowel movement frequency to death was  $8.0 \pm 3.5$  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 dorsomedial and ventromedial quadrants, the difference between those with <1 and 1 bowel movement per day did not reach statistical significance. In contrast, compared to men with <1 bowel movement per day, those with 1 per day had a 71% excess in neuron density in the ventrolateral quadrant ( $p=0.012$ ), while there was a 91% excess in those with >1 bowel movement per day ( $p=0.004$ ).

### Discussion

Although constipation is common in PD and can precede its motor symptoms by 12 years or more years<sup>1</sup>, 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.<sup>35</sup> Evidence for this includes findings of dopaminergic

neuron depletion in the colon and LB in the myenteric plexus in decedents who had PD.<sup>6,17</sup>

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.<sup>34</sup> The mechanism by which cigarette smoking is associated with higher SN neuron density needs further study. This finding could support a true neuroprotective role for smoking rather than an avoidance of smoking due to preclinical personality or other constitutional characteristics. This relationship will be explored in all decedents and in subgroups with and without Lewy pathology when a sufficient number of cases become available.

Among non-smokers, our data suggest that nigral neuron counts are lower in individuals with a history of infrequent bowel movements. The relationship seems strongest in the ventrolateral quadrant where there is a preponderance of SN injury in PD<sup>21,36</sup> but it was also present in the ventromedial and dorsomedial quadrants. Conversely, the dorsolateral quadrant did not show this relationship. The dorsal tier, however, is most affected by neuron fallout with aging and is least affected in PD according to a report by Fearnley and Lees in 1991.<sup>21</sup> This indicates that neuron loss associated with a low frequency of bowel movements occurs preferentially in the regions most vulnerable to Lewy pathology. Although we have shown in a separate



report that low frequency of bowel movements is also associated with incidental LB, the relationship with low SN neuron density appears to be independent of the presence of LB in the SN or LC and a 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.

Among these decedents without clinical PD or incidental LB there were 29 who received the Unified Parkinson's Disease Rating Scale (UPDRS) at the time of bowel movement assessment. 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. Low SN neuron density in decedents without incidental LB could be due to a different neurodegenerative process, or it is possible that neuronal loss may begin in the SN before synuclein deposition.

A possible limitation of our study is the question of generalizability of findings in this cohort of elderly, Japanese men. Age or cultural factors could influence bowel habits either directly or through dietary differences. Arguing against this is evidence

that frequency of bowel movements in the HAAS is similar to other populations. In the National Health and Nutrition Examination Survey, 64 to 74% had daily bowel movements compared to 61.6% (255/414) in the HAAS.<sup>37</sup> In an industrial community, 5.1% reported having <5 bowel movements/week, 68% reported 5 to 7/week, and 26% reported 2/day.<sup>38</sup> The latter corresponds well with the 30.4% (126/414) of HAAS men who reported having more than one movement/day. Regarding the finding that constipation can precede the diagnosis of PD, there have been two retrospective studies to date, one in the U.S. (Nebraska)<sup>2</sup> and the other in Israel<sup>11</sup> indicating that this relationship also exists in other populations. 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 independently of LB in the SN or LC. 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.

### Acknowledgement

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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/mm<sup>2</sup>) according to frequency of daily bowel movements and mid-life smoking status within quadrants of the substantia nigra.

Quadrant	<u>Bowel movements/day</u>			<u>p-value for comparing</u>	
	<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/mm<sup>2</sup>) 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)



### **Abstract**

#### **Pattern of Lewy Pathology Progression Suggested by Braak Staging System is Supported by Analysis of a Population-based Cohort of Patients**

John E. Duda, MD, Joseph V. Noorigian, BS, Helen Petrovitch, MD, Lon R. White, MD, MPH, G. Webster Ross, MD

**Objective:** To assess the validity of the Braak Lewy body (LB) staging system in a population-based cohort of patients spanning the spectrum of LB disorders with a large number of incidental LB cases.

**Background:** Braak LB staging has provided an intriguing model of the progression of Lewy pathology accumulation in affected brains and deserves further confirmation in similar cohorts enriched with large numbers of brains with incidental Lewy pathology.

