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14. ABSTRACT
Results from the Honolulu-Asia Aging Study (HAAS) supported by this project showed Parkinson's disease (PD) risk increased with milk consumption, olfactory dysfunction, excessive daytime sleepiness, and low LDL cholesterol. Olfactory dysfunction and constipation were found to be associated with incidental Lewy bodies. A polymorphism of the MDR1 transporter gene was associated with PD. The supplement aim was to measure organochlorine levels in all brains from the HAAS archive and to examine the association of brain organochlorine levels with clinical and pathological endpoints obtained through prior and ongoing research. Frozen occipital blocks from 421 brains were sent to Dr. E.D. Pellizzari of Research Triangle Institute for organochlorine level assays. These are complete. Preliminary analyses demonstrate that measurable A-chlordane, T-nonachlor, or Methoxychlor levels were found significantly more frequently in brains with Lewy bodies (including PD cases) than in those without Lewy bodies ($p<0.04$). A meeting is planned for February 2008 to finalize plans for presentation and publication of these data.

15. SUBJECT TERMS
Parkinson's Disease; Organochlorine

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INTRODUCTION

This is a report covering April 1, 2005 to March 31, 2007 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 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 Parkinson's disease (1) and parkinsonism (2) using existing data from the longitudinal HHP/HAAS. The work seeks to confirm previous reports [1-6] of an association of pesticide exposure with Parkinson's disease 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 Parkinson's disease 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.

The neuropathological component, (#3) currently has access to over 737 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 552 brains with completed microscopic evaluations, there are 105 brains that have Lewy bodies in either the substantia nigra or the locus ceruleus from participants that had no history of Parkinson's disease during life (incidental Lewy bodies). Markers of brain injury such as glial fibrillary acidic protein and levels of organochlorine compounds are being evaluated using frozen brain tissue.

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. [7-14] Results from this study were negative. We have recently begun a collaboration with the Parkinson's institute to evaluate transporter gene polymorphisms.

The Supplement to this research awarded in 2005 was to determine levels of organochlorine compounds in the brains of all available autopsy cases in our brain bank.

BODY
(Numbers refer to items in the Statement of Work)

1. Evaluation of Epidemiological Risk Factors and Preclinical Indicators as Predictors of Parkinson's Disease

1. Milk and Calcium: A manuscript was published in *Neurology* in 2005 [15] 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. See Appendix A.
2. Olfactory Dysfunction: A manuscript is in press in *Annals of Neurology* shows 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. See Appendix B.
3. 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).[16] See Appendix A.
4. 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 for the incident PD group. [17]
5. Low LDL Cholesterol: An abstract was published in *Movement Disorders* in 2006[18] 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 Parkinson's disease for men at the 80th versus the 20th percentile of LDL-C (135 versus 85 mg/dL) was 0.4 (95% confidence interval: 0.2, 0.9).
6. 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.

2. Evaluation of Epidemiological Risk Factors for Parkinsonism and Movement Abnormalities

1. 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.[19] See Appendix A.

3. Evaluation of Epidemiological Risk Factors and Clinical Features as Predictors of Lewy bodies

1. 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 the highest tertile of oder identification to 11.9% in the mid tertile, and to 17.4 in the lowest tertile (p=0.019).[20] See Appendix A.
2. Bowel movement frequency and incidental Lewy bodies: A manuscript was published in *Movement Disorders* in 2007[21] 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. See Appendix A.
3. Parkinsonian signs and incidental Lewy bodies: In an abstract published in the *Journal of the Neurological Sciences* in 2005. Results indicated that the five signs most closely associated with incidental Lewy bodies were slow hand movements, slow rapid alternating movements of hands, rigidity, body bradykinesia, and action or postural tremor of hands. In those who had 1 or fewer of these signs there were no cases of ILB (0/11). As the number of parkinsonian signs increased, the percent of men with incidental Lewy bodies rose significantly to 31.6% (6/19) in men with 5 signs (p=0.004). Associations were unaltered after adjustments for age at death, past use of coffee and cigarettes, and cognitive function (p=0.006).

4. Genetics Study

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). Ongoing analysis and preparation of manuscripts is planned for next year.

5. Organochlorine Supplement

Preliminary findings indicate that A-chlordane, T-nonachlor, and Methoxychlor found in brain tissue at death may be associated with Parkinson's disease or Lewy bodies. In February 2008, Drs. G. Webster Ross, Helen Petrovitch, and Rob Abbott plan to host a meeting in Honolulu for Dr. Edo Pelizarri (who performed the brain organochlorine evaluations). Drs. Diane Miller, and Jim O'Callaghan, colleagues from the National Institute of Occupational Safety and Health, and experts in this area will also attend this two day meeting. Final plans to present and publish these data will be completed during this meeting. See table below.

