BMJ open

Hypometabolism in the supplementary and anterior cingulate cortices is related to dysphagia in Parkinson's disease: a cross-sectional and 3-year longitudinal cohort study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-002249
Article Type:	Research
Date Submitted by the Author:	22-Oct-2012
Complete List of Authors:	Kikuchi, Akio; Tohoku University Graduate School of Medicine, Department of Neurology Baba, Toru; Tohoku University Graduate School of Medicine, Department of Neurology Hasegawa, Takafumi; Tohoku University Graduate School of Medicine, Department of Neurology Kobayashi, Michiko; Tohoku University Graduate School of Medicine, Department of Neurology Sugeno, Naoto; Tohoku University Graduate School of Medicine, Department of Neurology Konno, Masatoshi; Tohoku University Graduate School of Medicine, Department of Neurology Miura, Emiko; Tohoku University Graduate School of Medicine, Department of Neurology Hosokai, Yoshiyuki; Tohoku University Graduate School of Medicine, Department of Behavioral Neurology and Cognitive Neuroscience Ishioka, Toshiyuki; Saitama Prefectural University, Department of Occupational Therapy Nishio, Yoshiyuki; Tohoku University Graduate School of Medicine, Department of Behavioral Neurology and Cognitive Neuroscience Hirayama, Kazumi; Yamagata Prefectural University of Health Sciences, Department of Occupational Therapy Suzuki, Kyoko; Yamagata University Graduate School of Medicine, Department of Clinical Neuroscience Aoki, Masashi; Tohoku University Graduate School of Medicine, Department of Neurology Takahashi, Shoki; Tohoku University Graduate School of Medicine, Department of Diagnostic Radiology Fukuda, Hiroshi; Tohoku University, Department of Nuclear Medicine and Radiology Itoyama, Yasuto; National Center of Neurology and Psychiatry, National Center Hospital Mori, Etsuro; Tohoku University Graduate School of Medicine, Department of Behavioral Neurology and Cognitive Neuroscience Takeda, Atsushi; Tohoku University Graduate School of Medicine, Department of Neurology
Primary Subject Heading:	Neurology

Secondary Subject Heading:	Neurology
Keywords:	PARKINSON'S DISEASE, DYSPHAGIA, PET

Hypometabolism in the supplementary and anterior cingulate cortices is related to dysphagia in Parkinson's disease: a cross-sectional and 3-year longitudinal cohort study

Akio Kikuchi,[1] Toru Baba,[1] Takafumi Hasegawa,[1] Michiko Kobayashi,[1,2] Naoto Sugeno,[1] Masatoshi Konno,[1] Emiko Miura,[1] Yoshiyuki Hosokai,[3] Toshiyuki Ishioka,[3,4] Yoshiyuki Nishio,[3] Kazumi Hirayama,[3,5] Kyoko Suzuki,[3,6] Masashi Aoki,[1] Shoki Takahashi,[7] Hiroshi Fukuda,[8] Yasuto Itoyama,[1,9] Etsuro Mori,[3] Atsushi Takeda[1]

[1] Department of Neurology, Tohoku University Graduate School of Medicine, Sendai, Japan

[2] Department of Neurology, Tohoku Employees' Pension Welfare Hospital, Sendai, Japan.

[3] Department of Behavioral Neurology and Cognitive Neuroscience, Tohoku University Graduate School of Medicine, Sendai, Japan

[4] Department of Occupational Therapy, School of Health and Social Services, Saitama Prefectural University, Saitama, Japan

[5] Department of Occupational Therapy, Yamagata Prefectural University of Health Sciences, Yamagata, Japan

[6] Department of Clinical Neuroscience, Yamagata University Graduate School of Medicine, Yamagata, Japan

[7] Department of Diagnostic Radiology, Tohoku University Graduate School of Medicine, Sendai, Japan

[8] Department of Nuclear Medicine and Radiology, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan

[9] National Center Hospital, National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan

Corresponding Author: Atsushi Takeda, M.D.
Department of Neurology, Tohoku University Graduate School of Medicine
1-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi, 980-8574, Japan
Phone: +81-22-717-7189 Fax: +81-22-717-7192
E-mail: atakeda@med.tohoku.ac.jp

Text word count: 2201
Abstract word count: 261
Number of references: 40
Number of tables: 1
Number of figures: 4

ABSTRACT

Background: Dysphagia is one of the cardinal symptoms of Parkinson's disease (PD) and is closely related to the quality of life and longevity of PD patients.

Objective: To study the pathophysiological mechanisms responsible for dysphagia in PD.

Design: A cross-sectional and longitudinal comparative study.

Setting: Tohoku university hospital.

Participants: Eight patients with dysphagia, 15 patients without dysphagia and 10 normal control subjects.

Main Outcome Measures: The time needed for swallowing initiation and changes in brain glucose metabolism at baseline and after a 3-year follow-up period.

Results: The time needed for swallowing initiation was significantly longer in the patients with dysphagia compared with the patients without dysphagia at baseline and after the 3-year follow-up period ($p<0.05$). The patients with dysphagia exhibited hypometabolism in the supplementary motor area (SMA) and anterior cingulate cortex (ACC) compared with the 10 normal control subjects at baseline (uncorrected $p<0.001$). After the 3-year follow-up period, the number of brain areas showing hypometabolism increased, involving not only the SMA and the ACC but also the bilateral medial frontal lobes, middle cingulate cortex, thalamus and right superior, middle, inferior and orbital frontal gyri (uncorrected $p<0.001$). In contrast, the patients without dysphagia showed virtually no regional hypometabolism at baseline (uncorrected $p<0.001$) and only a small degree of hypometabolism in the SMA and ACC after the 3-year follow-up period (uncorrected $p<0.001$).

Conclusions: These results suggest that dysphagia in PD patients is mainly related to a difficulty in swallowing initiation that is based on a combination of poor movement planning due to SMA dysfunction and impaired cognitive processing due to ACC dysfunction.

ARTICLE SUMMARY

Article focus

- Cortical hypometabolism associated with dysphagia in PD were statistically examined at baseline and after a 3-year follow-up period.

Key messages

- The multiple cortical impairments, mainly in the SMA and ACC, might be responsible for the dysphagia in PD.
- The time needed for swallowing initiation was significantly longer in the patients with dysphagia.
- Dysphagia in PD patients is mainly related to a difficulty in swallowing initiation that is based on a combination of poor movement planning due to SMA dysfunction and impaired cognitive processing due to ACC dysfunction.

Strengths and limitations of this study

- Strength: this study is the first to statistically examine the associations between cortical hypometabolism and dysphagia in PD patients as a cross-sectional and longitudinal comparative study.
- Limitation: the findings may not be related to dysphagia alone because ^{18}F-fluorodeoxyglucose-PET cannot be used for a dynamic scanning during swallowing. Weakness: the absence of videofluoroscopy as swallowing evaluation.

INTRODUCTION

Parkinson's disease (PD) is primarily characterized by motor dysfunctions, some of which, such as tremor, rigidity, and bradykinesia, respond well to dopamine replacement therapy, whereas others, such as postural instability, dysarthria, and dysphagia, remain intractable and often impair quality of life in advanced cases. The involvement of non-dopaminergic systems is implied in such dopamine-refractory symptoms; however, the detailed pathophysiological mechanisms of these systems are still elusive. In particular, dysphagia is directly associated with malnutrition and difficulty in drug taking; moreover, this symptom sometimes results in aspiration pneumonia, the main cause of death in PD patients.[1-3] The voluntary transport of food through the oral cavity, pharynx, and esophagus to the stomach requires sequential motor events. Deglutition occurs through five consecutive phases: anticipatory (cognitive), preparatory (masticatory), oral, pharyngeal, and esophageal. Dysphagia is associated with all of these stages.[4-8]

In general, dysphagia in PD is thought to reflect impaired function of the medullary swallowing center. The involvement of higher central nervous system areas is also implied, but remains to be elucidated. In the present study, we investigated the swallowing functions of PD patients and compared them with changes in cortical metabolism using ^{18}F-fluorodeoxyglucose (FDG)-PET. Moreover, we investigated clinical and imaging data not only at baseline, but also after a 3-year follow-up period, making this research a longitudinal study.

METHODS

Participants

All of the 43 PD patients were diagnosed based on the United Kingdom PD Brain Bank criteria for idiopathic PD.[9] The extent of dementia was evaluated in all of the subjects, using the clinical dementia rating (CDR). PD patients with a CDR score greater than 0.5 were excluded to minimize artifacts related to cognitive impairment. We defined a score of 0 as no dysphagia and a score of >1 as dysphagia, according to Part II of the unified Parkinson's disease rating scale (UPDRS). The UPDRS total scores, mini-mental state examination (MMSE), and PET studies were evaluated during the "on" state, i.e., with the administration of anti-parkinsonian drugs. Ten age-matched control subjects (4 women and 6 men; mean age 64.4±4.12 years; mean MMSE score 28.7±1.49) were collected to compare with PD patients with and without dysphagia for PET analysis. All of the procedures were approved by the Ethical Committee of Tohoku University Graduate School of Medicine. Written informed consent was obtained from each subject after a full explanation of the entire 3-year longitudinal study.

PET procedure

All of the subjects fasted for at least 5 hours prior to PET scanning. PET scans were acquired 60 minutes after intravenous injection of 185-218 MBq FDG. Dynamic PET scans were taken in three-dimensional mode using a Biograph™ Duo PET scanner (Siemens Medical Systems, Inc., Iselin, NJ, USA). To minimize the effects of external stimuli during the FDG uptake period, the subjects stayed in a quiet room wearing an eye mask. The in-plane and axial resolutions of the scanner were 3.38 mm and 3.38

mm, respectively. Attenuation correction was performed with a computed tomography scan. Image reconstructions were performed using ordered subset expectation maximization algorithms (16 subsets x 6 iterations) using a Gaussian filter with full-width at half-maximum = 2.0 mm in a 256 x 256 matrix, with a pixel size of 1.33 x 1.33 mm and a slice thickness of 2.0 mm. The FDG-PET scans acquired at the follow-up were performed in an identical manner.

Data analysis

Statistical parametric mapping (SPM) was used for group comparisons of PET images. First, all of the PET images were spatially normalized with linear and nonlinear parameters using SPM2 software (Welcome Department of Imaging Neuroscience, London, UK) implemented in MATLAB® (The MathWorks, Inc., Sherborn, MA, USA). A three-dimensional Gaussian filter of 10 mm was used to smooth each image. Next, the regional metabolic abnormalities in the PD patients with and without dysphagia at baseline and after the 3-year follow-up period were estimated by comparison with 10 age-matched control subjects using proportional scaling. The statistical threshold was $p<0.001$ (uncorrected) with an extent threshold of 40 voxels.

Evaluation of swallowing

We evaluated the time needed for swallowing initiation and the 30-second swallowing frequency. The index and ring finger pads of the investigator were placed on the thyroid cartilage and laryngeal prominence. The time needed for swallowing initiation was defined as the time until movement of the thyroid cartilage and laryngeal

prominence following the guidance cue to begin swallowing. The time of swallowing initiation across three trials was averaged. We also measured the swallowing frequency by asking the patients to swallow as many times as possible in 30 seconds and counting the movements of the thyroid cartilage and laryngeal prominence.[10, 11]

The patient profiles were statistically analyzed using two-sample t-tests. For the swallowing function, the within-group differences of the patients with and without dysphagia were assessed using paired t-tests.

RESULTS

Of the 43 potentially eligible cases, 16 were excluded from the analysis because of dementia. The 27 patients who fulfilled the above entry criteria included 9 patients with dysphagia and 18 patients without dysphagia. The mean (±standard deviation) age was 68.2±3.23 years in the patients with dysphagia and 65.8±3.94 years in the patients without dysphagia. All of the patients were right-handed. Eight of 9 PD patients with dysphagia and 15 of 18 without dysphagia at baseline participated in the 3-year follow-up study. The details of the excluded patients were as follows: 2 patients without dysphagia went to other hospitals, and 1 patient without dysphagia and 1 patient with dysphagia were unable to be evaluated during the follow-up period because of the introduction of a feeding tube (figure 1).

Clinical features and medications were summarized in table 1. No significant differences were found in the age, Hoehn-Yahr stage, dosage of anti-parkinsonian agents, including L-dopa (table 1), or 30-second swallowing frequency (figure 2B), but significant differences were found between the two groups in disease duration,

UPDRS motor score, and time needed for swallowing initiation at baseline ($p<0.05$, table 1, figure 2A). After the 3-year follow-up period, the differences in the time needed for swallowing initiation were still clear, but no significant differences became evident in the UPDRS motor scores (table 1, figure 2A). Significant differences were revealed in the swallowing frequency within 30 seconds between the two groups ($p<0.05$, table 1, figure 2B).

A comparison of the regional cerebral glucose of PD patients with that of normal controls demonstrated hypometabolism in the supplementary motor area (SMA) and the anterior cingulate cortex (ACC) in the patients with dysphagia at baseline (uncorrected $p<0.001$, figure 3A). These metabolic changes showed no significant correlations with the severity of the Hoehn-Yahr stages (uncorrected $p<0.001$, data not shown) or the UPDRS motor scores (uncorrected $p<0.001$, data not shown). Furthermore, no regional hypermetabolism was found in the PD patients with dysphagia compared with the normal control subjects (uncorrected $p<0.001$, data not shown). Additionally, no relationships were observed between the changes in the regional cerebral glucose metabolism and the doses of anti-parkinsonian agents, including L-dopa ($p>0.05$, data not shown). After the 3-year follow-up period, the areas of hypometabolism included not only the SMA and the ACC but also the bilateral medial frontal lobes, middle cingulate cortex, thalamus and right superior, middle, inferior and orbital frontal gyri (uncorrected $p<0.001$, figure 3B). In contrast, PD patients without dysphagia showed virtually no regional hypometabolism at baseline (uncorrected $p<0.001$, figure 4A) and only a small degree of hypometabolism in the SMA and the ACC after the 3-year follow-up period (uncorrected $p<0.001$,

figure 4B) compared with normal controls.