**Methods:** A total of 126 cases from the brain bank of the Honolulu Asian Aging Study were examined including 23 cases of Parkinson's disease (PD), 7 cases of dementia with Lewy bodies (DLB) and 96 cases with no clinical history of Parkinsonism. This cohort did not exclude patients with concomitant Alzheimer's disease pathology and was not a random sample in that 35 cases of ILB, which were previously determined by hematoxylin and eosin staining of the pons and midbrain, were included in the sample. Immunohistochemical staining for  $\alpha$ -synuclein and semi-quantitative pathology density analyses were performed on available tissue samples collected from 15 brain regions including the olfactory bulb, medulla, pons, midbrain, hippocampus, amygdala, striatum at the level of the nucleus accumbens, basal forebrain, and 7 different neocortical regions. From these regions 16 specific foci were quantified representing each of the six Braak stages. At least two foci from each stage were quantified, including: olfactory bulb and dorsal motor nucleus of the vagus (Stage 1); pontine raphe nucleus and locus ceruleus (Stage 2); substantia nigra pars compacta, nucleus basalis of Meynert and basolateral nuclear complex of the amygdala (Stage 3); Ammon's horn and entorhinal cortex (Stage 4); insular, anterior cingulate, dorsolateral prefrontal and supramarginal cortices (Stage 5); and motor, primary sensory and middle temporal cortices (Stage 6).

**Results:** Among 126 autopsied cases, 81 cases were found to have some Lewy pathology, including all 30 cases of PD or DLB and 51 cases of ILB. All DLB cases had Braak LB stages of 5 and 6, PD cases were either Braak LB stages 3, 5 or 6, and ILBD cases were found representing all stages 1-6. Of these 126 cases, 119 (94.4%) were found to be consistent with the progression of pathological distribution outlined by Braak, defined as cases that had Lewy pathology in at least one foci for each stage affected. Seven cases were found to be clearly inconsistent with the Braak LB staging system with no evidence of pathology in any representative foci for a stage preceding the last stage with pathology. Six of these cases were ILB cases and one was a case of PD. The most common reasons for inconsistency was the absence of pathology in foci representative of Braak LB stages 2 and 4.

**Conclusions:** The general pattern of progression outlined in the Braak LB staging system is upheld in 94% of cases from this population-based cohort.

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**One-third of elderly men without a history of Parkinson's disease or dementia with Lewy bodies have Lewy pathology in the olfactory bulb**

*J.E. Duda, J.V. Nooragian, H. Petrovitch, L.R. White, G.W. Ross (Philadelphia, Pennsylvania)*

**Objective:** To determine the prevalence of Lewy pathology in the olfactory bulb of elderly decedent Japanese-American males with and without Lewy body disorders.

**Background:** The significance of Lewy pathology that occurs in the brain of individuals with no clinical Parkinsonism or dementia is still unclear. Recent observations concerning the progression of Lewy pathology suggest that deposition may occur in the olfactory system before typical neurodegenerative disease symptoms occur. Olfactory dysfunction has been documented in 62% of 80-97 year-old individuals and Lewy pathology in the olfactory system is a possible contributing factor.

**Methods:** Olfactory bulbs were obtained from 187 elderly decedent male participants in the Honolulu-Asia Aging Study including 21 with Parkinson's disease (PD), 8 with dementia with Lewy bodies (DLB) and 158 with neither disorder. Lewy pathology was identified with sensitive alpha-synuclein immunostaining of one olfactory bulb from each case. Distribution of Lewy pathology was characterized in all layers of the olfactory bulb and tract that could be distinguished in each case.

**Results:** All PD and DLB cases had Lewy pathology in the olfactory bulb. Lewy pathology was identified in 51 (32%) olfactory bulbs from the 158 cases with no clinical history of PD or DLB, so that they were defined as having incidental Lewy pathology (ILP). The likelihood of having moderate to severe pathology density was highest in patients with DLB and lowest in patients with ILP. Analysis of the distribution of Lewy pathology in cases with lower burdens of pathology revealed a fairly consistent pattern with the internal and external plexiform layers and the olfactory tract most often showing pathology, followed by the intrabulbar anterior olfactory nucleus and granular cell layer and least occurrence in the mitral cell layer and glomerular cell layer.