Association of Specific Organochlorine Residues in Post-Mortem Brains and Lewy Bodies (LB) (n=326: 263 normal, 41 LB, 22 PD)

		Measurement		p-value***
		Absent/ low* (N)	Present** (N)	Absent/ low* vs. Present**
A-chlordane	No LB	200	63	0.042
	PD+LB	40	23	
T-nonachlor	No LB	67	196	0.003
	PD+LB	5	58	
Methoxychlor	No LB	256	3	0.002
	PD+LB	56	5	
Oxychlordane.	No LB	247	16	0.937
	PD+LB	59	4	
Heptachlor	No LB	200	63	0.576
	PD+LB	50	13	

*Low = below limit of calibration
** Present = above limit of calibration
* **p-values for Chi-square tests comparing the group with LB to group without LB.

KEY RESEARCH ACCOMPLISHMENTS

1. Milk drinkers have higher risk of developing PD.
2. Olfactory Dysfunction is a predictor of future PD
3. Excessive Daytime Sleepiness is a predictor of PD
4. Depressive symptoms were not at statistically significant predictor of PD
5. Low LDL Cholesterol is a predictor of PD among men aged 71-75 at baseline.
6. Higher exposure to any metal and specifically mercury was associated with abnormal facial expression.
7. Olfactory dysfunction was associated with and incidental Lewy bodies.
8. Infrequent bowel movement frequency was associated with incidental Lewy bodies.
9. Parkinsonian signs were associated with incidental Lewy bodies.
10. A polymorphism of the MDR1 transporter gene with Parkinson's disease.
11. A-chlordane, T-nonachlor, and Methoxychlor found in brain tissue at death may be associated with Parkinson's disease or Lewy bodies.

REPORTABLE OUTCOMES

Published 2005-2007

1. Park M, Ross GW, Petrovitch H, White LR, Masaki KH, Nelson JS, Tanner CM, Curb JD, Blanchette PL, Abbott RD. Consumption of milk and calcium in midlife and the future risk of Parkinson disease. *Neurology* 2005;64(6):1047-51.

2. Abbott RD, Ross GW, White LR, Tanner CM, Masaki KH, Nelson J, Curb JD, Petrovitch H. Excessive daytime sleepiness and subsequent development of Parkinson disease. *Neurology*. 2005 Nov 8;65(9):1442-6

3. Charles LE, Burchfiel CM, Fekedulegn D, Kashon ML, Ross GW, Petrovitch H, Sanderson WT. Occupational Exposures and Movement Abnormalities among Japanese-American Men: The Honolulu-Asia Aging Study. *Neuroepidemiology.* 2006 Jan 26;26(3):130-139

4. Ross GW. Association of Olfactory Dysfunction With Incidental Lewy Bodies. *Movement Disorders* 2006;21:2062-2067.

5. Abbott RD. Bowel Movement Frequency in Late-Life and Incidental Lewy Bodies. *Movement Disorder* 2007;22;1581-1586.

In Press

1. Ross GW. Association of olfactory dysfunction with risk of future Parkinson's disease. *Annals of Neurology*. In Press

Published Abstracts

1. Abbott RD, Ross GW, White LR, Tanner CM, Nelson JS, Petrovitch H. Excessive daytime sleepiness and the future risk of Parkinson's disease. *Movement Disorders* 2005;20 (Suppl 10) S101

2. Ross W, Petrovitch H, Abbott RD, Tanner CM, Popper J, Masaki K, White LR. Association of olfactory dysfunction with risk of future Parkinson's disease*. Movement Disorders* 2005; 20 (Suppl 10) S129

3. Ross, GW, Abbott RD, Petrovitch H, Tanner C, Hardman J, White L. Parkinsonian signs and incidental Lewy bodies*. Journal of the Neurological Sciences*, 2005; Suppl to Vol. 238; S48.

4. Ross GW, Abbott RD, Petrovitch H, Davis D, Tanner CM, White LR. Bowel movement frequency in mid-life and incidental Lewy bodies. *Movement Disorders,* 2006; 21 (suppl 15) S659 [Abstract]

5. Petrovitch H, Fujikami G, Makski KH, Fong K, White LR, Blanchette P, Ross W. Depressive symptoms and Parkinson's disease: The Honolulu-Asia Aging Study. *Movement Disorders* 2006, 21 (15): S646

6. Huang X, Abbott RD, Petrovitch H, Mailman RB, Ross G. Low LDL cholesterol and increased risk of Parkinson's disease: prospective results from Honolulu aging study. *Movement Disorders* 2006, 21 (15): S507

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.

CONCLUSIONS

The work accomplished with our DoD funding has led to numerous opportunities for dissemination of our research findings at scientific meetings, symposia, and workshops over this period (April 1, 2005 to March 31, 2007).

We have previously reported that coffee drinking may be protective against PD.[23] The new finding that milk consumption is associated with an increased risk of PD may indicate that dietary factors or neurotoxins are related to PD risk.