DISCUSSION

The present results suggested that, although several motor cortical areas control deglutition, multiple cortical impairments, mainly in the SMA and ACC, might be responsible for the dysphagia in PD. These results were in agreement with the findings of previous activation studies of normal deglutition.[12-19] Impairments in these areas did not appear to be closely associated with the degree of general motor dysfunction and cognitive impairments, as no significant correlations were found between the UPDRS motor scores or the MMSE scores and the degree of hypometabolism in these areas (uncorrected $p<0.001$, data not shown). Moreover, although there were no significant changes in the UPDRS motor scores and the MMSE scores between baseline and 3-year follow-up (table 1), cortical hypometabolism in the medial frontal lobes was markedly extended, especially in the cases with dysphagia (figure 3). Interestingly, in the patients with dysphagia, both of the swallowing indices showed tendencies toward exacerbation during the 3-year follow-up period, although the differences were not significant (figure 2).

Major subcortical inputs to the SMA via the thalamus arise from the globus pallidus and the substantia nigra.[20] The SMA also receives limbic inputs from the cingulate cortex.[21] The SMA is known to be important in mediating and preparing complex sequences of movement[22] and in movement planning and execution.[23-27] PET and functional magnetic resonance imaging studies demonstrated SMA activation during the swallowing task.[12, 28] Activation of the SMA preceding the onset of

volitional swallowing can also be demonstrated by the assessment of the Bereitschaftspotential, one of the premotor potentials that is considered to reflect the activities of the SMA.[29] The amplitude of the Bereitschaftspotential[30] was shown to be significantly lower in PD patients compared with age-matched controls.[31] PET and single-photon emission computed tomography studies also demonstrated dysfunction of the SMA in PD.[32-36] Therefore, the SMA dysfunction in PD patients with dysphagia is likely to be related to the impaired volitional initiation of swallowing.

The ACC is connected to both the insula[37] and the amygdale,[38] which are implicated in autonomic function and somato- and viscerosensory input. Intense stimulation of the esophagus[8, 39] and changes in gastrointestinal motility[38] were shown to activate the ACC. The ACC is thought to play an important role in receiving sensory stimuli from the alimentary tract. The functions of the rostral ACC are autonomic regulation and visceromotor control.[14] In fact, the rostral ACC is activated during automatic swallowing.[14] In contrast, the more dorsal and caudal regions of the ACC function in skeletomotor control, including movement regulation and premotor function, response selection, attention to willed action, and nociception.[25] The intermediate and caudal regions of the ACC are activated during voluntary swallowing.[14, 19, 40] As PD patients with dysphagia showed hypometabolism in the intermediate and caudal regions of the ACC, corresponding to Brodmann's area 24 as shown in figure 3A, the dysphagia in PD patients appears to be associated with difficulties in the processing of voluntary swallowing. In fact, the mean time needed to initiate swallowing was longer in cases with dysphagia (figure 2).

In this study, the time needed for swallowing initiation, which shows high

repeatability and reflects the time from the anticipatory to the pharyngeal stages of deglutition, was measured, but other stages were not evaluated. Additional evaluation, such as videofluoroscopy, is needed to understand the relationship between brain hypometabolism and dysphagia in PD patients. Interestingly, in 4 of 8 patients with dysphagia, the time needed for swallowing initiation was above average (2.35 sec), and, in fact, percutaneous endoscopic gastrostomies were performed in 2 of these 4 patients within 4 years of the baseline study. Thus, by measuring the time needed for swallowing initiation, we may be able to predict the long-term prognosis of dysphagia.

CONCLUSION

In conclusion, the data presented in this study suggested that dysphagia in PD patients was related to dysfunctions in the SMA and the ACC, resulting in poor movement planning of voluntary swallowing. The results also implied that training exercises, possibly involving tongue movements, may activate broader cortical areas, including the SMA and ACC,[17] and may be useful in dysphagia rehabilitation.

Contributors: Conception and design: Akio Kikuchi, Atsushi Takeda. Analysis and interpretation of the data: Akio Kikuchi, Toru Baba, Atsushi Takeda. Drafting of the article: Akio Kikuchi, Atsushi Takeda. Collection and assembly of data: All authors.
Funding: This study was supported by a grant for a Symposium on Catecholamine and Neurological Disorders and a Grant-in-Aid for Scientific Research (C) (23591266) from The Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
Competing interests: None.
Patient consent: Standardised patient consent forms were signed by all patients and controls.
Ethics approval: Ethics approval was provided by the Ethical Committee of Tohoku

University Graduate School of Medicine.

Provenance and peer review: Not commissioned; externally peer reviewed.

Data sharing statement: No additional data are available.

REFERENCES

1. Nakashima K, Maeda M, Tabata M, et al. Prognosis of Parkinson's disease in Japan. Tottori University Parkinson's Disease Epidemiology (TUPDE) Study Group. Eur Neurol 1997;38 Suppl 2:60-3.
2. Hely MA, Morris JG, Traficante R, et al. The sydney multicentre study of Parkinson's disease: progression and mortality at 10 years. J Neurol Neurosurg Psychiatry 1999;67:300-7.
3. Fall PA, Saleh A, Fredrickson M, et al. Survival time, mortality, and cause of death in elderly patients with Parkinson's disease: a 9-year follow-up. Mov Disord 2003;18:1312-6.
4. Bushmann M, Dobmeyer SM, Leeker L, et al. Swallowing abnormalities and their response to treatment in Parkinson's disease. Neurology 1989;39:1309-14.
5. Bird MR, Woodward MC, Gibson EM, et al. Asymptomatic swallowing disorders in elderly patients with Parkinson's disease: a description of findings on clinical examination and videofluoroscopy in sixteen patients. Age Ageing 1994;23:251-4.
6. Leopold NA, Kagel MC. Prepharyngeal dysphagia in Parkinson's disease. Dysphagia 1996;11:14-22.
7. Leopold NA, Kagel MC. Pharyngo-esophageal dysphagia in Parkinson's disease. Dysphagia 1997;12:11-8; discussion 9-20.
8. Aziz Q, Andersson JL, Valind S, et al. Identification of human brain loci processing esophageal sensation using positron emission tomography. Gastroenterology 1997;113:50-9.
9. Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988;51:745-52.
10. Oguchi K, Saitoh E, Mizuno M, et al. The repetitive saliva swallowing test (RSST) as a screening test of functional dysphagia (1) normal values of RSST. Jpn J Rehabil Med 2000;37:375-82.
11. Tamura F, Mizukami M, Ayano R, et al. Analysis of feeding function and jaw stability in bedridden elderly. Dysphagia 2002;17:235-41.
12. Hamdy S, Rothwell JC, Brooks DJ, et al. Identification of the cerebral loci processing human swallowing with H2(15)O PET activation. J Neurophysiol 1999;81:1917-26.
13. Kern MK, Jaradeh S, Arndorfer RC, et al. Cerebral cortical representation of reflexive and volitional swallowing in humans. Am J Physiol Gastrointest Liver Physiol 2001;280:G354-60.
14. Martin RE, Goodyear BG, Gati JS, et al. Cerebral cortical representation of automatic and volitional swallowing in humans. J Neurophysiol 2001;85:938-50.
15. Martin R, Barr A, MacIntosh B, et al. Cerebral cortical processing of swallowing in older adults. Exp Brain Res 2007;176:12-22.
16. Mosier K, Bereznaya I. Parallel cortical networks for volitional control of swallowing in humans. Exp Brain Res 2001;140:280-9.
17. Martin RE, MacIntosh BJ, Smith RC, et al. Cerebral areas processing swallowing and tongue movement are overlapping but distinct: a functional magnetic resonance imaging study. J Neurophysiol 2004;92:2428-43.
18. Harris ML, Julyan P, Kulkarni B, et al. Mapping metabolic brain activation during human volitional swallowing: a positron emission tomography study using [18F]fluorodeoxyglucose. J Cereb Blood Flow Metab 2005;25:520-6.
19. Toogood JA, Barr AM, Stevens TK, et al. Discrete functional contributions of cerebral cortical foci in voluntary swallowing: a functional magnetic resonance imaging (fMRI) "Go, No-Go" study. Exp Brain Res 2005;161:81-90.

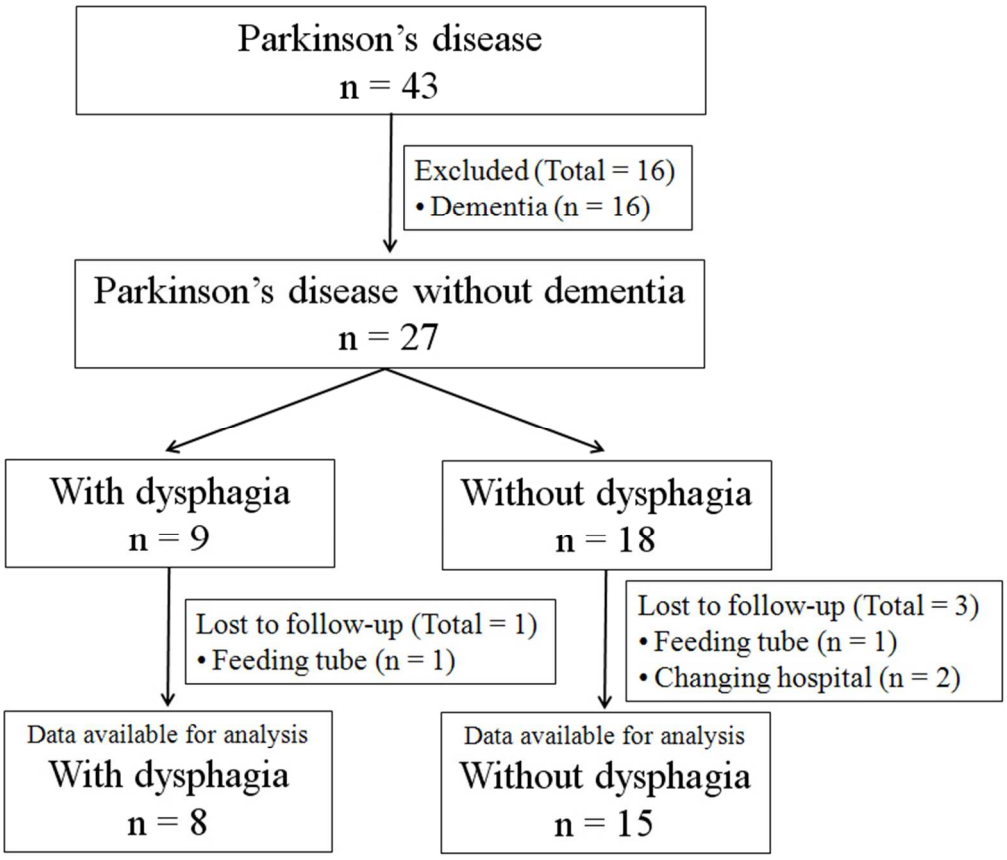
20. Hoover JE, Strick PL. Multiple output channels in the basal ganglia. Science 1993;259:819-21.
21. Tanji J. The supplementary motor area in the cerebral cortex. Neurosci Res 1994;19:251-68.
22. Fried I, Katz A, McCarthy G, et al. Functional organization of human supplementary motor cortex studied by electrical stimulation. J Neurosci 1991;11:3656-66.
23. Dum RP, Strick PL. The origin of corticospinal projections from the premotor areas in the frontal lobe. J Neurosci 1991;11:667-89.
24. Dum RP, Strick PL. Motor areas in the frontal lobe of the primate. Physiol Behav 2002;77:677-82.
25. Devinsky O, Morrell MJ, Vogt BA. Contributions of anterior cingulate cortex to behaviour. Brain 1995;118 (Pt 1):279-306.
26. Deiber MP, Ibanez V, Sadato N, et al. Cerebral structures participating in motor preparation in humans: a positron emission tomography study. J Neurophysiol 1996;75:233-47.
27. Richter W, Andersen PM, Georgopoulos AP, et al. Sequential activity in human motor areas during a delayed cued finger movement task studied by time-resolved fMRI. Neuroreport 1997;8:1257-61.
28. Mosier K, Patel R, Liu WC, et al. Cortical representation of swallowing in normal adults: functional implications. Laryngoscope 1999;109:1417-23.
29. Huckabee ML, Deecke L, Cannito MP, et al. Cortical control mechanisms in volitional swallowing: the Bereitschaftspotential. Brain Topogr 2003;16:3-17.
30. Kornhuber HH, Deecke L. [Changes In The Brain Potential In Voluntary Movements And Passive Movements In Man: Readiness Potential And Reafferent Potentials.]. Pflugers Arch Gesamte Physiol Menschen Tiere 1965;284:1-17.
31. Dick JP, Rothwell JC, Day BL, et al. The Bereitschaftspotential is abnormal in Parkinson's disease. Brain 1989;112 (Pt 1):233-44.
32. Playford ED, Jenkins IH, Passingham RE, et al. Impaired mesial frontal and putamen activation in Parkinson's disease: a positron emission tomography study. Ann Neurol 1992;32:151-61.
33. Grafton ST, Waters C, Sutton J, et al. Pallidotomy increases activity of motor association cortex in Parkinson's disease: a positron emission tomographic study. Ann Neurol 1995;37:776-83.
34. Jahanshahi M, Jenkins IH, Brown RG, et al. Self-initiated versus externally triggered movements. I. An investigation using measurement of regional cerebral blood flow with PET and movement-related potentials in normal and Parkinson's disease subjects. Brain 1995;118 (Pt 4):913-33.
35. Brooks DJ. PET and SPECT studies in Parkinson's disease. Baillieres Clin Neurol 1997;6:69-87.
36. Kikuchi A, Takeda A, Kimpara T, et al. Hypoperfusion in the supplementary motor area, dorsolateral prefrontal cortex and insular cortex in Parkinson's disease. J Neurol Sci 2001;193:29-36.
37. Mesulam MM, Mufson EJ. Insula of the old world monkey. III: Efferent cortical output and comments on function. J Comp Neurol 1982;212:38-52.
38. Pandya DN, Van Hoesen GW, Domesick VB. A cingulo-amygdaloid projection in the rhesus monkey. Brain Res 1973;61:369-73.
39. Kern MK, Birn RM, Jaradeh S, et al. Identification and characterization of cerebral cortical response to esophageal mucosal acid exposure and distention. Gastroenterology 1998;115:1353-62.
40. Paus T, Otaky N, Caramanos Z, et al. In vivo morphometry of the intrasulcal gray matter

in the human cingulate, paracingulate, and superior-rostral sulci: hemispheric asymmetries, gender differences and probability maps. J Comp Neurol 1996;376:664-73.