**Conclusions:** Incidental Lewy pathology in the elderly is more common than previously recognized. Nearly one-third of elderly Japanese-American males without a history of PD or DLB have Lewy pathology in the olfactory bulb. It is plausible that a proportion of olfactory impairment in the elderly is caused by Lewy pathology in the olfactory system.

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**Xeomin® is stable without refrigeration and is not affected by short-term temperature stress**

*S. Grein, G.J. Mander, H.V. Taylor (Frankfurt am Main, Germany)*

**Objective:** Xeomin® is a botulinum neurotoxin type A preparation, which, in contrast to other commercially available BTX-A preparations, only contains the pure 150 kD neurotoxin without complexing proteins. Xeomin® has not yet been approved for marketing in the USA. The stability of Xeomin® at elevated temperatures was assessed in comprehensive long-term stability studies as well as in short-term temperature stress studies.

**Background:** Native botulinum toxin type A (BTX-A) is a high molecular weight complex of approximately 900 kD, which is composed of the biologically active 150 kD neurotoxin and several hemagglutinins and other irrelevant proteins. Although these complexing proteins do not have any therapeutic effect, it has been speculated that they might be required to achieve long-term stability of botulinum neurotoxin type A preparations.

**Methods:** In long-term (real-time) and accelerated stability studies according to ICH guidelines, samples of Xeomin® were stored at temperatures ranging from 4°C to 40°C. In the short-term temperature stress study, samples of Xeomin® were stored between 45°C

and 80°C for up to 6 months. All samples were tested with fully validated or standardized pharmacopoeia analytical methods, which are also used for release testing of Xeomin®.

**Results:** Long-term (real-time) and accelerated stability studies have shown no detrimental effects on the quality of Xeomin® at ambient as well as elevated temperatures, and have accounted for its shelf-life of three years without the need for refrigeration. Moreover, the results of the short-term temperature stress study clearly demonstrate that Xeomin® is not negatively affected by storage at temperatures between 40°C and 60°C for up to one month.

**Conclusions:** Xeomin®, a preparation of Botulinum neurotoxin type A free of complexing proteins, can be safely stored without refrigeration and is not affected by short-term temperature stress. Consequently, the complexing proteins in pharmaceutical preparations of Botulinum neurotoxin A are neither required for stability nor for the therapeutic effect.

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**Effect of different patterns of GPi DBS on bradykinesia in the non-human primate model of Parkinson's disease**

*J. Zhang, W. Xu, K. Baker, J. Minnich, E. Bynum, J.L. Vitek (Cleveland, Ohio)*

**Objective:** To evaluate the effects of different patterns and frequencies of globus pallidus internus (GPi) stimulation on bradykinesia in primates made parkinsonian with the neurotoxin MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine).

**Background:** Deep brain stimulation (DBS) is an effective therapy for the motor symptoms associated with Parkinson's disease (PD). Present devices deliver a continuous train of stimulus pulses and little is known about the effect of varying stimulation patterns on parkinsonian motor signs.

**Methods:** A rhesus monkey was made parkinsonian by intracarotid injection of MPTP and a DBS lead was implanted. Therapeutic stimulation parameters were determined by behavioral testing. Bradykinesia was assessed by measuring the time required to retrieve raisins from three, 2 cm diameter, 0.3 cm deep wells (redesigned Kluver-board). A programmable pulse generator (Irel II, Medtronic Inc.) and an external stimulator (Grass Technologies, Model S88) were used to deliver stimulation. For the latter, stimuli were delivered via a stimulus isolation unit (Model PSIU6) and controlled using Lab-View software.

**Results:** 1) Continuous DBS at 135 and 80Hz significantly improved bradykinesia, with retrieval times improving from 6.35 sec. to 4.35 sec. ( $p < 0.01$ ) and 4.43 sec. ( $p < 0.01$ ), respectively. 2) Regular and irregular bursting patterns of 80Hz (20 Hz bursts of 4 pulses each) improved retrieval time to 3.99 sec ( $p < 0.01$ ) and 4.42 ( $p < 0.01$ ), respectively. The regular bursting pattern improved bradykinesia to a greater extent than the 135Hz continuous stimulation pattern while cutting energy output by 40%. 3) An irregular 80Hz continuous stimulation pattern was not effective, with retrieval time of 6.22 sec. ( $p > 0.05$  compared to no stimulation).