In the past we have shown that increased triceps skinfold thickness, a measure of peripheral adiposity[24], longer QT[25] interval on ECG and constipation[26] during mid-life may portend the onset of the motor syndrome of PD by years. We have now added associations of olfactory dysfunction, excessive daytime sleepiness, and low blood levels of LDL-cholesterol 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.

Additionally, the findings that parkinsonian signs may be found in individuals with incidental Lewy bodies indicates that incidental Lewy bodies may represent preclinical PD. The finding that olfactory dysfunction and constipation (along with increased reaction time reported in the past,[27]) are predictors of incidental Lewy bodies suggests that these characteristics may be useful in the early detection of Parkinson's disease. 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 three organochlorines are detected more frequently in brains of men who also had Lewy bodies at death. The organochlorine exposure in most of these brains took place as long as 30 years ago. This year we will begin to examine the association of these levels with clinical endpoints (Parkinson's disease, parkinsonism, Alzheimer's disease, cognitive impairment) and continue 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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18. Huang X, Abbott RD, Petrovitch H, Mailman RB, Ross G. Low LDL cholesterol and increased risk of Parkinson's disease: prospective results from Honolulu aging study. *Movement Disorders* 2006, 21 (15): S507

19. Charles LE, Burchfiel CM, Fekedulegn D, Kashon ML, Ross GW, Petrovitch H, Sanderson WT. Occupational Exposures and Movement Abnormalities among Japanese-American Men: The Honolulu-Asia Aging Study. *Neuroepidemiology.* 2006 Jan 26;26(3):130-139

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25. White LR, Petrovitch H, Abbott RD, Masaki KH,Yano K, Grandinetti A, Popper JS, Ross GW Prolonged QT interval in midlife is a risk factor for subsequent development of Parkinson's disease. Presented at the 5th International Conference on Progress in Alzheimer's and Parkinson's Disease, Kyoto, Japan, April 2001

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APPENDIX A

Published Manuscripts:

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

Excessive daytime sleepiness and subsequent development of Parkinson disease

Occupational exposures and movement abnormalities among Japanese-American Men: The Honolulu-Asia Aging Study

Association of Olfactory Dysfunction with incidental lewy bodies

Bowel Movement frequency in late-life and incidental lewy bodies

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

M. Park, MD, PhD; G.W. Ross, MD; H. Petrovitch, MD; L.R. White, MD; K.H. Masaki, MD; J.S. Nelson, MD; C.M. Tanner, MD, PhD; J.D. Curb, MD; P.L. Blanchette, MD; and R.D. Abbott, PhD

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.

NEUROLOGY 2005;64:1047–1051

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.[1] 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.[1] 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.[2,3] 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.[4] Dietitians used standardized methods to obtain individual recall of food intake through the use of food models and serving utensils to illustrate portion sizes.[4,5] Collected data were validated against a 7-day diet record in 329 of the 8,006 men in the original cohort.[6] There were

From the Korea University Genomic Institute (Drs. Park and Abbott) and Department of Neurology (Dr. Park), College of Medicine, Korea University, Ansan-Si, Republic of Korea; and Pacific Health Research Institute (Drs. Ross, Petrovitch, White, Masaki, Nelson, Curb, and Abbott), Department of Veterans Affairs (Drs. Ross, Petrovitch, and White), Kuakini Medical Center and Honolulu-Asia Aging Study (Drs. Ross, Petrovitch, White, Masaki, Curb, and Abbott), and Departments of Geriatric Medicine and Medicine (Drs. Ross, Petrovitch, White, Masaki, Curb, and Blanchette), University of Hawaii John A. Burns School of Medicine, Honolulu, Parkinson's Institute (Drs. Ross and Tanner), Sunnyvale, CA, and Division of Biostatistics and Epidemiology (Dr. Abbott), University of Virginia School of Medicine, Charlottesville.

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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.[6] 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.[7] 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 nondietary 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.[8] 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.[9,10] Further description of the data collection methods for the other risk factors has been published elsewhere.[2,3]

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.[11] 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.[12] 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.[12] Further description of the diagnosis of PD is provided elsewhere.[13,14]

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.[15] Age-adjusted risk factors were also derived across the ranges of dietary intake.[15] To test for an effect of milk and calcium intake on the risk of PD, proportional hazards regression models were examined.[16] 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 ± 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 ± 7.5 years (range 54 to 89 years), and the average time to a diagnosis was 18.4 ± 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
Dairy sources				
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*			$p = 0.063$	$p = 0.046$
Nondairy sources				
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† (1.3,4.1)	2.6‡ (1.1,6.4)
Test for trend§	$p = 0.007$	$p = 0.085$

* Adjusted for age, coffee intake, pack-years of smoking, physical activity, tricep skinfold thickness, total kilocalories and fat intake, and years worked on a plantation.
† Excess risk of PD vs men who consumed no milk ($p < 0.01$).
‡ 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