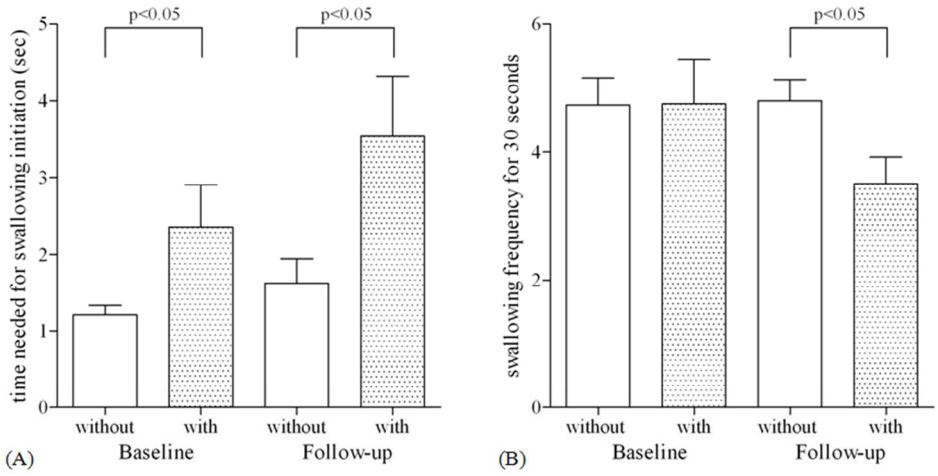
Table 1 Profiles of PD patients

Parameters	Baseline		Follow-up	
	With dysphagia	Without dysphagia	With dysphagia	Without dysphagia
Number of patients	8	15		
Sex (M / W)	6/ 2	4 /11		
Age (yrs)				
Mean	67.8±3.11	65.2±4.02		
Range	63-71	60-74		
Disease duration (yrs)	6.75±3.73*	3.53±3.58		
MMSE	28.5±1.93	28.5±1.51	27.9±2.80	28.4±1.60
Hoehn-Yahr stage	2.75±0.27	2.37±0.67	3.06±0.42	2.93±0.53
UPDRS motor score	23.4±7.05*	14.8±7.49	21.0±5.93	17.4±11.1
L-dopa equivalent dose (mg/day)	400±311	267±321	590±155	514±311

Values are mean±standard deviation or number of patients.
*Significant difference $p<0.05$ between with dysphagia and without dysphagia.
PD=Parkinson's disease; M=men; W=women; MMSE=Mini Mental State Examination; UPDRS=unified Parkinson's disease rating scale



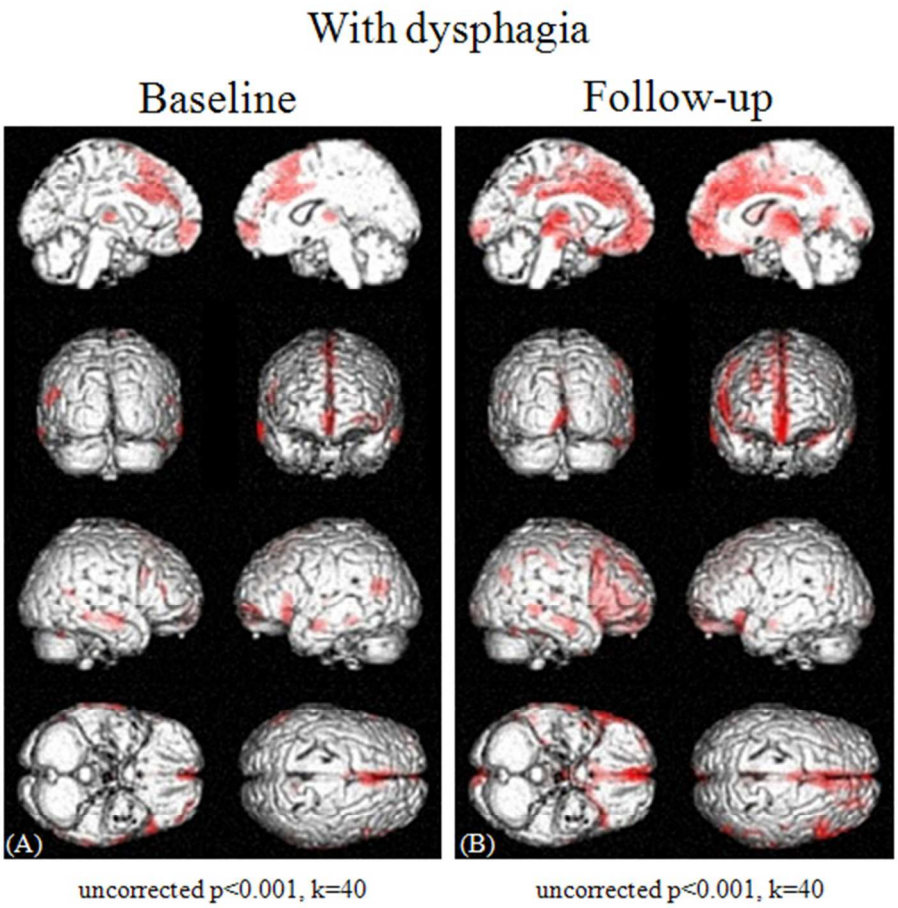
PD patients and follow-up flow diagram.
284x243mm (72 x 72 DPI)



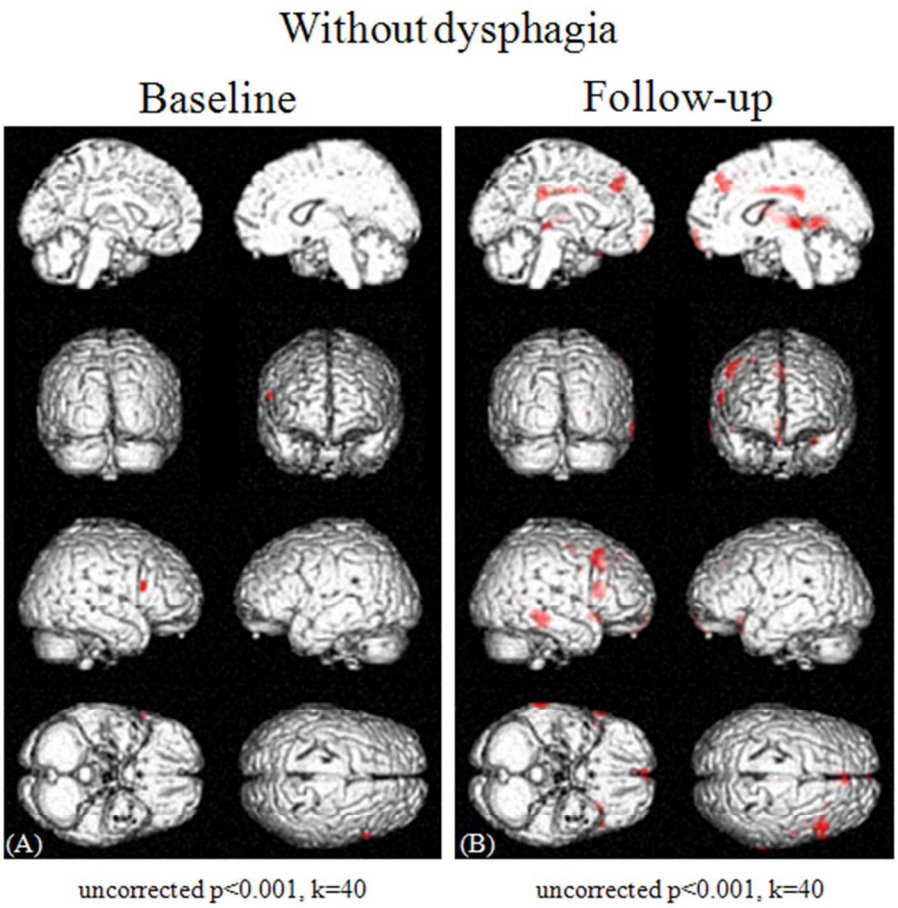
The time needed for swallowing initiation and the 30-second swallowing frequency in PD patients with and without dysphagia at baseline and after a 3-year follow-up period.

There were significant differences between PD patients with and without dysphagia in the time needed for swallowing initiation at baseline and after a 3-year follow-up period ($p<0.05$) (A). In the 30-second swallowing frequency, there was no significant difference between the PD patients with and without dysphagia at baseline ($p>0.05$), but a significant difference between the two groups was evident after the 3-year follow-up period ($p<0.05$) (B).

322x162mm (72 x 72 DPI)



Cross-sectional analyses of brain maps showing the differences between PD patients with dysphagia and normal control subjects at baseline (A) and after a 3-year follow-up period (B). Various areas showed differences in the standardized PET data, and areas with a height threshold of p<0.001 (uncorrected) and an extent threshold of 40 voxels are illustrated. A comparison of regional cerebral glucose metabolism values demonstrated hypometabolism in the SMA and ACC in the PD patients with dysphagia compared with normal control subjects at baseline (uncorrected p<0.001, threshold=40 voxels) (A). After the 3-year follow-up period, the areas of hypometabolism included not only the SMA and the ACC but also the bilateral medial frontal lobes, middle cingulate cortex, thalamus and right superior, middle, inferior, and orbital frontal gyri (uncorrected p<0.001, threshold=40 voxels) (B).
212x201mm (72 x 72 DPI)



Cross-sectional analyses of brain maps showing differences between PD patients without dysphagia and normal control subjects at baseline (A) and after a 3-year follow-up period (B). Various areas showed differences in the standardized PET data, and areas with a height threshold of p<0.001 (uncorrected) and an extent threshold of 40 voxels are illustrated. The PD patients without dysphagia showed virtually no hypometabolism at baseline (uncorrected p<0.001, threshold=40 voxels) (A) and only a small degree of hypometabolism in the SMA and ACC after the 3-year follow-up period (uncorrected p<0.001, threshold=40 voxels) (B) compared with normal control subjects.
212x201mm (72 x 72 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-7
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	5-7
		(*c*) Explain how missing data were addressed	5
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	5

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	7
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7-8
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	7
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-10
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ open

Hypometabolism in the supplementary and anterior cingulate cortices is related to dysphagia in Parkinson's disease: a cross-sectional and 3-year longitudinal cohort study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-002249.R1
Article Type:	Research
Date Submitted by the Author:	19-Dec-2012
Complete List of Authors:	Kikuchi, Akio; Tohoku University Graduate School of Medicine, Department of Neurology Baba, Toru; Tohoku University Graduate School of Medicine, Department of Neurology Hasegawa, Takafumi; Tohoku University Graduate School of Medicine, Department of Neurology Kobayashi, Michiko; Tohoku University Graduate School of Medicine, Department of Neurology Sugeno, Naoto; Tohoku University Graduate School of Medicine, Department of Neurology Konno, Masatoshi; Tohoku University Graduate School of Medicine, Department of Neurology Miura, Emiko; Tohoku University Graduate School of Medicine, Department of Neurology Hosokai, Yoshiyuki; Tohoku University Graduate School of Medicine, Department of Behavioral Neurology and Cognitive Neuroscience Ishioka, Toshiyuki; Saitama Prefectural University, Department of Occupational Therapy Nishio, Yoshiyuki; Tohoku University Graduate School of Medicine, Department of Behavioral Neurology and Cognitive Neuroscience Hirayama, Kazumi; Yamagata Prefectural University of Health Sciences, Department of Occupational Therapy Suzuki, Kyoko; Yamagata University Graduate School of Medicine, Department of Clinical Neuroscience Aoki, Masashi; Tohoku University Graduate School of Medicine, Department of Neurology Takahashi, Shoki; Tohoku University Graduate School of Medicine, Department of Diagnostic Radiology Fukuda, Hiroshi; Tohoku University, Department of Nuclear Medicine and Radiology Itoyama, Yasuto; National Center of Neurology and Psychiatry, National Center Hospital Mori, Etsuro; Tohoku University Graduate School of Medicine, Department of Behavioral Neurology and Cognitive Neuroscience Takeda, Atsushi; Tohoku University Graduate School of Medicine, Department of Neurology
Primary Subject Heading:	Neurology

Secondary Subject Heading:	Neurology
Keywords:	PARKINSON'S DISEASE, DYSPHAGIA, PET

Hypometabolism in the supplementary and anterior cingulate cortices is related to dysphagia in Parkinson's disease: a cross-sectional and 3-year longitudinal cohort study

Akio Kikuchi,[1] Toru Baba,[1] Takafumi Hasegawa,[1] Michiko Kobayashi,[1,2] Naoto Sugeno,[1] Masatoshi Konno,[1] Emiko Miura,[1] Yoshiyuki Hosokai,[3] Toshiyuki Ishioka,[3,4] Yoshiyuki Nishio,[3] Kazumi Hirayama,[3,5] Kyoko Suzuki,[3,6] Masashi Aoki,[1] Shoki Takahashi,[7] Hiroshi Fukuda,[8] Yasuto Itoyama,[1,9] Etsuro Mori,[3] Atsushi Takeda[1]

[1] Department of Neurology, Tohoku University Graduate School of Medicine, Sendai, Japan
[2] Department of Neurology, Tohoku Employees' Pension Welfare Hospital, Sendai, Japan.
[3] Department of Behavioral Neurology and Cognitive Neuroscience, Tohoku University Graduate School of Medicine, Sendai, Japan
[4] Department of Occupational Therapy, School of Health and Social Services, Saitama Prefectural University, Saitama, Japan
[5] Department of Occupational Therapy, Yamagata Prefectural University of Health Sciences, Yamagata, Japan
[6] Department of Clinical Neuroscience, Yamagata University Graduate School of Medicine, Yamagata, Japan
[7] Department of Diagnostic Radiology, Tohoku University Graduate School of Medicine, Sendai, Japan
[8] Department of Nuclear Medicine and Radiology, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan
[9] National Center Hospital, National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan

Corresponding Author: Atsushi Takeda, M.D.
Department of Neurology, Tohoku University Graduate School of Medicine
1-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi, 980-8574, Japan
Phone: +81-22-717-7189 Fax: +81-22-717-7192
E-mail: atakeda@med.tohoku.ac.jp

Text word count: 2201
Abstract word count: 261
Number of references: 40
Number of tables: 1
Number of figures: 4

ABSTRACT

Background: Dysphagia is one of the cardinal symptoms of Parkinson's disease (PD) and is closely related to the quality of life and longevity of PD patients.

Objective: To study the pathophysiological mechanisms responsible for dysphagia in PD.

Design: A cross-sectional and longitudinal comparative study.

Setting: Tohoku university hospital.

Participants: Eight patients with dysphagia, 15 patients without dysphagia and 10 normal control subjects.