**Conclusions:** Although therapeutic stimulation frequencies used for PD are typically  $\geq 130$  Hz delivered in a continuous pattern, lower frequency stimulation delivered in a regular bursting pattern may be equally or more effective and require lower energy prolonging battery life. Improvement of bradykinesia with bursting patterns of stimulation brings into question the role of bursting activity in the pathogenesis of PD motor signs.

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**Supranormal gait in mice following brief exposure to isoflurane**

*B.R. Theriault, I. Amende, C. Gomez, T.G. Hannon (Boston, Massachusetts)*

**Objective:** To determine how gait is affected in mice recovering from isoflurane anesthesia.

maintenance of mitochondrial membrane potential (MMP) and energy homeostasis. Recent findings suggest that UCP5 has neuroprotective effects but the underlying mechanisms are unclear.

**Methods:** A stably UCP5-overexpressing SH-SY5Y cell line was established by transfection with pcDNA3.1 and selected by G418. The effects of UCP5 overexpression were explored by measuring ATP levels (with and without the addition of ADP), cell survival, MMP and reactive oxygen species (ROS) in cells exposed to 1 mM MPP<sup>+</sup> or 200  $\mu$ M dopamine, and compared with untreated cells in vector controls and UCP5-overexpressing cells. Cell survival rates were measured using <sup>3</sup>H-thymidine uptake. Changes in MMP and ROS levels were measured by flow cytometry using JC-1 and dihydroethidium (DHE) respectively. Changes in UCP5 mRNA and protein levels in vector controls and UCP5-overexpressing cells exposed to MPP<sup>+</sup> or dopamine (with and without addition of 5  $\mu$ g/ml actinomycin) were assessed using quantitative RT-PCR and Western analysis respectively.

**Results:** ATP levels and MMP in UCP5-overexpressing cells were significantly reduced compared to vector control under normal and ADP-treated conditions, indicating UCP5 has an uncoupling effect. Cells overexpressing UCP5 also showed increase cell survival rate, preserved MMP and ATP levels, and reduced ROS level after MPP<sup>+</sup> and dopamine-induced cytotoxicity compared to their vector controls. Upregulation in UCP5 mRNA levels in vector controls and UCP5-overexpressing cells were observed after MPP<sup>+</sup> and dopamine exposure, and was abolished after addition of actinomycin, indicating that the upregulation of UCP5 expression in response to MPP<sup>+</sup> and dopamine cytotoxicity was due an increase in transcriptional activity.

**Conclusions:** UCP5 is an uncoupler, and is neuroprotective in MPP<sup>+</sup> and dopamine-induced cytotoxicity by preserving ATP levels and MMP, and reducing ROS levels. Upregulation of UCP5 expression may be an important protective cellular response to toxic insults.

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#### Association of olfactory dysfunction in the elderly with Lewy pathology in the olfactory bulb

*W. Ross, J.E. Duda, R.D. Abbott, H. Petrovitch, J.V. Noorigian, C.M. Tanner, K. Masaki, A. McMurtry, J. Ueyhara-Lock, L.R. White (Honolulu, Hawaii)*

**Objective:** To determine the association of olfactory dysfunction during late life with Lewy pathology in the olfactory bulb postmortem.

**Background:** Olfactory dysfunction is common in Parkinson's disease (PD) and may occur prior to the cardinal motor features. Studies indicate that Lewy pathology occurs in the olfactory bulb of PD patients and that the olfactory bulb is one of the first structures to be involved as the underlying process develops. However, it is unknown whether Lewy pathology in the olfactory bulb is responsible for olfactory dysfunction in PD.