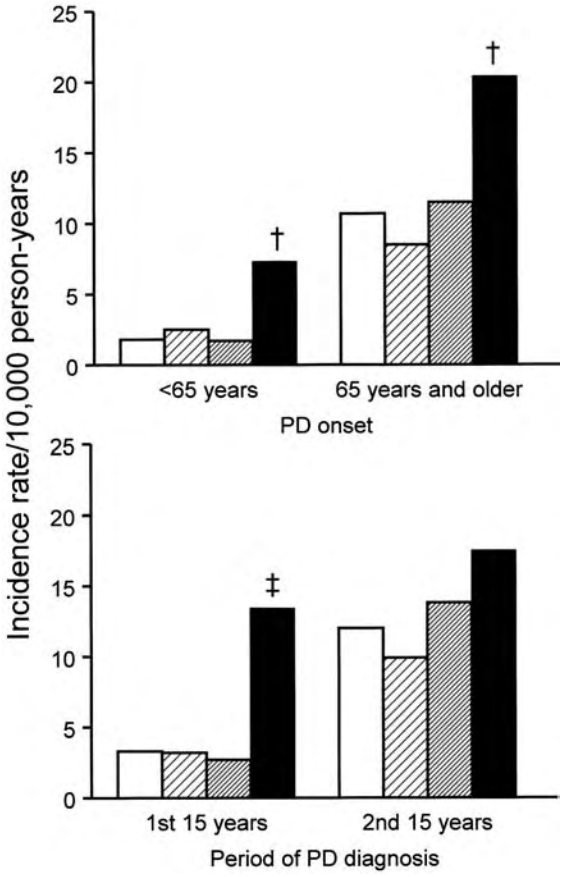

Figure. Age-adjusted incidence of early and late Parkinson disease (PD) onset (<65 and ≥65 years, respectively) and the incidence of PD in the first and second 15 years of follow-up according to milk intake during midlife. Milk intake increases from 0 (open columns), >0 to 8 (wide-hatched columns), >8 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.[1] 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,[1] 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,[1] 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.[1] 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.[1] 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.[17-19] 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,[20-24] and substantia nigra organochlorine levels have been found to be higher in cases of PD than in cases of Alzheimer disease and controls.[25,26] Other contaminants that have been found in milk include tetrahydroisoquinoline (used in the synthesis of pesticides),[27,28] which is known to induce parkinsonism in primates.[29,30]

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.[22-24] 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.[31,32] Whether heptachlor contamination occurred prior to 1981 is uncertain.[23,24]

Whereas the findings from the Honolulu Heart Program are consistent with recent observations of an association between dairy products and PD,[1] 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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HIGH LIGHT
CME

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,[1-8] 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.[9,10] 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.[11] 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.[12] Men who reported being sleepy most of the day were defined as having EDS.[13,14] Similar questionnaires have been used elsewhere.[3,4,15,16] 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 too 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.[17] 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.[18] 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.[19-21]

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.[22] 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.[23] Average sleep features and additional factors were also derived and age-adjusted in those with and without EDS.[23] 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.[24] 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.[25,26] 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 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.

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	p 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 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; $p = 0.004$). 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; $p = 0.021$).

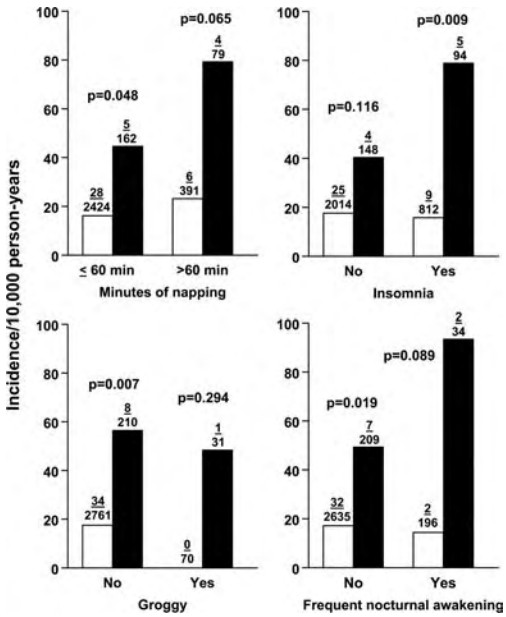

Figure. Incidence of Parkinson disease (PD) in men with and without excessive daytime sleepiness (EDS) within strata of other sleep-related features. Solid bars represent the incidence of PD for men with EDS. Clear bars represent the incidence of PD for men without EDS. p values are for a test of an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

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.[1-8,27] 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.[28] Others report that RBD can predate clinical PD by an average of 10 to 12 years.[29-33] 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.[34] 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[3,4,15,16,35] 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.[1,3,4,15,16,28,35] Others have also suggested that scales used in the diagnosis of PD should include more questions on sleep-related disorders.[1]