Main Outcome Measures: The time needed for swallowing initiation and changes in brain glucose metabolism at baseline and after a 3-year follow-up period.

Results: The time needed for swallowing initiation was significantly longer in the patients with dysphagia compared with the patients without dysphagia at baseline and after the 3-year follow-up period ($p<0.05$). The patients with dysphagia exhibited hypometabolism in the supplementary motor area (SMA) and anterior cingulate cortex (ACC) compared with the 10 normal control subjects at baseline (uncorrected $p<0.001$). After the 3-year follow-up period, the number of brain areas showing hypometabolism increased, involving not only the SMA and the ACC but also the bilateral medial frontal lobes, middle cingulate cortex, thalamus and right superior, middle, inferior and orbital frontal gyri (uncorrected $p<0.001$). In contrast, the patients without dysphagia showed virtually no regional hypometabolism at baseline (uncorrected $p<0.001$) and only a small degree of hypometabolism in the SMA and ACC after the 3-year follow-up period (uncorrected $p<0.001$).

Conclusions: These results suggest that dysphagia in PD patients is mainly related to a difficulty in swallowing initiation that is based on a combination of poor movement planning due to SMA dysfunction and impaired cognitive processing due to ACC dysfunction.

ARTICLE SUMMARY

Article focus

- Cortical hypometabolism associated with dysphagia in PD were statistically examined at baseline and after a 3-year follow-up period.

Key messages

- The multiple cortical impairments, mainly in the SMA and ACC, might be responsible for the dysphagia in PD.
- The time needed for swallowing initiation was significantly longer in the patients with dysphagia.
- Dysphagia in PD patients is mainly related to a difficulty in swallowing initiation that is based on a combination of poor movement planning due to SMA dysfunction and impaired cognitive processing due to ACC dysfunction.

Strengths and limitations of this study

- Strength: this study is the first to statistically examine the associations between cortical hypometabolism and dysphagia in PD patients as a cross-sectional and longitudinal comparative study.
- Limitation: the findings may not be related to dysphagia alone because ^{18}F-fluorodeoxyglucose-PET cannot be used for a dynamic scanning during swallowing. Weakness: the absence of videofluoroscopy as swallowing evaluation.

INTRODUCTION

Parkinson's disease (PD) is primarily characterized by motor dysfunctions, some of which, such as tremor, rigidity, and bradykinesia, respond well to dopamine replacement therapy, whereas others, such as postural instability, dysarthria, and dysphagia, remain intractable and often impair quality of life in advanced cases. The involvement of non-dopaminergic systems is implied in such dopamine-refractory symptoms; however, the detailed pathophysiological mechanisms of these systems are still elusive. In particular, swallowing difficulty is directly associated with malnutrition and difficulty in drug taking; moreover, this symptom sometimes results in aspiration pneumonia, the main cause of death in PD patients.[1-3] The voluntary transport of food through the oral cavity, pharynx, and esophagus to the stomach requires sequential motor events. Deglutition occurs through five consecutive phases: anticipatory (cognitive), preparatory (masticatory), oral, pharyngeal, and esophageal. Dysphagia is associated with all of these stages.[4-8]

In general, dysphagia in PD is thought to reflect impaired function of the medullary swallowing center[9]. The involvement of higher central nervous system areas is also implied, but remains to be elucidated. In the present study, we investigated the swallowing functions of PD patients and compared them with changes in cortical metabolism using ^{18}F-fluorodeoxyglucose (FDG)-PET. Moreover, we investigated clinical and imaging data not only at baseline, but also after a 3-year follow-up period, making this research a longitudinal study.

METHODS

Participants

All of the 43 PD patients were diagnosed based on the United Kingdom PD Brain Bank

criteria for idiopathic PD.[10] The extent of dementia was evaluated in all of the subjects, using the clinical dementia rating (CDR). PD patients with a CDR score greater than 0.5 were excluded to minimize artifacts related to cognitive impairment. We defined a score of 0 as no dysphagia and a score of >1 as dysphagia, according to Part II of the unified Parkinson's disease rating scale (UPDRS). The UPDRS total scores, mini-mental state examination (MMSE), evaluation of swallowing, and PET studies were evaluated during the "on" state, i.e., with the administration of anti-parkinsonian drugs without L-dopa induced dyskinesia. Ten age-matched control subjects (4 women and 6 men; mean age 64.4±4.12 years; mean MMSE score 28.7±1.49) were collected to compare with PD patients with and without dysphagia for PET analysis. All of the procedures were approved by the Ethical Committee of Tohoku University Graduate School of Medicine. Written informed consent was obtained from each subject after a full explanation of the entire 3-year longitudinal study.

PET procedure

The FDG-PET scans were performed in all 23 PD patients and 10 age-matched control subjects. All of the subjects fasted for at least 5 hours prior to PET scanning. To minimize the effects of external stimuli during a 1-hour FDG uptake period after intravenous injection of 185-218 MBq FDG, the subjects stayed in a quiet room wearing an eye mask under resting conditions. PET scans were acquired for 10 minutes under resting conditions. Dynamic PET scans were taken in 3D mode using a Biograph^{TM} Duo PET scanner (Siemens Medical Systems, Inc., Iselin, NJ, USA). The in-plane and axial resolutions of the scanner were 3.38 mm and 3.38 mm, respectively. Attenuation correction was performed with a computed tomography scan. Image reconstructions were performed using ordered subset expectation maximization algorithms (16 subsets x 6 iterations) using a Gaussian filter with full-width at

half-maximum = 2.0 mm in a 256 x 256 matrix, with a pixel size of 1.33 x 1.33 mm and a slice thickness of 2.0 mm, and a field of view of 340 mm. The FDG-PET scans acquired at the follow-up were performed in an identical manner.

Data analysis

Statistical parametric mapping (SPM) was used for group comparisons of PET images. First, all of the PET images were spatially normalized with linear and nonlinear parameters using SPM2 software (Welcome Department of Imaging Neuroscience, London, UK) implemented in MATLAB® (The MathWorks, Inc., Sherborn, MA, USA). A three-dimensional Gaussian filter of 10 mm was used to smooth each image. Global normalization was performed using SPM's "proportional scaling," and proportional threshold masking was set at 0.8. Next, the regional metabolic abnormalities in the PD patients with and without dysphagia at baseline and after the 3-year follow-up period were estimated by comparison with 10 age-matched control subjects using proportional scaling. The statistical threshold was $p<0.001$ (uncorrected) with an extent threshold of 40 voxels.

Evaluation of swallowing

We evaluated the time needed for swallowing initiation and the 30-second swallowing frequency in all 23 PD patients and 10 healthy volunteers. The index and ring finger pads of the investigator were placed on the thyroid cartilage and laryngeal prominence. The time needed for swallowing initiation was defined as the time until movement of the thyroid cartilage and laryngeal prominence following the verbal guidance cue to begin swallowing using a timer. The time of swallowing initiation across three trials was averaged. We also measured the swallowing frequency by asking the patients to swallow as many times as

possible in 30 seconds and counting the movements of the thyroid cartilage and laryngeal prominence.[11, 12]

The patient profiles were statistically analyzed using two-sample t-tests. For the swallowing function, the within-group differences of the patients with and without dysphagia were assessed using paired t-tests.

RESULTS

Of the 43 potentially eligible cases, 16 were excluded from the analysis because of dementia. The 27 patients who fulfilled the above entry criteria included 9 patients with dysphagia and 18 patients without dysphagia. The mean (±standard deviation) age was 68.2±3.23 years in the patients with dysphagia and 65.8±3.94 years in the patients without dysphagia. All of the patients were right-handed. Eight of 9 PD patients with dysphagia and 15 of 18 without dysphagia at baseline participated in the 3-year follow-up study. The details of the excluded patients were as follows: 2 patients without dysphagia went to other hospitals, and 1 patient without dysphagia and 1 patient with dysphagia were unable to be evaluated during the follow-up period because of the introduction of a feeding tube (figure 1).

Clinical features and medications were summarized in table 1. No significant differences were found in the age, Hoehn-Yahr stage, dosage of anti-parkinsonian agents, including L-dopa (table 1), or 30-second swallowing frequency (figure 2B), but significant differences were found between the two groups in disease duration, UPDRS motor score, and time needed for swallowing initiation at baseline ($p<0.05$, table 1, figure 2A). After the 3-year follow-up period, the differences in the time needed for swallowing initiation were still clear, but no significant differences became evident in the UPDRS motor scores (table 1, figure 2A). Significant differences were revealed in the swallowing frequency within 30 seconds between

the two groups ($p<0.05$, table 1, figure 2B). The time needed for swallowing initiation (1.02±0.36 sec) and the 30-second swallowing frequency (5.10±2.42) in 10 healthy volunteers were the almost same as those of PD without dysphagia. There was no significant difference in the L-dopa equivalent dose between baseline and 3-year follow-up in PD with dysphagia using paired t-test ($p>0.05$), while a significant difference was found in PD without dysphagia ($p<0.05$). No significant differences were found in the UPDRS motor score between baseline and 3-year follow-up within groups using paired t-tests ($p>0.05$).

A comparison of the regional cerebral glucose of PD patients with that of normal controls demonstrated hypometabolism in the supplementary motor area (SMA) and the anterior cingulate cortex (ACC) in the patients with dysphagia at baseline (uncorrected $p<0.001$, figure 3A). These metabolic changes showed no significant correlations with the severity of the Hoehn-Yahr stages (uncorrected $p<0.001$, data not shown) or the UPDRS motor scores (uncorrected $p<0.001$, data not shown). Furthermore, no regional hypermetabolism was found in the PD patients with dysphagia compared with the normal control subjects (uncorrected $p<0.001$, data not shown). Additionally, no relationships were observed between the changes in the regional cerebral glucose metabolism and the doses of anti-parkinsonian agents, including L-dopa ($p>0.05$, data not shown). After the 3-year follow-up period, the areas of hypometabolism included not only the SMA and the ACC but also the bilateral medial frontal lobes, middle cingulate cortex, thalamus and right superior, middle, inferior and orbital frontal gyri (uncorrected $p<0.001$, figure 3B). Only a small degree of hypermetabolism in the left middle and right superior occipital lobes, left middle temporal lobe, left supramarginal gyrus, and left calcarine cortex was found in PD patients with dysphagia compared to the normal control subjects (uncorrected $p<0.001$, data not shown). In contrast, PD patients without dysphagia showed virtually no regional hypometabolism at

baseline (uncorrected p<0.001, figure 4A) and only a small degree of hypometabolism in the SMA and the ACC after the 3-year follow-up period (uncorrected p<0.001, figure 4B) compared with normal controls. On the other hand, in PD patients without dysphagia, no regional hypermetabolism was found at baseline (uncorrected p<0.001, data not shown) and only a small degree of hypermetabolism in the left supramarginal gyrus, left postcentral gyrus, and left middle and superior lobes was found after the 3-year follow-up period (uncorrected p<0.001, data not shown). Medullary hypometabolism was not found at baseline nor after a 3-year follow-up period (uncorrected p<0.001, figures 3, 4).

DISCUSSION

The present results suggested that, although several motor cortical areas control deglutition, multiple cortical impairments, mainly in the SMA and ACC, might be responsible for the dysphagia in PD. These results were in agreement with the findings of previous activation studies of normal deglutition such as functional MRI[13-18] and PET[19, 20]. Impairments in these areas did not appear to be closely associated with the degree of general motor dysfunction and cognitive impairments, as no significant correlations were found between the UPDRS motor scores or the MMSE scores and the degree of hypometabolism in these areas (uncorrected p<0.001, data not shown). Moreover, although there were no significant changes in the UPDRS motor scores and the MMSE scores, except for the L-dopa equivalent dose in PD without dysphagia, between baseline and 3-year follow-up in each group (table 1), cortical hypometabolism in the medial frontal lobes was markedly extended, especially in the cases with dysphagia (figure 3). Interestingly, in the patients with dysphagia, both of the swallowing indices showed tendencies toward exacerbation during the 3-year follow-up period, although the differences were not significant (figure 2). Although no significant

difference was found in the UPDRS motor score between PD with and without dysphagia after a 3-year follow-up, the time needed for swallowing initiation was worsening in PD with dysphagia. Bradykinesia did not appear to be directly related to the outcome measurement results for evaluation of swallowing. We could not find any compensatory mechanisms for dysphagia because regional hypermetabolism was not found in PD with and without dysphagia at baseline.

Major subcortical inputs to the SMA via the thalamus arise from the globus pallidus and the substantia nigra.[21] The SMA also receives limbic inputs from the cingulate cortex.[22] The SMA is known to be important in mediating and preparing complex sequences of movement[23] and in movement planning and execution.[24-28] PET and functional magnetic resonance imaging studies demonstrated SMA activation during the swallowing task.[19, 29] Activation of the SMA preceding the onset of volitional swallowing can also be demonstrated by the assessment of the Bereitschaftspotential, one of the premotor potentials that is considered to reflect the activities of the SMA.[30] The amplitude of the Bereitschaftspotential[31] was shown to be significantly lower in PD patients compared with age-matched controls.[32] PET and single-photon emission computed tomography studies also demonstrated dysfunction of the SMA in PD.[33-37] Therefore, the SMA dysfunction in PD patients with dysphagia is likely to be related to the impaired volitional initiation of swallowing.

The ACC is connected to both the insula[38] and the amygdale,[39] which are implicated in autonomic function and somato- and viscerosensory input. Intense stimulation of the esophagus[8, 40] and changes in gastrointestinal motility[39] were shown to activate the ACC. The ACC is thought to play an important role in receiving sensory stimuli from the alimentary tract. The functions of the rostral ACC are autonomic regulation and visceromotor control.[14]

In fact, the rostral ACC is activated during automatic swallowing.[14] In contrast, the more dorsal and caudal regions of the ACC function in skeletomotor control, including movement regulation and premotor function, response selection, attention to willed action, and nociception.[26] The intermediate and caudal regions of the ACC are activated during voluntary swallowing.[14, 18, 41] As PD patients with dysphagia showed hypometabolism in the intermediate and caudal regions of the ACC, corresponding to Brodmann's area 24 as shown in figure 3A, the dysphagia in PD patients appears to be associated with difficulties in the processing of voluntary swallowing. In fact, the mean time needed to initiate swallowing was longer in cases with dysphagia (figure 2).