**Methods:** Olfaction was assessed using the 12 item Brief Smell Identification Test (BSIT) administered from 1991 to 1996 to elderly men in the Honolulu-Asia Aging Study. Among those who died and underwent autopsy, one olfactory bulb was examined from each brain using sensitive alpha-synuclein immunostaining to identify Lewy pathology, including Lewy bodies and Lewy neurites. Percent of bulbs with Lewy pathology was determined across tertiles of odor identification.

**Results:** There were 103 autopsied men without prevalent dementia or nasal congestion at the time of olfaction testing. Lewy pathology was identified in 33 bulbs (32%) including 9 diagnosed with PD or dementia with Lewy bodies (DLB) prior to death. Average time from testing to death was 3.6 years (range 1 month to 8.4 years) and mean age at death was 85 years (range 75 to 99). The percent of bulbs with Lewy pathology in the highest BSIT score tertile (8-12) was 15.4 compared to 43.5 for the middle tertile (score 6-7) and 41.5 for the lowest tertile (score 0-5). The percent of decedents with Lewy pathology in the olfactory bulb decreased significantly with increasing BSIT score without ( $p=0.04$ ) and with ( $p=0.015$ ) adjustment for age at olfaction testing, time from testing to death, midlife smoking,

and coffee intake. Results were similar after removing 9 cases with PD or dementia with Lewy bodies.

**Conclusions:** Olfactory dysfunction is associated with Lewy pathology in the olfactory bulb. The process responsible for alpha-synuclein deposition in the olfactory structures may cause the olfactory deficits that occur in PD as well as in some non-diseased elderly.

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#### The potential of minocycline for neuroprotection in nigra of zitter mutant rat

*A. Nakamura, T. Kadowaki, K. Hashimoto, S.-i. Sakakibara, K. Nakadate, K. Hirata, S. Ueda (Mibu, Tochigi, Japan)*

**Objective:** To evaluate the efficacy of minocycline in a tyrosine hydroxylase (TH) cell of substantia nigra of zitter mutant rat.

**Background:** The homozygous zitter rat (zi/zi) is an autosomal recessive mutant derived from the Sprague-Dawley (SD) rat, and is characterized by curled body hair, bent whiskers, fine tremor and flaccid paresis. The mutant rat has abnormal metabolism of oxygen species. In this mutant rat, there is also a loss of dopaminergic neurons with age and there is a corresponding loss of dopamine (DA) fibers and presence of abnormal DA fibers similar to Parkinson's disease. Previous studies have demonstrated that minocycline has been shown to display neuroprotective properties in various models of neurodegenerative diseases. Minocycline has been shown to block microglial activation of 6-hydroxydopamine and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned parkinsonism animal models and protect against nigrostriatal dopaminergic neurodegeneration. However, the efficacy of minocycline in the mutant rat is unknown.

**Methods:** One-, 2-, 4- and 6-month-old homozygote (zi/zi) zitter rats and age-matched SD rats were used. We gave minocycline to zitter rat for six months. Animals were perfused. Then, brain was quickly removed and homogenized. Free-floating frozen sections were stained for TH. We compared TH cells of nigra in zitter rat with minocycline administrated zitter rat, then we reviewed it.

**Results:** We showed that there were not decrease TH cells of nigra in minocycline administrated zitter rats in comparison with zitter rats.

**Conclusions:** The present data may thus suggest that minocycline has been shown to display neuroprotective properties in these administrated rat. These results suggest that minocycline may slow the dopaminergic cell loss in zitter mutant rat. Thus, the zitter rat may represent a good model for studying the dopaminergic cell death.

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#### Individual dopaminergic neurons show raised iron levels in Parkinson's disease

*A.E. Oakley, J.F. Collingwood, J. Dobson, G. Love, H.R. Perrott, J.A. Edwardson, M. Elstner, C.M. Morris (Stoke on Trent, United Kingdom)*

**Objective:** Evidence suggests that abnormal iron metabolism is associated with Parkinson's disease (PD), with raised iron levels found in pathologically affected areas. It is unknown if this elevated iron is actually associated with neurons or reactive glia. We addressed this issue by determining if raised iron was present in single dopaminergic neurons.