We are also not aware of a study that has conducted polysomography 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[29-31] or in comparisons with controls without polysomnographic assessments.[32,33] The general population prevalence of RBD is also uncertain because polysmonography 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.[28] 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.[3,4] In one report, snoring and sleeping time were unrelated to PD.[1] Others observed that nighttime sleep disorders were similar in PD patients with and without EDS.[2]

The prevalence of EDS in the elderly may also be poorly underestimated because excessive somnolence is often unrecognized in subjective reporting.[5] Although the prevalence of EDS in the Honolulu sample (8%) is lower than the 17% prevalence in men enrolled in the Cardiovascular Health Study,[15] variation among study samples can be appreciable, ranging from 1% to 47% in healthy controls or in subjects without PD.[2-5] For those with PD, reported prevalence varies from 15% to 76%.[2-5] 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.[36,37] 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.[38] 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.[1-6,27-33]

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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.

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].

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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 recoded; if a subject received a score ≥ 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[1]										
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

[1] 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. 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 ≤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 exposure 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 ≥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 ≥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 ≤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[a]	Pesticide		Solvent		Metal		Manganese		Mercury		Overall
		n	mean ± SD	n	mean ± SD	n	mean ± SD	n	mean ± SD	n	mean ± SD	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_{trend}		<0.0001		<0.0001		<0.0001		<0.0001		0.0049	
BMI, kg/m^2	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_{trend}		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_{trend}		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_{trend}		<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_{trend}		0.0137		<0.0001		<0.0001		0.0004		0.1430	
Pack-years of smoking[b]	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_{trend}		0.7110		0.2741		0.3665		0.1279		0.1017	
Alcohol consumption[c] 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_{trend}		0.5911		0.8712		0.6624		0.5221		0.0333	

[a] 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.

[b] Pack-years of smoking include only current and former smokers.

[c] 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[a] 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[b]		
		OR	95% CI	OR	95% CI	OR	95% CI	p_{trend}
Pesticides								
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	
Solvents								
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	
Metals								
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[b]		
		OR	95% CI	OR	95% CI	OR	95% CI	p_{trend}
Gait								
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	
Facial expression								
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	
Manganese								
Rapid alternating hand movements								
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	
Posture								
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	
Gait								
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	
Facial expression								
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	
Mercury								
Rapid alternating hand movements								
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	
Posture								
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	
Gait								
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	
Facial expression								
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	

[a] 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.

[b] 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, reported 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-

sessment 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 becoming 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 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.

Key words: olfaction; Lewy bodies; epidemiology; Parkinson's disease

Olfactory dysfunction, whether measured by odor identification, recognition, or threshold, is associated with Parkinson's disease (PD)[1,2] 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.[3] 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.[4] With establishment of the HAAS, research on diseases of aging including PD was initiated at the 1991–1993 examination,[5] and all cases of PD in the cohort were identified.[6,7] Additional cases of PD were identified during follow-up examinations in 1994–1996, 1997–1999, and 2000–2001 and by examination of medical records.[7] 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.[8,9] 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).[5,10] 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.[11] 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.[12]

Standardized gross and microscopic examinations of multiple brain regions are performed routinely. Details have been previously described.[13,14] 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.[15] Crude and age-adjusted prevalence of ILB were also estimated across ranges of odor identification with standard analysis of covariance models.[16] 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.[17] 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[a]	6.3 ± 3.1[b]
Age at olfaction testing (yr)	83.5 ± 6.1 (77–93)[c]	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)[d]	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.
[a]Mean ± standard deviation.
[b]Significant difference from cases ($P = 0.011$).
[c]Range.
[d]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)[a]	18.2[b] (10/55)[c]	17.4[d]	11.0 (1.3–526)[e]
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	$P = 0.006$	$P = 0.019$	

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

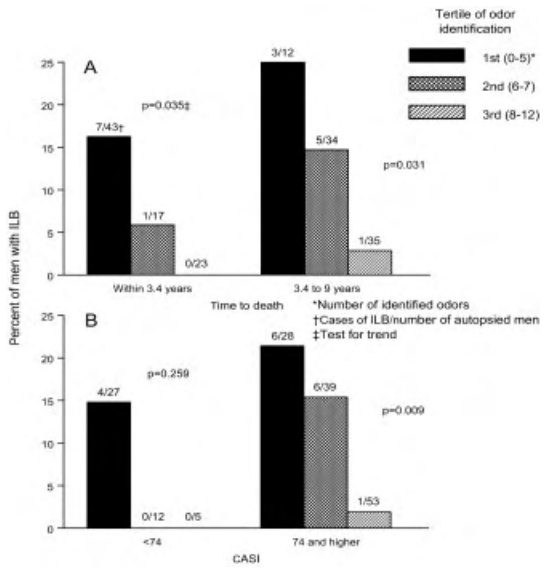

FIG. 1. Percent of autopsied men with ILB within tertiles of odor identification, according to time from olfaction testing to death (**A**) and to CASI score (**B**).