In this study, the time needed for swallowing initiation and swallowing frequency for 30 seconds showed high reproducibility. The time needed for swallowing initiation was thought to reflect the time from the anticipatory to the pharyngeal stages of deglutition, was measured, but other stages were not evaluated. Additional evaluation, such as videofluoroscopy, is needed to understand the relationship between brain hypometabolism and dysphagia in PD patients. Interestingly, in 4 of 8 patients with dysphagia, the time needed for swallowing initiation was above average (2.35 sec), and, in fact, percutaneous endoscopic gastrostomies were performed in 2 of these 4 patients within 4 years of the baseline study. Thus, by measuring the time needed for swallowing initiation, we may be able to predict the long-term prognosis of swallowing difficulty.

CONCLUSION

In conclusion, the data presented in this study suggested that dysphagia in PD patients was related to dysfunctions in the SMA and the ACC, resulting in poor movement planning of voluntary swallowing. The results also suggest that training exercises, which can activate

broader cortical areas including the SMA and ACC,[17] may be useful to alleviate swallowing difficulty.

Contributors: Conception and design: Akio Kikuchi, Atsushi Takeda. Analysis and interpretation of the data: Akio Kikuchi, Toru Baba, Atsushi Takeda. Drafting of the article: Akio Kikuchi, Atsushi Takeda. Collection and assembly of data: All authors.

Funding: This study was supported by a grant for a Symposium on Catecholamine and Neurological Disorders and a Grant-in-Aid for Scientific Research (C) (23591266) from The Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: None.

Patient consent: Standardised patient consent forms were signed by all patients and controls.

Ethics approval: Ethics approval was provided by the Ethical Committee of Tohoku University Graduate School of Medicine.

Provenance and peer review: Not commissioned; externally peer reviewed.

Data sharing statement: No additional data are available.

REFERENCES

1. Nakashima K, Maeda M, Tabata M, et al. Prognosis of Parkinson's disease in Japan. Tottori University Parkinson's Disease Epidemiology (TUPDE) Study Group. Eur Neurol 1997;38 Suppl 2:60-3.
2. Hely MA, Morris JG, Traficante R, et al. The sydney multicentre study of Parkinson's disease: progression and mortality at 10 years. J Neurol Neurosurg Psychiatry 1999;67:300-7.
3. Fall PA, Saleh A, Fredrickson M, et al. Survival time, mortality, and cause of death in elderly patients with Parkinson's disease: a 9-year follow-up. Mov Disord 2003;18:1312-6.
4. Bushmann M, Dobmeyer SM, Leeker L, et al. Swallowing abnormalities and their response to treatment in Parkinson's disease. Neurology 1989;39:1309-14.
5. Bird MR, Woodward MC, Gibson EM, et al. Asymptomatic swallowing disorders in elderly patients with Parkinson's disease: a description of findings on clinical examination and videofluoroscopy in sixteen patients. Age Ageing 1994;23:251-4.
6. Leopold NA, Kagel MC. Prepharyngeal dysphagia in Parkinson's disease. Dysphagia 1996;11:14-22.
7. Leopold NA, Kagel MC. Pharyngo-esophageal dysphagia in Parkinson's disease. Dysphagia 1997;12:11-8; discussion 9-20.
8. Aziz Q, Andersson JL, Valind S, et al. Identification of human brain loci processing esophageal sensation using positron emission tomography. Gastroenterology 1997;113:50-9.
9. Hunter PC, Crameri J, Austin S, et al. Response of parkinsonian swallowing dysfunction to dopaminergic stimulation. J Neurol Neurosurg Psychiatry 1997;63:579-83.
10. Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988;51:745-52.
11. Oguchi K, Saitoh E, Mizuno M, et al. The repetitive saliva swallowing test (RSST) as a screening test of functional dysphagia (1) normal values of RSST. Jpn J Rehabil Med 2000;37:375-82.
12. Tamura F, Mizukami M, Ayano R, et al. Analysis of feeding function and jaw stability in bedridden elderly. Dysphagia 2002;17:235-41.
13. Kern MK, Jaradeh S, Arndorfer RC, et al. Cerebral cortical representation of reflexive and volitional swallowing in humans. Am J Physiol Gastrointest Liver Physiol 2001;280:G354-60.
14. Martin RE, Goodyear BG, Gati JS, et al. Cerebral cortical representation of automatic and volitional swallowing in humans. J Neurophysiol 2001;85:938-50.
15. Martin R, Barr A, MacIntosh B, et al. Cerebral cortical processing of swallowing in older adults. Exp Brain Res 2007;176:12-22.
16. Mosier K, Bereznaya I. Parallel cortical networks for volitional control of swallowing in humans. Exp Brain Res 2001;140:280-9.
17. Martin RE, MacIntosh BJ, Smith RC, et al. Cerebral areas processing swallowing and tongue movement are overlapping but distinct: a functional magnetic resonance imaging study. J Neurophysiol 2004;92:2428-43.
18. Toogood JA, Barr AM, Stevens TK, et al. Discrete functional contributions of cerebral cortical foci in voluntary swallowing: a functional magnetic resonance imaging (fMRI) "Go, No-Go" study. Exp Brain Res 2005;161:81-90.
19. Hamdy S, Rothwell JC, Brooks DJ, et al. Identification of the cerebral loci processing human swallowing with H2(15)O PET activation. J Neurophysiol 1999;81:1917-26.
20. Harris ML, Julyan P, Kulkarni B, et al. Mapping metabolic brain activation during

human volitional swallowing: a positron emission tomography study using [18F]fluorodeoxyglucose. J Cereb Blood Flow Metab 2005;25:520-6.
21. Hoover JE, Strick PL. Multiple output channels in the basal ganglia. Science 1993;259:819-21.
22. Tanji J. The supplementary motor area in the cerebral cortex. Neurosci Res 1994;19:251-68.
23. Fried I, Katz A, McCarthy G, et al. Functional organization of human supplementary motor cortex studied by electrical stimulation. J Neurosci 1991;11:3656-66.
24. Dum RP, Strick PL. The origin of corticospinal projections from the premotor areas in the frontal lobe. J Neurosci 1991;11:667-89.
25. Dum RP, Strick PL. Motor areas in the frontal lobe of the primate. Physiol Behav 2002;77:677-82.
26. Devinsky O, Morrell MJ, Vogt BA. Contributions of anterior cingulate cortex to behaviour. Brain 1995;118 (Pt 1):279-306.
27. Deiber MP, Ibanez V, Sadato N, et al. Cerebral structures participating in motor preparation in humans: a positron emission tomography study. J Neurophysiol 1996;75:233-47.
28. Richter W, Andersen PM, Georgopoulos AP, et al. Sequential activity in human motor areas during a delayed cued finger movement task studied by time-resolved fMRI. Neuroreport 1997;8:1257-61.
29. Mosier K, Patel R, Liu WC, et al. Cortical representation of swallowing in normal adults: functional implications. Laryngoscope 1999;109:1417-23.
30. Huckabee ML, Deecke L, Cannito MP, et al. Cortical control mechanisms in volitional swallowing: the Bereitschaftspotential. Brain Topogr 2003;16:3-17.
31. Kornhuber HH, Deecke L. [Changes In The Brain Potential In Voluntary Movements And Passive Movements In Man: Readiness Potential And Reafferent Potentials.]. Pflugers Arch Gesamte Physiol Menschen Tiere 1965;284:1-17.
32. Dick JP, Rothwell JC, Day BL, et al. The Bereitschaftspotential is abnormal in Parkinson's disease. Brain 1989;112 (Pt 1):233-44.
33. Playford ED, Jenkins IH, Passingham RE, et al. Impaired mesial frontal and putamen activation in Parkinson's disease: a positron emission tomography study. Ann Neurol 1992;32:151-61.
34. Grafton ST, Waters C, Sutton J, et al. Pallidotomy increases activity of motor association cortex in Parkinson's disease: a positron emission tomographic study. Ann Neurol 1995;37:776-83.
35. Jahanshahi M, Jenkins IH, Brown RG, et al. Self-initiated versus externally triggered movements. I. An investigation using measurement of regional cerebral blood flow with PET and movement-related potentials in normal and Parkinson's disease subjects. Brain 1995;118 (Pt 4):913-33.
36. Brooks DJ. PET and SPECT studies in Parkinson's disease. Baillieres Clin Neurol 1997;6:69-87.
37. Kikuchi A, Takeda A, Kimpara T, et al. Hypoperfusion in the supplementary motor area, dorsolateral prefrontal cortex and insular cortex in Parkinson's disease. J Neurol Sci 2001;193:29-36.
38. Mesulam MM, Mufson EJ. Insula of the old world monkey. III: Efferent cortical output and comments on function. J Comp Neurol 1982;212:38-52.
39. Pandya DN, Van Hoesen GW, Domesick VB. A cingulo-amygdaloid projection in the rhesus monkey. Brain Res 1973;61:369-73.

40. Kern MK, Birn RM, Jaradeh S, et al. Identification and characterization of cerebral cortical response to esophageal mucosal acid exposure and distention. Gastroenterology 1998;115:1353-62.
41. Paus T, Otaky N, Caramanos Z, et al. In vivo morphometry of the intrasulcal gray matter in the human cingulate, paracingulate, and superior-rostral sulci: hemispheric asymmetries, gender differences and probability maps. J Comp Neurol 1996;376:664-73.

Table 1 Profiles of PD patients

Parameters	Baseline		Follow-up	
	With dysphagia	Without dysphagia	With dysphagia	Without dysphagia
Number of patients	8	15		
Sex (M / W)	6/ 2	4 /11		
Age (yrs)				
Mean	67.8±3.11	65.2±4.02		
Range	63-71	60-74		
Disease duration (yrs)	6.75±3.73*	3.53±3.58		
MMSE	28.5±1.93	28.5±1.51	27.9±2.80	28.4±1.60
Hoehn-Yahr stage	2.75±0.27	2.37±0.67	3.06±0.42	2.93±0.53
UPDRS motor score	23.4±7.05*	14.8±7.49	21.0±5.93	17.4±11.1
L-dopa equivalent dose (mg/day)	400±311	267±321	590±155	514±311

Values are mean±standard deviation or number of patients.
*Significant difference $p<0.05$ between with dysphagia and without dysphagia.
PD=Parkinson's disease; M=men; W=women; MMSE=Mini Mental State Examination; UPDRS=unified Parkinson's disease rating scale

Hypometabolism in the supplementary and anterior cingulate cortices is related to dysphagia in Parkinson's disease: a cross-sectional and 3-year longitudinal cohort study

Akio Kikuchi,[1] Toru Baba,[1] Takafumi Hasegawa,[1] Michiko Kobayashi,[1,2] Naoto Sugeno,[1] Masatoshi Konno,[1] Emiko Miura,[1] Yoshiyuki Hosokai,[3] Toshiyuki Ishioka,[3,4] Yoshiyuki Nishio,[3] Kazumi Hirayama,[3,5] Kyoko Suzuki,[3,6] Masashi Aoki,[1] Shoki Takahashi,[7] Hiroshi Fukuda,[8] Yasuto Itoyama,[1,9] Etsuro Mori,[3] Atsushi Takeda[1]

[1] Department of Neurology, Tohoku University Graduate School of Medicine, Sendai, Japan
[2] Department of Neurology, Tohoku Employees' Pension Welfare Hospital, Sendai, Japan.
[3] Department of Behavioral Neurology and Cognitive Neuroscience, Tohoku University Graduate School of Medicine, Sendai, Japan
[4] Department of Occupational Therapy, School of Health and Social Services, Saitama Prefectural University, Saitama, Japan
[5] Department of Occupational Therapy, Yamagata Prefectural University of Health Sciences, Yamagata, Japan
[6] Department of Clinical Neuroscience, Yamagata University Graduate School of Medicine, Yamagata, Japan
[7] Department of Diagnostic Radiology, Tohoku University Graduate School of Medicine, Sendai, Japan
[8] Department of Nuclear Medicine and Radiology, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan
[9] National Center Hospital, National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan

Corresponding Author: Atsushi Takeda, M.D.
Department of Neurology, Tohoku University Graduate School of Medicine
1-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi, 980-8574, Japan
Phone: +81-22-717-7189 Fax: +81-22-717-7192
E-mail: atakeda@med.tohoku.ac.jp

Text word count: 2201
Abstract word count: 261
Number of references: 40
Number of tables: 1
Number of figures: 4

Formatted: Numbering: Restart each page

ABSTRACT

Background: Dysphagia is one of the cardinal symptoms of Parkinson's disease (PD) and is closely related to the quality of life and longevity of PD patients.

Objective: To study the pathophysiological mechanisms responsible for dysphagia in PD.

Design: A cross-sectional and longitudinal comparative study.

Setting: Tohoku university hospital.

Participants: Eight patients with dysphagia, 15 patients without dysphagia and 10 normal control subjects.

Main Outcome Measures: The time needed for swallowing initiation and changes in brain glucose metabolism at baseline and after a 3-year follow-up period.

Results: The time needed for swallowing initiation was significantly longer in the patients with dysphagia compared with the patients without dysphagia at baseline and after the 3-year follow-up period ($p<0.05$). The patients with dysphagia exhibited hypometabolism in the supplementary motor area (SMA) and anterior cingulate cortex (ACC) compared with the 10 normal control subjects at baseline (uncorrected $p<0.001$). After the 3-year follow-up period, the number of brain areas showing hypometabolism increased, involving not only the SMA and the ACC but also the bilateral medial frontal lobes, middle cingulate cortex, thalamus and right superior, middle, inferior and orbital frontal gyri (uncorrected $p<0.001$). In contrast, the patients without dysphagia showed virtually no regional hypometabolism at baseline (uncorrected $p<0.001$) and only a small degree of hypometabolism in the SMA and ACC after the 3-year follow-up period (uncorrected $p<0.001$).

Conclusions: These results suggest that dysphagia in PD patients is mainly related to a difficulty in swallowing initiation that is based on a combination of poor movement planning due to SMA dysfunction and impaired cognitive processing due to ACC dysfunction.

ARTICLE SUMMARY

Article focus

- Cortical hypometabolism associated with dysphagia in PD were statistically examined at baseline and after a 3-year follow-up period.

Key messages

- The multiple cortical impairments, mainly in the SMA and ACC, might be responsible for the dysphagia in PD.
- The time needed for swallowing initiation was significantly longer in the patients with dysphagia.
- Dysphagia in PD patients is mainly related to a difficulty in swallowing initiation that is based on a combination of poor movement planning due to SMA dysfunction and impaired cognitive processing due to ACC dysfunction.