**Background:** Evidence indicates that genetic factors play a considerable role in the development of PD. Mutations in  $\alpha$ -synuclein or proteasomal pathway proteins lead to rare familial forms of PD, but the pathways to sporadic PD are unclear, and it is not evident why such dysmetabolism should lead to preferential substantia nigra (SN) zona compacta (SNzc) neuron death. One explanation for this selective vulnerability is iron-mediated neurotoxicity. The SN is iron-rich, and iron is extensively used by neurons for mitochondrial oxidative metabolism and in the synthesis of dopamine. Elevated iron has been indicated in PD SN using MRI, and bulk analysis of postmortem PD SN reveals raised nonheme iron, though this is associated almost exclusively with glia and not neurons. If iron plays a primary role in

Fos-ir neurons decreased to below control levels, likely reflecting a state of excessive depolarization/inactivation. This inverted U-shaped profile was mirrored by a VP output structure, the medial STN, which showed a 289% increase in Fos-ir neurons with intra-VP injections of 0.45  $\mu\text{g}$  NMDA. The response was halved following intra-VP injections of 0.9  $\mu\text{g}$  NMDA. Of the 12 other brain regions measured, three showed VP NMDA-induced enhancements in Fos-ir: the frontal cortex, entopeduncular nucleus and substantia nigra pars reticulata, all regions associated with the basal ganglia. This profile was altered in the PD-like rat, where the Fos-ir response to intra-VP NMDA unaffected in the frontal cortex, entopeduncular nucleus and substantia nigra pars reticulata, and significantly reduced by 62% in the pedunculopontine nucleus, 33% in the basolateral amygdala and 42% in the mSTN.

Conclusions: These findings indicate that the influence of the VP on the basal ganglia and the limbic system is altered in PD and these alterations may underlie mood and mnemonic disorders associated with the disease.

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#### Lewy pathology in the olfactory bulb is associated with decreased neuron density in the substantia nigra

H. Petrovitch, J.E. Duda, R.D. Abbott, J. Nelson, J.V. Noorigian, C.M. Tanner, K. Masaki, L.R. White, J. Ueyhara-Lock, W. Ross (Honolulu, Hawaii)

Objective: To examine the association of Lewy pathology in the olfactory bulb with substantia nigra (SN) neuron density.

Background: Evidence suggests that the process leading to Lewy pathology in the brain occurs in the olfactory bulb before the substantia nigra when the classic motor features of Parkinson's disease (PD) become evident. Lewy pathology in the olfactory bulb, however, has never been shown to be related to neuron density in the SN.

Methods: SN neuron counts were performed on single transverse caudal midbrain sections from deceased Honolulu-Asia Aging Study participants. Area of the SN was determined and density reported as neurons/ $\text{mm}^2$ . Brains were also examined for Lewy bodies (LB) in the SN and locus ceruleus (LC) with hematoxylin and eosin stain. Olfactory bulbs were examined using sensitive alpha-synuclein immunostaining to identify LBs and Lewy neurites.

Results: Mean age at death was 85 years (range 73-99). The mean substantia nigra neuron density was highest among 124 brains with no Lewy pathology (18.9/ $\text{mm}^2$ ) compared to 30 individuals without PD who had Lewy pathology restricted to the olfactory bulb (15.6/ $\text{mm}^2$ ,  $p=.02$ ) and 40 individuals with Lewy pathology in the LC or SN (14.8/ $\text{mm}^2$ ,  $p=.001$ ). Density was lowest in 20 PD cases (7.7/ $\text{mm}^2$ ). There were 154 brains that had either no Lewy pathology ( $N=124$ ) or Lewy pathology restricted to the olfactory bulb ( $N=30$ ). In this group percent of brains with olfactory bulb Lewy pathology was 33.3% in the lowest quartile of SN neuron density, 18% in the 2<sup>nd</sup> quartile, and 13.2% in the 3<sup>rd</sup> and top quartiles. The percent of decedents with Lewy pathology in the olfactory bulb decreased significantly as SN neuron density increased with and without adjustment for age at death, midlife pack-years of cigarette smoking, and midlife intake of coffee ( $p=0.025$ ).

Conclusions: Findings suggest that Lewy pathology in the olfactory bulb is associated with lower neuron density in the SN in individuals who do not have Parkinson's disease. In some instances, there is already significant neuronal loss in the SN at the earliest stages of alpha-synuclein deposition in the brain.