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[18,19] and that 80% of striatal dopamine is lost before symptoms of PD become apparent.[20] 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.[21] 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,[14] validating the earlier work of Fearnley and Lees.[18] 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.[22] Olfactory deficits have been found in untreated patients with early PD.[23,24] 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.[25] Declines in striatal dopamine transporter binding as measured by [^{123}I] beta-CIT single photon emission tomography (SPECT) have been reported in hyposmic relatives of PD patients while normosmic relatives had normal binding.[26] 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 [^{123}I] beta-CIT binding ratios at the baseline examination.[27] 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 ^{123}I-FP-CIT. Together, these studies provide strong evidence that impaired olfaction is a preclinical marker of PD.[28]

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.[29] 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.[29] Furthermore, pathological studies of ILB cases using α-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.[3,30] Impaired olfactory identification has been found to correlate significantly with dopamine transporter binding in the putamen on [^{99m}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.[31]

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.[32] 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.[33] 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.[34] This may explain why levodopa does not reverse olfactory deficits in PD patients.[23,35,36]

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.[21] 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.[3] Use of α-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 α-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.[37] 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.[1,2] It is speculated that when these regions are affected, impairments can occur in colonic transit.[1,2] 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).[3] 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.[4-7] 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.[6-8] 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.[9,10] 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.[11] 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,[12] 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.[13-16] 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.[17,18] 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.[19] 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.[7,20] 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.[21] 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.[22] 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.[23] 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[a]	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.

[a]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)[a]	4.3[b] (1.2, 17.1)[c]
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[d] (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[e]		
<1	23.7	4.5[f] (1.1, 23.4)
1	14.0	2.3 (0.9, 6.9)
>1	6.6	Reference
Test for trend	$P = 0.016$	

[a]Number with Lewy bodies/sample size.
[b]Excess percent with Lewy bodies versus men with >1 bowel movement/day ($P = 0.036$).
[c]95% confidence interval.
[d]Excess percent with Lewy bodies versus men with >1 bowel movement/day ($P = 0.034$).
[e]Adjusted for age, time to death, mid-life pack-years of smoking and coffee intake, physical activity, and the cognitive abilities screening instrument score.
[f]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.[24] Although constipation is a common symptom of PD and can predate its clinical onset by many years,[24,25] 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.[4,6] 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.[5,6] 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.[6,7] 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.[2,3] 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.[1] 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.[3]

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.[26,27] Lewy bodies and Lewy neurites may occur in Meissner's and Auerbach's plexi in the gastrointestinal tract of non-parkinsonian individuals.[2] 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.[27-30] 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.[2]

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.[24] 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% $^{3}/_{12}$) versus men whose bowel movements were more frequent (11.9% $^{27}/_{226}$).

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.[31] 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.[32] 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.[32]

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.[33-37] In the National Health and Nutrition Examination Survey, 64 to 74% recorded daily defecation compared to 60.4% in the current sample.[33] 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.[34] 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.[35]

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.[2,3] 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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APPENDIX B

In Press Manuscript:

Association of olfactory dysfunction with risk of future Parkinson's disease

IN PRESS: ANNALS OF NEUROLOGY

Association of olfactory dysfunction with risk of future Parkinson's disease

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Running Head: Olfaction and PD (16 characters)

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Title characters = 77; word count abstract = 247; word count text = 3395

Abstract

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 (HAAS) cohort members.

Methods: Olfaction was assessed from 1991 to 1996 in 2,267 men in the HAAS aged 71 to 95 years and 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 developed PD (24.6/10,000 person-years). The average age at the time of diagnosis was 82.9 ± 3.8 years (range: 76-93), and the average time to a diagnosis was 4.0 ± 1.9 years (range: 1-8). During the first four 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 to 25.6) compared to the top two quartiles. This relationship was not evident beyond four years of follow-up.

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

Introduction

Olfactory dysfunction is associated with Parkinson's disease (PD), whether measured by odor identification, recognition, or threshold.[1,2] Evidence is accumulating that impaired olfaction may precede the classic 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[3,4] Asymptomatic first degree relatives of patients with PD are more likely than those without a family history to have impaired olfaction.[5] In an important study but one using a selected sample of relatives of PD patients, olfactory deficits were shown to precede PD.[6] Recent neuropathological advances suggest that the olfactory system is among the earliest brain regions involved in PD,[7] and olfactory deficits are associated with the presence of incidental Lewy bodies in the brains of decedents without parkinsonism or dementia during life.[8]

Despite these findings, it is not known whether olfactory 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 of PD in the population-based, longitudinal Honolulu-Asia Aging Study (HAAS).