Strengths and limitations of this study

- Strength: this study is the first to statistically examine the associations between cortical hypometabolism and dysphagia in PD patients as a cross-sectional and longitudinal comparative study.
- Limitation: the findings may not be related to dysphagia alone because ^{18}F-fluorodeoxyglucose-PET cannot be used for a dynamic scanning during swallowing. Weakness: the absence of videofluoroscopy as swallowing evaluation.

INTRODUCTION

Parkinson's disease (PD) is primarily characterized by motor dysfunctions, some of which, such as tremor, rigidity, and bradykinesia, respond well to dopamine replacement therapy, whereas others, such as postural instability, dysarthria, and dysphagia, remain intractable and often impair quality of life in advanced cases. The involvement of non-dopaminergic systems is implied in such dopamine-refractory symptoms; however, the detailed pathophysiological mechanisms of these systems are still elusive. In particular, swallowing difficulty~~dysphagia~~ is directly associated with malnutrition and difficulty in drug taking; moreover, this symptom sometimes results in aspiration pneumonia, the main cause of death in PD patients.[1-3] The voluntary transport of food through the oral cavity, pharynx, and esophagus to the stomach requires sequential motor events. Deglutition occurs through five consecutive phases: anticipatory (cognitive), preparatory (masticatory), oral, pharyngeal, and esophageal. Dysphagia is associated with all of these stages.[4-8]

In general, dysphagia in PD is thought to reflect impaired function of the medullary swallowing center[9]. The involvement of higher central nervous system areas is also implied, but remains to be elucidated. In the present study, we investigated the swallowing functions of PD patients and compared them with changes in cortical metabolism using ^{18}F-fluorodeoxyglucose (FDG)-PET. Moreover, we investigated clinical and imaging data not only at baseline, but also after a 3-year follow-up period, making this research a longitudinal study.

Field Code Changed

Field Code Changed

Formatted: Font color: Red

METHODS

Participants

All of the 43 PD patients were diagnosed based on the United Kingdom PD Brain Bank

criteria for idiopathic PD.[109] The extent of dementia was evaluated in all of the subjects, using the clinical dementia rating (CDR). PD patients with a CDR score greater than 0.5 were excluded to minimize artifacts related to cognitive impairment. We defined a score of 0 as no dysphagia and a score of >1 as dysphagia, according to Part II of the unified Parkinson's disease rating scale (UPDRS). The UPDRS total scores, mini-mental state examination (MMSE), evaluation of swallowing, and PET studies were evaluated during the "on" state, i.e., with the administration of anti-parkinsonian drugs without L-dopa induced dyskinesia. Ten age-matched control subjects (4 women and 6 men; mean age 64.4±4.12 years; mean MMSE score 28.7±1.49) were collected to compare with PD patients with and without dysphagia for PET analysis. All of the procedures were approved by the Ethical Committee of Tohoku University Graduate School of Medicine. Written informed consent was obtained from each subject after a full explanation of the entire 3-year longitudinal study.

PET procedure

The FDG-PET scans were performed in all 23 PD patients and 10 age-matched control subjects. All of the subjects fasted for at least 5 hours prior to PET scanning. ~~PET scans were acquired 60 minutes after intravenous injection of 185-218 MBq FDG. Dynamic PET scans were taken in three-dimensional mode using a Biograph™ Duo PET scanner (Siemens Medical Systems, Inc., Iselin, NJ, USA).~~ To minimize the effects of external stimuli during a 1-hour~~the~~ FDG uptake period after intravenous injection of 185-218 MBq FDG, the subjects stayed in a quiet room wearing an eye mask under resting conditions. PET scans were acquired for 10 minutes under resting conditions. Dynamic PET scans were taken in 3D mode using a Biograph™ Duo PET scanner (Siemens Medical Systems, Inc., Iselin, NJ, USA). The in-plane and axial resolutions of the scanner were 3.38 mm and 3.38 mm,

Field Code Changed

Formatted: Font color: Red

respectively. Attenuation correction was performed with a computed tomography scan. Image reconstructions were performed using ordered subset expectation maximization algorithms (16 subsets x 6 iterations) using a Gaussian filter with full-width at half-maximum = 2.0 mm in a 256 x 256 matrix, with a pixel size of 1.33 x 1.33 mm and a slice thickness of 2.0 mm, and a field of view of 340 mm. The FDG-PET scans acquired at the follow-up were performed in an identical manner.

Data analysis

Statistical parametric mapping (SPM) was used for group comparisons of PET images. First, all of the PET images were spatially normalized with linear and nonlinear parameters using SPM2 software (Welcome Department of Imaging Neuroscience, London, UK) implemented in MATLAB® (The MathWorks, Inc., Sherborn, MA, USA). A three-dimensional Gaussian filter of 10 mm was used to smooth each image. Global normalization was performed using SPM's "proportional scaling," and proportional threshold masking was set at 0.8. Next, the regional metabolic abnormalities in the PD patients with and without dysphagia at baseline and after the 3-year follow-up period were estimated by comparison with 10 age-matched control subjects using proportional scaling. The statistical threshold was $p<0.001$ (uncorrected) with an extent threshold of 40 voxels.

Evaluation of swallowing

We evaluated the time needed for swallowing initiation and the 30-second swallowing frequency in all 23 PD patients and 10 healthy volunteers. The index and ring finger pads of the investigator were placed on the thyroid cartilage and laryngeal prominence. The time needed for swallowing initiation was defined as the time until movement of the thyroid

cartilage and laryngeal prominence following the verbal guidance cue to begin swallowing using a timer. The time of swallowing initiation across three trials was averaged. We also measured the swallowing frequency by asking the patients to swallow as many times as possible in 30 seconds and counting the movements of the thyroid cartilage and laryngeal prominence.[11, 12~~10, 11~~]

Field Code Changed

The patient profiles were statistically analyzed using two-sample t-tests. For the swallowing function, the within-group differences of the patients with and without dysphagia were assessed using paired t-tests.

RESULTS

Of the 43 potentially eligible cases, 16 were excluded from the analysis because of dementia. The 27 patients who fulfilled the above entry criteria included 9 patients with dysphagia and 18 patients without dysphagia. The mean (±standard deviation) age was 68.2±3.23 years in the patients with dysphagia and 65.8±3.94 years in the patients without dysphagia. All of the patients were right-handed. Eight of 9 PD patients with dysphagia and 15 of 18 without dysphagia at baseline participated in the 3-year follow-up study. The details of the excluded patients were as follows: 2 patients without dysphagia went to other hospitals, and 1 patient without dysphagia and 1 patient with dysphagia were unable to be evaluated during the follow-up period because of the introduction of a feeding tube (figure 1).

Clinical features and medications were summarized in table 1. No significant differences were found in the age, Hoehn-Yahr stage, dosage of anti-parkinsonian agents, including L-dopa (table 1), or 30-second swallowing frequency (figure 2B), but significant differences were found between the two groups in disease duration, UPDRS motor score, and time needed for swallowing initiation at baseline ($p<0.05$, table 1, figure 2A). After the 3-year

follow-up period, the differences in the time needed for swallowing initiation were still clear, but no significant differences became evident in the UPDRS motor scores (table 1, figure 2A). Significant differences were revealed in the swallowing frequency within 30 seconds between the two groups ($p<0.05$, table 1, figure 2B). The time needed for swallowing initiation (1.02±0.36 sec) and the 30-second swallowing frequency (5.10±2.42) in 10 healthy volunteers were the almost same as those of PD without dysphagia. There was no significant difference in the L-dopa equivalent dose between baseline and 3-year follow-up in PD with dysphagia using paired t-test ($p>0.05$), while a significant difference was found in PD without dysphagia ($p<0.05$). No significant differences were found in the UPDRS motor score between baseline and 3-year follow-up within groups using paired t-tests ($p>0.05$).

A comparison of the regional cerebral glucose of PD patients with that of normal controls demonstrated hypometabolism in the supplementary motor area (SMA) and the anterior cingulate cortex (ACC) in the patients with dysphagia at baseline (uncorrected $p<0.001$, figure 3A). These metabolic changes showed no significant correlations with the severity of the Hoehn-Yahr stages (uncorrected $p<0.001$, data not shown) or the UPDRS motor scores (uncorrected $p<0.001$, data not shown). Furthermore, no regional hypermetabolism was found in the PD patients with dysphagia compared with the normal control subjects (uncorrected $p<0.001$, data not shown). Additionally, no relationships were observed between the changes in the regional cerebral glucose metabolism and the doses of anti-parkinsonian agents, including L-dopa ($p>0.05$, data not shown). After the 3-year follow-up period, the areas of hypometabolism included not only the SMA and the ACC but also the bilateral medial frontal lobes, middle cingulate cortex, thalamus and right superior, middle, inferior and orbital frontal gyri (uncorrected $p<0.001$, figure 3B). Only a small degree of hypermetabolism in the left middle and right superior occipital lobes, left middle temporal

lobe, left supramarginal gyrus, and left calcarine cortex was found in PD patients with dysphagia compared to the normal control subjects (uncorrected p<0.001, data not shown). In contrast, PD patients without dysphagia showed virtually no regional hypometabolism at baseline (uncorrected p<0.001, figure 4A) and only a small degree of hypometabolism in the SMA and the ACC after the 3-year follow-up period (uncorrected p<0.001, figure 4B) compared with normal controls. On the other hand, in PD patients without dysphagia, no regional hypermetabolism was found at baseline (uncorrected p<0.001, data not shown) and only a small degree of hypermetabolism in the left supramarginal gyrus, left postcentral gyrus, and left middle and superior lobes was found after the 3-year follow-up period (uncorrected p<0.001, data not shown). Medullary hypometabolism was not found at baseline nor after a 3-year follow-up period (uncorrected p<0.001, figures 3, 4).

DISCUSSION

The present results suggested that, although several motor cortical areas control deglutition, multiple cortical impairments, mainly in the SMA and ACC, might be responsible for the dysphagia in PD. These results were in agreement with the findings of previous activation studies of normal deglutition such as functional MRI[13-18] and PET[19, 20] [13-20] [12-19]. Impairments in these areas did not appear to be closely associated with the degree of general motor dysfunction and cognitive impairments, as no significant correlations were found between the UPDRS motor scores or the MMSE scores and the degree of hypometabolism in these areas (uncorrected p<0.001, data not shown). Moreover, although there were no significant changes in the UPDRS motor scores and the MMSE scores, except for the L-dopa equivalent dose in PD without dysphagia, between baseline and 3-year follow-up in each group (table 1), cortical hypometabolism in the medial frontal lobes was markedly extended, especially in the

Formatted: Superscript
Formatted: Font color: Red
Field Code Changed

cases with dysphagia (figure 3). Interestingly, in the patients with dysphagia, both of the swallowing indices showed tendencies toward exacerbation during the 3-year follow-up period, although the differences were not significant (figure 2). Although no significant difference was found in the UPDRS motor score between PD with and without dysphagia after a 3-year follow-up, the time needed for swallowing initiation was worsening in PD with dysphagia. Bradykinesia did not appear to be directly related to the outcome measurement results for evaluation of swallowing. We could not find any compensatory mechanisms for dysphagia because regional hypermetabolism was not found in PD with and without dysphagia at baseline.

Major subcortical inputs to the SMA via the thalamus arise from the globus pallidus and the substantia nigra.[21,20] The SMA also receives limbic inputs from the cingulate cortex.[22,21] The SMA is known to be important in mediating and preparing complex sequences of movement[23,22] and in movement planning and execution.[24-28,23-27] PET and functional magnetic resonance imaging studies demonstrated SMA activation during the swallowing task.[19, 29,12, 28] Activation of the SMA preceding the onset of volitional swallowing can also be demonstrated by the assessment of the Bereitschaftspotential, one of the premotor potentials that is considered to reflect the activities of the SMA.[30,29] The amplitude of the Bereitschaftspotential[31,30] was shown to be significantly lower in PD patients compared with age-matched controls.[32,31] PET and single-photon emission computed tomography studies also demonstrated dysfunction of the SMA in PD.[33-37,32-36] Therefore, the SMA dysfunction in PD patients with dysphagia is likely to be related to the impaired volitional initiation of swallowing.

The ACC is connected to both the insula[38,37] and the amygdale,[39,38] which are implicated in autonomic function and somato- and viscerosensory input. Intense stimulation of the

Field Code Changed
Field Code Changed
Field Code Changed
Field Code Changed
Field Code Changed
Field Code Changed
Field Code Changed
Field Code Changed
Field Code Changed
Field Code Changed

esophagus[8, 40~~8, 39~~] and changes in gastrointestinal motility[39~~38~~] were shown to activate the ACC. The ACC is thought to play an important role in receiving sensory stimuli from the alimentary tract. The functions of the rostral ACC are autonomic regulation and visceromotor control.[14] In fact, the rostral ACC is activated during automatic swallowing.[14] In contrast, the more dorsal and caudal regions of the ACC function in skeletomotor control, including movement regulation and premotor function, response selection, attention to willed action, and nociception.[26~~25~~] The intermediate and caudal regions of the ACC are activated during voluntary swallowing.[14, 18, 41~~14, 19, 40~~] As PD patients with dysphagia showed hypometabolism in the intermediate and caudal regions of the ACC, corresponding to Brodmann's area 24 as shown in figure 3A, the dysphagia in PD patients appears to be associated with difficulties in the processing of voluntary swallowing. In fact, the mean time needed to initiate swallowing was longer in cases with dysphagia (figure 2).

Field Code Changed
Field Code Changed
Field Code Changed
Field Code Changed
Field Code Changed

In this study, the time needed for swallowing initiation and swallowing frequency for 30 seconds showed high reproducibility. ~~the~~ The time needed for swallowing initiation was thought to~~, which shows high repeatability and~~ reflect~~s~~ the time from the anticipatory to the pharyngeal stages of deglutition, was measured, but other stages were not evaluated. Additional evaluation, such as videofluoroscopy, is needed to understand the relationship between brain hypometabolism and dysphagia in PD patients. Interestingly, in 4 of 8 patients with dysphagia, the time needed for swallowing initiation was above average (2.35 sec), and, in fact, percutaneous endoscopic gastrostomies were performed in 2 of these 4 patients within 4 years of the baseline study. Thus, by measuring the time needed for swallowing initiation, we may be able to predict the long-term prognosis of swallowing difficulty~~dysphagia~~.