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#### Directional control of foot force in Parkinson's disease

K.G. Gruben, R.S. Giachetti, J.A. Lazarus (Madison, Wisconsin)

Objective: To characterize the preferred coordination of force production with the leg in a human with Parkinson's disease (PD).

Background: Non-disabled humans that are seated demonstrate a preference to maintain a specific foot force direction while increasing

magnitude above resting levels at a given posture. The preferred force direction varies with limb posture so as to be directed near the center of mass (CM), a characteristic important for upright postural stability, but irrelevant in a seated task. We hypothesize that humans with postural instability and adequate leg strength have deficits in the control of foot force direction. While these deficits are masked by compensatory behaviors required in upright tasks, they may be detected during a seated task. We have shown that stroke causes a systematic disruption in force directional control and aimed to determine if PD may also alter this control, contributing to the postural instability observed in this population.

Methods: A single participant with Hoehn & Yahr Stage I PD (left side affected) performed pushing efforts to a force target of 200N against pedals, free to rotate about pivots placed at one of five points in the anterior-posterior direction. The foot was placed at one of three positions at each pivot: pivot under heel, mid-foot and first metatarsal head for a total of 15 postural conditions. The subject sat with their torso reclined 35 degrees from vertical, with hip angle at 120 degrees, ankle angle at 90 deg and the limb axis at 80% of its full length. Foot force was measured with two AMTI force plates operating at 200 Hz. The location of the CM was measured empirically.

Results: The added forces were highly linear as has been found in control subjects and those with stroke. The force directions varied with limb posture so as to direct the force approximately at the CM. However, the force direction for the most extreme limb postures in the left leg did not follow the same pattern as observed in the right leg and in non-disabled humans. The affected side also showed much less variability in preferred force direction from trial to trial in comparison to the other side.

Conclusions: The abnormal coordination of leg muscles observed in PD may contribute to postural difficulties, and may represent a potential target for therapy aimed at improving postural stability.

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#### Characterization of LRRK2 kinase activity

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Objective: To compare the effects of wild type and the G2019S mutation on *in vitro* kinase activity of full-length and various truncated forms of LRRK2.

Background: Leucine-rich repeat kinase 2 (LRRK2) belongs to a Ras/GTPase superfamily of multi-domain proteins containing an N-terminal leucine rich repeat domain, a Roc (Ras of complex) domain, a COR (C-terminal of Roc) domain, a mixed lineage kinase domain and a C-terminal WD40 motif. The G2019S substitution in the activation loop of LRRK2 kinase domain is the most prevalent mutation and genetic determinant of autosomal dominant PD identified to date.

Methods: Kinase activity of wild type and mutant (G2019S and kinase dead) LRRK2 was assessed using myelin basic protein and LRRKtide as the substrate or by autokinase activity analysis. LRRK2 kinase activity was compared using PAGE, filter binding and Transcreener assay methods.

Results: Overall the data indicate the following rank order for the kinase activity among the various constructs: [1326-2527]>[1462-2167]>[1849-2527]>[1314-2154]>[1849-2154]>full-length protein. Comparison of full-length or [1326-2527] wild-type LRRK2 with the corresponding G2019S mutant variants expressed and purified from either Sf9 cells or HEK293 cells demonstrated approximately 1.7-3.0 fold augmentation in kinase activity in the mutant protein. This increase in kinase activity was observed in both autophosphorylation and myelin basic protein phosphorylation assays.

Conclusions: The data provide evidence that whereas minimum catalytic activity is retained in the kinase domain fragment the maximum catalytic activity requires an intact Roc, COR and kinase domain as well as a WD40 motif without the N-terminal containing the LRR. Further, our data confirm that a gain of G2019S mediated kinase function is only detected with full-length protein or [1326-2527]

## Abstract Preview - Step 3/4

- print version -

Category: Neuropathology

Title: THE ASSOCIATION OF PRECLINICAL INDICATORS OF PARKINSON'S DISEASE WITH EARLY STAGES OF SYNUCLEIN DEPOSITION

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Text: **Background:** Constipation, excessive daytime sleepiness and olfactory impairment may occur years before classic motor features of Parkinson's disease (PD). Pathological observations indicate that the earliest sites of alpha-synuclein deposition are the dorsal motor nucleus of the vagus nerve and the olfactory bulb, followed by other brainstem regions including the locus ceruleus (LC), the raphe nuclei and the magnocellular portions of the reticular formation, all of which might produce symptoms prior to onset of the cardinal motor signs of PD that appear when Lewy pathology reaches the SN.