Methods

Study Sample

From 1965 to 1968, the Honolulu Heart Program began following 8,006 men of Japanese ancestry for development of cardiovascular disease.[9] 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 1991-93 full cohort exam and continuing with follow-up examinations 1994-96, 1997-99, and 1999-2000.[10,11] Procedures for all exams were in accordance with institutional guidelines and approved by an 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.[12,13] 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 B-SIT 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.[11] During the 1994-1996 examination 2705 men were examined and all received the olfactory testing. For those who had testing at both exams, the earliest available odor identification score was used. Overall, there were 2906 men who 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 of future PD. These included age at the time of olfactory assessment, mid-life cigarette smoking and coffee intake, bowel movement frequency, excessive daytime sleepiness, and cognitive function. 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 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)[11,14] 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.

PD case finding and diagnosis

Efforts to identify all PD cases in the cohort began in 1991 and have continued through all subsequent exams. Detailed case finding methods have been previously published.[10,15,16] During each exam 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 neurologic examination that included the Unified Parkinson's Disease Rating Scale.[17] 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. The criteria required that the subject have: (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.[18] Cases of parkinsonism related to progressive supranuclear palsy, multiple system atrophy, cerebrovascular disease, drug induced parkinsonism, post-encephalitic parkinsonism, or post-traumatic parkinsonism were not included among the cases of PD. Up to 8 years of follow-up were available on 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.[19] 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 of time,[6,20] separate analyses were performed for the first and second four years of follow-up. Average values and percents of potential confounders were also derived and age-adjusted across the odor identification quartiles. Since the number of PD cases was small, logistic regression models were examined to assess the association of the odor identification score with the risk of PD based on exact testing methods.[21] 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.[22,23] After adjustment for age and the other study characteristics, odds ratios for PD and 95% confidence intervals were estimated comparing the risk of PD in men in each of the bottom two odor identification quartiles to men in the top two quartiles (reference group). All P-values were based on two-sided tests of significance.

Results

Among the 2906 men who received olfaction testing at baseline, 58 with prevalent PD (i.e. 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 ± 4.1 years (range: 71-95). During the course of follow-up, 35 men developed PD (24.6/10,000 person-years). The average age at the time of diagnosis was 82.9 ± 3.8 years (range: 76-93), and the average time to a diagnosis was 4.0 ± 1.9 years (range: 1-8).

Table 1 displays the study characteristics across quartiles of odor identification. Decreased odor identification was associated with older age, higher pack years of smoking, more mid-life 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 and second four years of follow-up. During the first four 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, mid-life cigarette smoking and coffee drinking, bowel movement frequency, excessive daytime sleepiness, and CASI score, the relative odds of developing PD using the highest two quartiles of odor identification as the reference group were 3.1 (95% confidence

interval (CI), 0.6 to 16.1) for the second quartile, and 5.2 (95% CI, 1.5 to 25.6) for the lowest quartile (P = 0.001 for trend).

For the second four years of follow-up, there was no apparent relationship between olfaction and incident PD (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 four years of follow-up were similar to those diagnosed in the second four years with respect to the clinical charactieristics 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 to 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 due to cognitive impairment or early undiagnosed dementia, we repeated the analysis for the first four 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[24]). This cut-off score corresponds to the 16th percentile of CASI scores and has been used in previous analyses involving the CASI in this cohort.[25] Age-adjusted incidence per 10,000 person-years was 54.6 (8 PD cases/418 at risk) for quartile 1, the lowest odor identification score quartile; 29.8 (5/455) for quartile 2; 9.1 (2/563) for quartile 3; and 4.5 (1/551) for quartile 4 (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 to 26.4) for quartile 2 and 6.1 (95% CI, 1.4 to 40.9) for quartile 1. The corresponding test for trend yielded a P value of 0.001. When a similar analysis was performed for the second four 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 quartile 2, 4.8 years in quartile 3, 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 predate the development of clinical PD in men by at least four years. These results remained significant when restricting the at risk population to those without cognitive impairment.

It is well established that olfactory deficits are very common in PD, occurring at about the same frequency as resting tremor,[2,26,27] 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 prior to diagnosis[4] and olfactory deficits have been found in untreated patients with early PD.[3,28] In one study, up to 90% of PD patients tested had lower odor identification scores than normal matched controls, and olfactory

deficits were unrelated to severity or duration of disease or use of medications.[26] 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 while motor signs continue to worsen through the later stages.[29] Consistent with this idea is a recent imaging study using a group of PD subjects early in their disease that found a significant positive correlation between odor identification and dopamine transporter binding on [^{99m}Tc] TRODAT-1 single photon emission tomography (SPECT) imaging in the putamen, but no correlation between dopamine transporter binding and motor function or symptom duration.[29]

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.[5] Hyposmic, nonparkinsonian relatives of PD patients are reported to have lower striatal dopamine transporter binding as measured by [^{123}I] beta-CIT SPECT imaging compared to normosmic relatives suggesting subclinical striatonigral degeneration in the hyposmic subjects.[30] In a follow-up report from the same prospective study, 4 of 40 hyposmic subjects with decreased [^{123}I] beta-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 subjects developed PD. Among the subjects who underwent a second scan, mean decline in beta-CIT binding was greater in the hyposmic subjects than in those who were normosmic.[6]

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 revealed that the average decline in olfactory identification scores was higher compared to the decline among those that did not develop PD. [20] Taken together, these studies provide strong evidence that impaired olfaction typically occurs prior to the classic motor features of PD.