CONCLUSION

In conclusion, the data presented in this study suggested that dysphagia in PD patients was related to dysfunctions in the SMA and the ACC, resulting in poor movement planning of voluntary swallowing. The results also suggest that training exercises, which can activate broader cortical areas including the SMA and ACC,[17] may be useful to alleviate swallowing difficulty. ~~The results also implied that training exercises, possibly involving tongue movements, may activate broader cortical areas, including the SMA and ACC,[17] and may be useful in dysphagia rehabilitation.~~

Field Code Changed

Contributors: Conception and design: Akio Kikuchi, Atsushi Takeda. Analysis and interpretation of the data: Akio Kikuchi, Toru Baba, Atsushi Takeda. Drafting of the article: Akio Kikuchi, Atsushi Takeda. Collection and assembly of data: All authors.

Funding: This study was supported by a grant for a Symposium on Catecholamine and Neurological Disorders and a Grant-in-Aid for Scientific Research (C) (23591266) from The Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: None.

Patient consent: Standardised patient consent forms were signed by all patients and controls.

Ethics approval: Ethics approval was provided by the Ethical Committee of Tohoku University Graduate School of Medicine.

Provenance and peer review: Not commissioned; externally peer reviewed.

Data sharing statement: No additional data are available.

REFERENCES

1. Nakashima K, Maeda M, Tabata M, et al. Prognosis of Parkinson's disease in Japan. Tottori University Parkinson's Disease Epidemiology (TUPDE) Study Group. Eur Neurol 1997;38 Suppl 2:60-3.
2. Hely MA, Morris JG, Traficante R, et al. The sydney multicentre study of Parkinson's disease: progression and mortality at 10 years. J Neurol Neurosurg Psychiatry 1999;67:300-7.
3. Fall PA, Saleh A, Fredrickson M, et al. Survival time, mortality, and cause of death in elderly patients with Parkinson's disease: a 9-year follow-up. Mov Disord 2003;18:1312-6.
4. Bushmann M, Dobmeyer SM, Leeker L, et al. Swallowing abnormalities and their response to treatment in Parkinson's disease. Neurology 1989;39:1309-14.
5. Bird MR, Woodward MC, Gibson EM, et al. Asymptomatic swallowing disorders in elderly patients with Parkinson's disease: a description of findings on clinical examination and videofluoroscopy in sixteen patients. Age Ageing 1994;23:251-4.
6. Leopold NA, Kagel MC. Prepharyngeal dysphagia in Parkinson's disease. Dysphagia 1996;11:14-22.
7. Leopold NA, Kagel MC. Pharyngo-esophageal dysphagia in Parkinson's disease. Dysphagia 1997;12:11-8; discussion 9-20.
8. Aziz Q, Andersson JL, Valind S, et al. Identification of human brain loci processing esophageal sensation using positron emission tomography. Gastroenterology 1997;113:50-9.
9. Hunter PC, Crameri J, Austin S, et al. Response of parkinsonian swallowing dysfunction to dopaminergic stimulation. J Neurol Neurosurg Psychiatry 1997;63:579-83.
10. Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988;51:745-52.
11. Oguchi K, Saitoh E, Mizuno M, et al. The repetitive saliva swallowing test (RSST) as a screening test of functional dysphagia (1) normal values of RSST. Jpn J Rehabil Med 2000;37:375-82.
12. Tamura F, Mizukami M, Ayano R, et al. Analysis of feeding function and jaw stability in bedridden elderly. Dysphagia 2002;17:235-41.
13. Kern MK, Jaradeh S, Arndorfer RC, et al. Cerebral cortical representation of reflexive and volitional swallowing in humans. Am J Physiol Gastrointest Liver Physiol 2001;280:G354-60.
14. Martin RE, Goodyear BG, Gati JS, et al. Cerebral cortical representation of automatic and volitional swallowing in humans. J Neurophysiol 2001;85:938-50.
15. Martin R, Barr A, MacIntosh B, et al. Cerebral cortical processing of swallowing in older adults. Exp Brain Res 2007;176:12-22.
16. Mosier K, Bereznaya I. Parallel cortical networks for volitional control of swallowing in humans. Exp Brain Res 2001;140:280-9.
17. Martin RE, MacIntosh BJ, Smith RC, et al. Cerebral areas processing swallowing and tongue movement are overlapping but distinct: a functional magnetic resonance imaging study. J Neurophysiol 2004;92:2428-43.
18. Toogood JA, Barr AM, Stevens TK, et al. Discrete functional contributions of cerebral cortical foci in voluntary swallowing: a functional magnetic resonance imaging (fMRI) "Go, No-Go" study. Exp Brain Res 2005;161:81-90.
19. Hamdy S, Rothwell JC, Brooks DJ, et al. Identification of the cerebral loci processing human swallowing with H2(15)O PET activation. J Neurophysiol 1999;81:1917-26.
20. Harris ML, Julyan P, Kulkarni B, et al. Mapping metabolic brain activation during

Formatted: Font: 12 pt

Formatted: Indent: Left: 0", First line: 0"

human volitional swallowing: a positron emission tomography study using [18F]fluorodeoxyglucose. J Cereb Blood Flow Metab 2005;25:520-6.
21. Hoover JE, Strick PL. Multiple output channels in the basal ganglia. Science 1993;259:819-21.
22. Tanji J. The supplementary motor area in the cerebral cortex. Neurosci Res 1994;19:251-68.
23. Fried I, Katz A, McCarthy G, et al. Functional organization of human supplementary motor cortex studied by electrical stimulation. J Neurosci 1991;11:3656-66.
24. Dum RP, Strick PL. The origin of corticospinal projections from the premotor areas in the frontal lobe. J Neurosci 1991;11:667-89.
25. Dum RP, Strick PL. Motor areas in the frontal lobe of the primate. Physiol Behav 2002;77:677-82.
26. Devinsky O, Morrell MJ, Vogt BA. Contributions of anterior cingulate cortex to behaviour. Brain 1995;118 (Pt 1):279-306.
27. Deiber MP, Ibanez V, Sadato N, et al. Cerebral structures participating in motor preparation in humans: a positron emission tomography study. J Neurophysiol 1996;75:233-47.
28. Richter W, Andersen PM, Georgopoulos AP, et al. Sequential activity in human motor areas during a delayed cued finger movement task studied by time-resolved fMRI. Neuroreport 1997;8:1257-61.
29. Mosier K, Patel R, Liu WC, et al. Cortical representation of swallowing in normal adults: functional implications. Laryngoscope 1999;109:1417-23.
30. Huckabee ML, Deecke L, Cannito MP, et al. Cortical control mechanisms in volitional swallowing: the Bereitschaftspotential. Brain Topogr 2003;16:3-17.
31. Kornhuber HH, Deecke L. [Changes In The Brain Potential In Voluntary Movements And Passive Movements In Man: Readiness Potential And Reafferent Potentials.]. Pflugers Arch Gesamte Physiol Menschen Tiere 1965;284:1-17.
32. Dick JP, Rothwell JC, Day BL, et al. The Bereitschaftspotential is abnormal in Parkinson's disease. Brain 1989;112 (Pt 1):233-44.
33. Playford ED, Jenkins IH, Passingham RE, et al. Impaired mesial frontal and putamen activation in Parkinson's disease: a positron emission tomography study. Ann Neurol 1992;32:151-61.
34. Grafton ST, Waters C, Sutton J, et al. Pallidotomy increases activity of motor association cortex in Parkinson's disease: a positron emission tomographic study. Ann Neurol 1995;37:776-83.
35. Jahanshahi M, Jenkins IH, Brown RG, et al. Self-initiated versus externally triggered movements. I. An investigation using measurement of regional cerebral blood flow with PET and movement-related potentials in normal and Parkinson's disease subjects. Brain 1995;118 (Pt 4):913-33.
36. Brooks DJ. PET and SPECT studies in Parkinson's disease. Baillieres Clin Neurol 1997;6:69-87.
37. Kikuchi A, Takeda A, Kimpara T, et al. Hypoperfusion in the supplementary motor area, dorsolateral prefrontal cortex and insular cortex in Parkinson's disease. J Neurol Sci 2001;193:29-36.
38. Mesulam MM, Mufson EJ. Insula of the old world monkey. III: Efferent cortical output and comments on function. J Comp Neurol 1982;212:38-52.
39. Pandya DN, Van Hoesen GW, Domesick VB. A cingulo-amygdaloid projection in the rhesus monkey. Brain Res 1973;61:369-73.

40. Kern MK, Birn RM, Jaradeh S, et al. Identification and characterization of cerebral cortical response to esophageal mucosal acid exposure and distention. Gastroenterology 1998;115:1353-62.

41. Paus T, Otaky N, Caramanos Z, et al. In vivo morphometry of the intrasulcal gray matter in the human cingulate, paracingulate, and superior-rostral sulci: hemispheric asymmetries, gender differences and probability maps. J Comp Neurol 1996;376:664-73.

1. Nakashima K, Maeda M, Tabata M, et al. Prognosis of Parkinson's disease in Japan. Tottori University Parkinson's Disease Epidemiology (TUPDE) Study Group. Eur Neurol 1997;38 Suppl 2:60-3.
2. Hely MA, Morris JG, Traficante R, et al. The sydney multicentre study of Parkinson's disease: progression and mortality at 10 years. J Neurol Neurosurg Psychiatry 1999;67:300-7.
3. Fall PA, Saleh A, Fredrickson M, et al. Survival time, mortality, and cause of death in elderly patients with Parkinson's disease: a 9-year follow-up. Mov Disord 2003;18:1312-6.
4. Bushmann M, Dobmeyer SM, Leeker L, et al. Swallowing abnormalities and their response to treatment in Parkinson's disease. Neurology 1989;39:1309-14.
5. Bird MR, Woodward MC, Gibson EM, et al. Asymptomatic swallowing disorders in elderly patients with Parkinson's disease: a description of findings on clinical examination and videofluoroscopy in sixteen patients. Age Ageing 1994;23:251-4.
6. Leopold NA, Kagel MC. Prepharyngeal dysphagia in Parkinson's disease. Dysphagia 1996;11:14-22.
7. Leopold NA, Kagel MC. Pharyngo-esophageal dysphagia in Parkinson's disease. Dysphagia 1997;12:11-8; discussion 9-20.
8. Aziz Q, Andersson JL, Valind S, et al. Identification of human brain loci processing esophageal sensation using positron emission tomography. Gastroenterology 1997;113:50-9.
9. Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988;51:745-52.
10. Oguchi K, Saitoh E, Mizuno M, et al. The repetitive saliva swallowing test (RSST) as a screening test of functional dysphagia (1) normal values of RSST. Jpn J Rehabil Med 2000;37:375-82.
11. Tamura F, Mizukami M, Ayano R, et al. Analysis of feeding function and jaw stability in bedridden elderly. Dysphagia 2002;17:235-41.
12. Hamdy S, Rothwell JC, Brooks DJ, et al. Identification of the cerebral loci processing human swallowing with H2(15)O PET activation. J Neurophysiol 1999;81:1917-26.
13. Kern MK, Jaradeh S, Arndorfer RC, et al. Cerebral cortical representation of reflexive and volitional swallowing in humans. Am J Physiol Gastrointest Liver Physiol 2001;280:G354-60.
14. Martin RE, Goodyear BG, Gati JS, et al. Cerebral cortical representation of automatic and volitional swallowing in humans. J Neurophysiol 2001;85:938-50.
15. Martin R, Barr A, MacIntosh B, et al. Cerebral cortical processing of swallowing in older adults. Exp Brain Res 2007;176:12-22.
16. Mosier K, Bereznaya I. Parallel cortical networks for volitional control of swallowing in humans. Exp Brain Res 2001;140:280-9.
17. Martin RE, MacIntosh BJ, Smith RC, et al. Cerebral areas processing swallowing and tongue movement are overlapping but distinct: a functional magnetic resonance

Formatted: Indent: Left: 0", Hanging: 0.5"

imaging study. J Neurophysiol 2004;92:2428-43.
18. Harris ML, Julyan P, Kulkarni B, et al. Mapping metabolic brain activation during human volitional swallowing: a positron emission tomography study using [18F]fluorodeoxyglucose. J Cereb Blood Flow Metab 2005;25:520-6.
19. Toogood JA, Barr AM, Stevens TK, et al. Discrete functional contributions of cerebral cortical foci in voluntary swallowing: a functional magnetic resonance imaging (fMRI) "Go, No-Go" study. Exp Brain Res 2005;161:81-90.
20. Hoover JE, Strick PL. Multiple output channels in the basal ganglia. Science 1993;259:819-21.
21. Tanji J. The supplementary motor area in the cerebral cortex. Neurosci Res 1994;19:251-68.
22. Fried I, Katz A, McCarthy G, et al. Functional organization of human supplementary motor cortex studied by electrical stimulation. J Neurosci 1991;11:3656-66.
23. Dum RP, Strick PL. The origin of corticospinal projections from the premotor areas in the frontal lobe. J Neurosci 1991;11:667-89.
24. Dum RP, Strick PL. Motor areas in the frontal lobe of the primate. Physiol Behav 2002;77:677-82.
25. Devinsky O, Morrell MJ, Vogt BA. Contributions of anterior cingulate cortex to behaviour. Brain 1995;118 (Pt 1):279-306.
26. Deiber MP, Ibanez V, Sadato N, et al. Cerebral structures participating in motor preparation in humans: a positron emission tomography study. J Neurophysiol 1996;75:233-47.
27. Richter W, Andersen PM, Georgopoulos AP, et al. Sequential activity in human motor areas during a delayed cued finger movement task studied by time-resolved fMRI. Neuroreport 1997;8:1257-61.
28. Mosier K, Patel R, Liu WC, et al. Cortical representation of swallowing in normal adults: functional implications. Laryngoscope 1999;109:1417-23.
29. Huckabee ML, Deecke L, Cannito MP, et al. Cortical control mechanisms in volitional swallowing: the Bereitschaftspotential. Brain Topogr 2003;16:3-17.
30. Kornhuber HH, Deecke L. [Changes In The Brain Potential In Voluntary Movements And Passive Movements In Man: Readiness Potential And Reafferent Potentials.]. Pflugers Arch Gesamte Physiol Menschen Tiere 1965;284:1-17.
31. Dick JP, Rothwell JC, Day BL, et al. The Bereitschaftspotential is abnormal in Parkinson's disease. Brain 1989;112 (Pt 1):233-44.
32. Playford ED, Jenkins IH, Passingham RE, et al. Impaired mesial frontal and putamen activation in Parkinson's disease: a positron emission tomography study. Ann Neurol 1992;32:151-61.
33. Grafton ST, Waters C, Sutton J, et al. Pallidotomy increases activity of motor association cortex in Parkinson's disease: a positron emission tomographic study. Ann Neurol 1995;37:776-83.
34. Jahanshahi M, Jenkins IH, Brown RG, et al. Self-initiated versus externally triggered movements. I. An investigation using measurement of regional cerebral blood flow with PET and movement-related potentials in normal and Parkinson's disease subjects. Brain 1995;118 (Pt 4):913-33.
35. Brooks DJ. PET and SPECT studies in Parkinson's disease. Baillieres Clin Neurol 1997;6:69-87.
36. Kikuchi A, Takeda A, Kimpara T, et al. Hypoperfusion in the supplementary motor area, dorsolateral prefrontal cortex and insular cortex in Parkinson's disease. J Neurol