**Objective:** Determine the association of preclinical indicators of PD with early stages of synuclein deposition in the brain

**Methods:** During repeat Honolulu-Asia Aging Study examinations, elderly Japanese-American men were asked about constipation and sleep problems. Grip strength and olfactory ability were measured. Autopsied decedents who died within 5 years of their last evaluation were included in this analysis. Alpha-synuclein immunostaining was used to identify Lewy pathology in olfactory bulbs. H&E staining was used to identify Lewy bodies in the LC and SN.

**Results:** There were 60 decedents who had no synuclein pathology (stage 0), 10 with synuclein pathology restricted to the olfactory bulb (early stage), 26 with involvement of the LC or SN but without clinical PD during life (mid-stage), and 30 with clinical PD during life (late stage). Those in early stage had significantly fewer bowel movements per day (1.6) than those in stage 0 (2.3) ( $p < 0.05$ ). Excessive daytime sleepiness and poor olfaction were more common in mid-stage than in earlier stages. Olfaction and grip strength decreased significantly across the stages from 0 to late stage ( $p < 0.04$ ).

**Conclusions:** Bowel movement frequency, grip strength, olfaction, and likelihood of excessive daytime sleepiness appear to be affected early in the course of synuclein deposition prior to the classical motor features of PD.

Conference: 6th International Congress on Mental Dysfunctions & Other Non-Motor features in PARKINSON'S DISEASE · Abstract:

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## Abstract Preview - Step 3/4

- print version -

Category: Mental manifestations of Parkinson's Disease

**Title:** EFFECTS OF LEWY BODIES, ALZHEIMER LESIONS, AND VASCULAR LESIONS ON COGNITION IN THE POPULATION BASED HONOLULU-ASIA AGING STUDY

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**Text:** **Background:** The effect of Lewy bodies on cognitive function in a community based population is unclear.

**Aims:** to examine the relative effects of Lewy bodies (LB), cerebrovascular lesions, and Alzheimer lesions on cognitive function among deceased Honolulu-Asia Aging Study (HAAS) participants without regard to clinical diagnosis.

**Methods:** There were 365 HAAS decedents who had cognitive screening within three years of death using the cognitive abilities screening instrument (CASI). Scores range from the lowest 0 to 100. A standardized protocol was used to identify brainstem LB and quantify LB in the limbic and cortical regions to determine LB score; and to quantify cerebral infarcts, microvascular lesions, neocortical neurofibrillary tangles (NFT) and neuritic plaques (NP), and atrophy. A general linear regression model adjusting for age at CASI time and education was used to determine the independent effects on CASI score of the neuropathologic features.

**Results:** Atrophy ( $P < 0.001$ ), NFT ( $P < 0.001$ ), LB score ( $P < 0.001$ ), microvascular lesions ( $P = 0.007$ ), and lacunes ( $P < 0.04$ ) were independently and inversely associated with CASI score. NP and large infarcts did not contribute independently to low CASI when NFT and microvascular lesions were considered. To better understand additive effects of the lesions, four groups were formed. In a subset with no AD or vascular pathology, progression of LB score from brainstem to cortical had no apparent effect on CASI score ( $P = 0.62$ ). In contrast, presence of limbic and cortical predominant LB was associated with lower CASI score compared to no LB or brainstem predominant LB in subsets with vascular lesions ( $P = 0.05$ ), NFT ( $P = 0.01$ ) or both ( $P = 0.003$ ).

**Conclusions:** Lewy body score is a significant independent predictor of cognitive function in the HAAS. While small numbers make interpretation difficult, it appears that Lewy bodies have the most apparent effect on cognition when AD or vascular lesions are also present.

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