Findings from the HAAS presented here demonstrate that impaired olfaction was not a strong predictor of PD when follow-up time from olfaction testing to development of PD was beyond four years. While 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 relationship of olfactory deficits to higher risk of future PD begins to weaken beyond a threshold of approximately four 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 above, olfaction was not a sensitive indicator of incident PD when measured seven or more years prior to onset of motor signs.[20] Follow-up was only two 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 develop PD.[6] Therefore, findings from these two studies suggest that olfactory impairment begins between 2 and 7 years prior to PD diagnosis. Lastly, while the exact time between disease onset and appearance of classic 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.[31-33]

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.[1,7] One study of seven PD cases and seven controls found a strong correlation between neuron loss in the anterior olfactory nucleus and duration of disease.[34] Another study of ten cases and controls 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 gender matched controls. Noting the neuroinhibitory role of dopamine in olfactory transmission, it was speculated that higher dopamine function suppresses olfaction.[35]

The work of Braak and colleagues who meticulously examined the brains of deceased persons without neurological disease suggests that the olfactory structures along with the dorsal motor nucleus of the vagus nerve are the earliest brain regions to be affected by Lewy degeneration,[7,36] 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.[8]

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.[37,38] Diminished olfactory neurogenesis in mice is associated with impaired fine olfactory discrimination.[39] 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 persons with PD.[40]

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 as well as Lewy pathology in the corticomedial 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.[41]

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.[42]

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, while the B-SIT 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 relationship 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 relationship 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.[43]

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 of developing PD within 4 years. This relationship seems to weaken beyond that time. Olfactory testing along 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 developing PD who could participate in trials of medications designed to prevent or slow disease progression.[44,45] More expensive but conceivably more specific tests such as transcranial echosonography or dopamine transporter imaging might narrow this at risk population even further.

Table 1. Mean age and age-adjusted average and percent of characteristics by quartile of odor identification score

Study characteristic	Quartile of odor identification score			
	1st (0-5)* (549)†	2nd (6-7) (515)	3rd (8-9) (622)	4th (10-12) (581)
Age in years§	81.2 ± 4.5‡	80.2 ± 4.2	79.2 ± 3.7	78.4 ± 3.3
Mid-life pack-years of smoking§	28.3 ± 28.4	27.0 ± 28.4	25.3 ± 26.4	20.7 ± 23.6
Mid-life coffee intake (oz/day)§	14.6 ± 12.9	13.8 ± 13.3	13.4 ± 12.8	12.6 ± 12.2
Bowel movements/day||	2.1 ± 0.6	2.2 ± 0.5	2.3 ± 0.5	2.3 ± 0.5
Excessive daytime sleepiness (%)¶	8.4	7.6	6.0	5.3
CASI||	80.4 ± 11.5	83.6 ± 8.7	84.9 ± 7.9	86.6 ± 7.0

*Number of odors recognized

†Sample size ‡Average ± standard deviation

§Significant decline with increased olfaction ($p<0.001$)

||Significant increase with increased olfaction ($p<0.001$)

¶Significant decline with increased olfaction ($p=0.029$)

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		Adjusted odds ratio†
	Crude	Age-adjusted	
First 4 years of follow-up			
1st (0-5)*	51.1|| (10/549)‡	54.5||	5.2¶ (1.5, 25.6)§
2nd (6-7)	25.9 (5/515)	26.6	3.1 (0.6, 16.1)
3rd (8-9)	8.4 (2/622)	8.2	reference
4th (10-12)	8.9 (2/581)	8.4	
Overall	22.3 (19/2267)		
Test for trend	p<0.001	p<0.001	p=0.001
Second 4 years of follow-up			
1st (0-5)	16.7 (2/389)	18.0	0.3 (0.0, 2.7)
2nd (6-7)	40.2 (5/409)	42.1	2.2 (0.5, 4.1)
3rd (8-9)	24.5 (4/526)	23.9	reference
4th (10-12)	30.4 (5/522)	28.6	
Overall	28.0 (16/1846)		
Test for trend	p=0.550	p=0.694	p=0.646

*Number of odors identified

†Adjusted for age, mid-life cigarette smoking and coffee drinking, bowel movement frequency, excessive daytime sleepiness, and the Cognitive Abilities Screening Instrument.

‡Cases of Parkinson's disease/sample at risk §95% confidence interval

||Significant excess risk of Parkinson's disease versus the reference (p=0.001).

¶Significant excess risk of Parkinson's disease versus the reference (p=0.007).

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