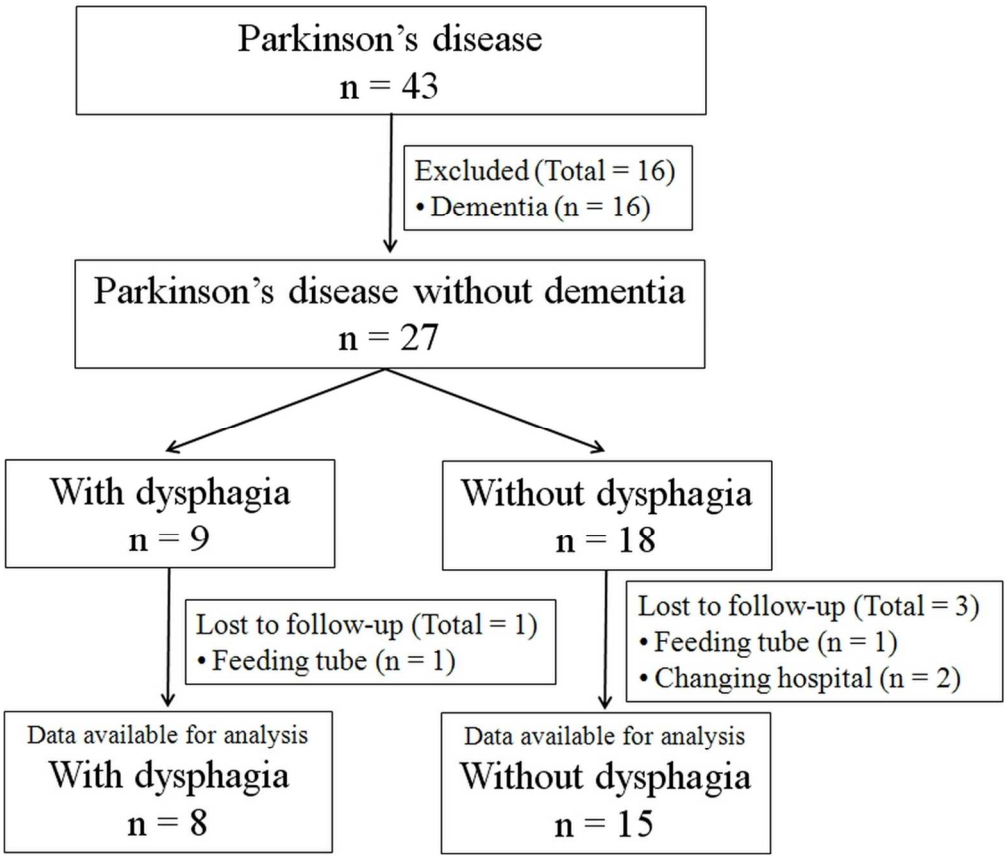
Sci 2001;193:29-36.
37. Mesulam MM, Mufson EJ. Insula of the old world monkey. III: Efferent cortical output and comments on function. J Comp Neurol 1982;212:38-52.
38. Pandya DN, Van Hoesen GW, Domesick VB. A cingulo-amygdaloid projection in the rhesus monkey. Brain Res 1973;61:369-73.
39. Kern MK, Birn RM, Jaradeh S, et al. Identification and characterization of cerebral cortical response to esophageal mucosal acid exposure and distention. Gastroenterology 1998;115:1353-62.
40. Paus T, Otaky N, Caramanos Z, et al. In vivo morphometry of the intrasulcal gray matter in the human cingulate, paracingulate, and superior rostral sulci: hemispheric asymmetries, gender differences and probability maps. J Comp Neurol 1996;376:664-73.

Table 1 Profiles of PD patients

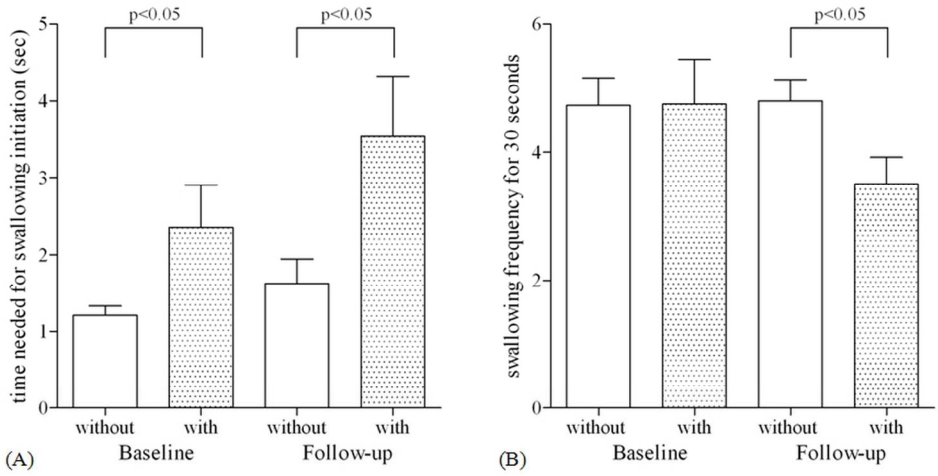
Parameters	Baseline		Follow-up	
	With dysphagia	Without dysphagia	With dysphagia	Without dysphagia
Number of patients	8	15		
Sex (M / W)	6/ 2	4 /11		
Age (yrs)				
Mean	67.8±3.11	65.2±4.02		
Range	63-71	60-74		
Disease duration (yrs)	6.75±3.73*	3.53±3.58		
MMSE	28.5±1.93	28.5±1.51	27.9±2.80	28.4±1.60
Hoehn-Yahr stage	2.75±0.27	2.37±0.67	3.06±0.42	2.93±0.53
UPDRS motor score	23.4±7.05*	14.8±7.49	21.0±5.93	17.4±11.1
L-dopa equivalent dose (mg/day)	400±311	267±321	590±155	514±311

Values are mean±standard deviation or number of patients.
*Significant difference $p<0.05$ between with dysphagia and without dysphagia.
PD=Parkinson's disease; M=men; W=women; MMSE=Mini Mental State Examination; UPDRS=unified Parkinson's disease rating scale

Formatted: Indent: Left: 0", First line: 0"



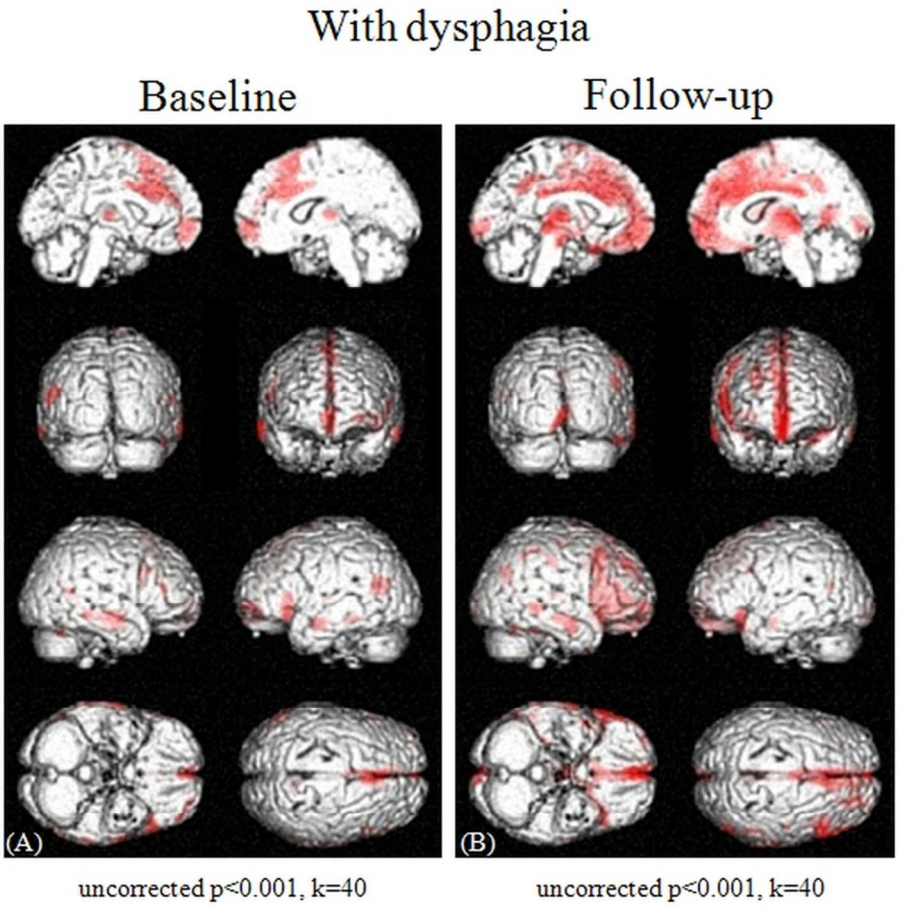
PD patients and follow-up flow diagram.
105x90mm (300 x 300 DPI)



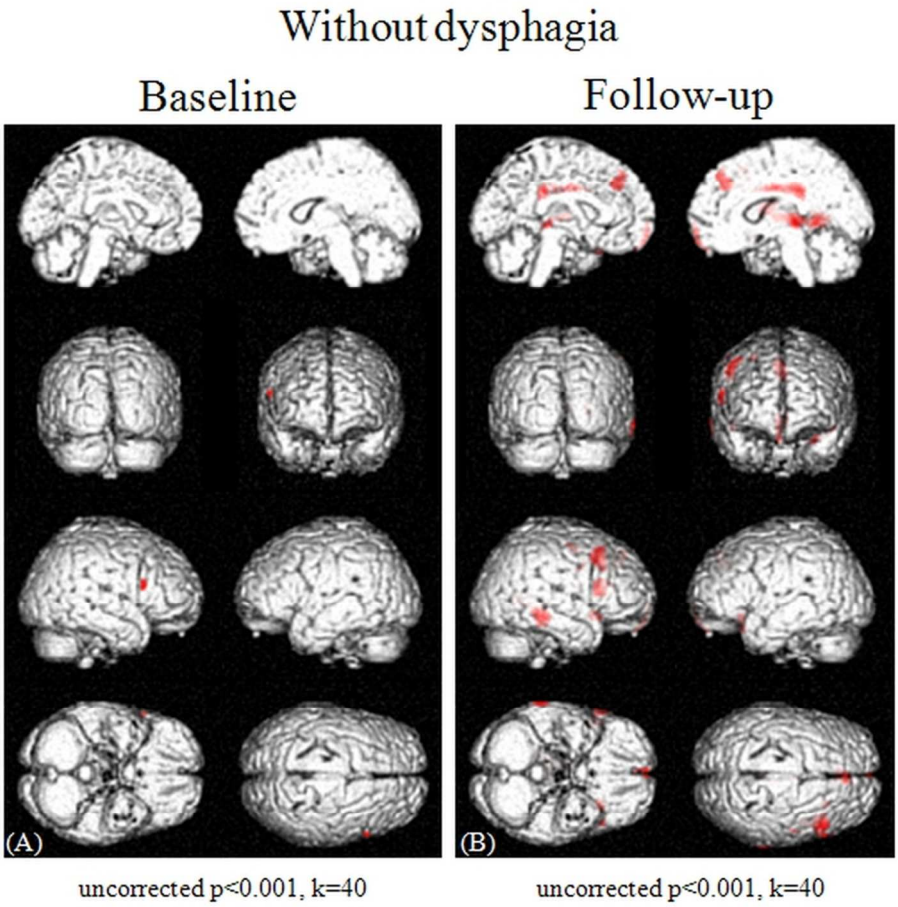
The time needed for swallowing initiation and the 30-second swallowing frequency in PD patients with and without dysphagia at baseline and after a 3-year follow-up period.

There were significant differences between PD patients with and without dysphagia in the time needed for swallowing initiation at baseline and after a 3-year follow-up period ($p<0.05$) (A). In the 30-second swallowing frequency, there was no significant difference between the PD patients with and without dysphagia at baseline ($p>0.05$), but a significant difference between the two groups was evident after the 3-year follow-up period ($p<0.05$) (B).

178x90mm (300 x 300 DPI)



Cross-sectional analyses of brain maps showing the differences between PD patients with dysphagia and normal control subjects at baseline (A) and after a 3-year follow-up period (B). Various areas showed differences in the standardized PET data, and areas with a height threshold of p<0.001 (uncorrected) and an extent threshold of 40 voxels are illustrated. A comparison of regional cerebral glucose metabolism values demonstrated hypometabolism in the SMA and ACC in the PD patients with dysphagia compared with normal control subjects at baseline (uncorrected p<0.001, threshold=40 voxels) (A). After the 3-year follow-up period, the areas of hypometabolism included not only the SMA and the ACC but also the bilateral medial frontal lobes, middle cingulate cortex, thalamus and right superior, middle, inferior, and orbital frontal gyri (uncorrected p<0.001, threshold=40 voxels) (B).
95x90mm (300 x 300 DPI)



Cross-sectional analyses of brain maps showing differences between PD patients without dysphagia and normal control subjects at baseline (A) and after a 3-year follow-up period (B). Various areas showed differences in the standardized PET data, and areas with a height threshold of p<0.001 (uncorrected) and an extent threshold of 40 voxels are illustrated. The PD patients without dysphagia showed virtually no hypometabolism at baseline (uncorrected p<0.001, threshold=40 voxels) (A) and only a small degree of hypometabolism in the SMA and ACC after the 3-year follow-up period (uncorrected p<0.001, threshold=40 voxels) (B) compared with normal control subjects.
95x90mm (300 x 300 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-7
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	5-7
		(*c*) Explain how missing data were addressed	5
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	5

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	7
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7-8
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	7
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-10
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.